Propofol Preferentially Relaxes Neurokinin Receptor-2-induced Airway Smooth Muscle Contraction in Guinea Pig Trachea

Neil R. Gleason, M.D.,* George Gallos, M.D.,§ Yi Zhang, M.D., † Charles W. Emala, M.D.‡

ABSTRACT

Background: Propofol is the anesthetic of choice for patients with reactive airway disease and is thought to reduce intubation- or irritant-induced bronchoconstriction. However, additional neurotransmitters, including neurokinins, play a role in irritant-induced bronchoconstriction. We questioned the mechanistic assumption that the clinically recognized protective effect of propofol against irritant-induced bronchoconstriction during intubation was due to attenuation of airway cholinergic reflexes.

Methods: Muscle force was continuously recorded from isolated guinea pig tracheal rings in organ baths. Rings were subjected to exogenous contractile agonists (acetylcholine, histamine, endothelin-1, substance P, acetyl-substance P, and neurokinin A) or to electrical field stimulation (EFS) to differentiate cholinergic or nonadrenergic, noncholinergic nerve-mediated contraction with or without cumulatively increasing concentrations of propofol, thiopental, etomidate, or ketamine.

Results: Propofol did not attenuate the cholinergic component of EFS-induced contraction at clinically relevant concentrations. In contrast, propofol relaxed nonadrenergic, noncholinergic-mediated EFS contraction at concentrations within the clinical range (20–100 μM, n = 9; P < 0.05), and propofol was more potent against an exogenous selective neurokinin-2 receptor versus neurokinin-1 receptor agonist contraction (n = 6, P < 0.001).

Conclusions: Propofol, at clinically relevant concentrations, relaxes airway smooth muscle contracted by nonadrenergic, noncholinergic-mediated EFS and exogenous neurokinins but not contractions elicited by the cholinergic component of EFS. These findings suggest that the mechanism of protective effects of propofol against irritant-induced bronchoconstriction involves attenuation of tachykinins released from nonadrenergic, noncholinergic nerves acting at neurokinin-2 receptors on airway smooth muscle.

What We Already Know about This Topic

● Propofol is preferred for anesthetic induction in patients with reactive airway disease. The presumed protection of propofol against bronchoconstriction has been decreased airway parasympathetic nerve acetylcholine release

What This Article Tells Us That Is New

● In guinea pig tracheal rings, clinically relevant concentrations of propofol did not block tracheal smooth muscle contraction by parasympathetic nerve acetylcholine release but did attenuate contraction by stimulated nonadrenergic, noncholinergic nerve neurotransmitter release, specifically neurokinin A

Propofol is recognized as the preferred intravenous anesthetic agent in patients with reactive airway disease requiring intubation, an event that can induce irritant-mediated reflex bronchoconstriction.1 The presumed mechanism of airway protection by propofol involves the attenuation of parasympathetic nerve acetylcholine release: the assumed mechanism of irritant-induced bronchoconstriction.2–4 Suprachlinal concentrations of propofol are required to block agonists directly contracting airway smooth muscle, which has lent support to this presumed neural cholinergic mechanism. Although propofol is known to act, in part, by potentiating endogenous γ-aminobutyric acid action at γ-aminobutyric acid receptors type A on airway smooth muscle,5 direct airway smooth muscle effects of propofol at concentrations above those achieved clinically have been attributed to modulation of L-type calcium channels6 and inositol phosphate signaling.7–9 Studies suggesting a vagal nerve-mediated mechanism for propofol have made
two important and perhaps incorrect assumptions of irritant-induced bronchoconstriction: (1) cholinergic nerves are the primary airway efferent nerve and (2) acetylcholine is the primary agonist.

Cholinergic irritant-induced bronchoconstriction is mediated by an irritant-activated afferent fiber signaling to the central nervous system to stimulate cholinergic outflow along the vagus nerve contracting airway smooth muscle via released acetylcholine acting at M3 muscarinic receptors. However, additional irritant-sensing afferent nerves are present in the airway. Non-adrenergic, noncholinergic (NANC) nerves are composed of the following three groups of airway afferent nerves: (1) unmyelinated nociceptive C fibers, (2) rapidly adapting or irritant mechanoreceptors, and (3) slowly adapting stretch receptors differentiated by airway location, physiochemical sensitivity, neurochemistry, and conduction velocities.10–13 Stimulated NANC nerves induce bronchoconstriction in animals and humans by liberated neurotransmitters (e.g., tachykinins).14–18 Tachykinins have multiple effects in airways, including bronchoconstriction, hyperemia, microvascular hyperpermeability, and mucus secretion by affecting airway smooth muscle, mucosal vasculature, submucosal glands, and mast cells through Gq-coupled neurokinin receptors with subtypes 1, 2, and 3.

In this study, we questioned the mechanistic presumption that propofol preferentially relaxes irritant-induced bronchoconstriction by attenuating the activity of efferent parasympathetic cholinergic nerves. To address this mechanistic question, we studied whether propofol was more effective than thiopental, etomidate, or ketamine in attenuating contractile responses were obtained, cumulatively increasing concentrations of propofol (0.5–100 μM), thiopental (0.5–200 μM), ketamine (0.5–50 μM), or etomidate (0.008–16 μM) were added to the baths.

Cholinergic and NANC EFS. Tracheal rings were contracted with cumulatively increasing concentrations of acetylcholine (0.1–100 μM) twice before resting tension was reestablished at 1 g, and either 10 μM thiorphan (acetylcholine experiments) or 1 μM atropine (NANC experiments) was added to the baths. Ten-second trains of square wave direct current EFS (50–50 Hz, 24 V, 0.5 ms pulse width) every 80 s to 20 min were applied through platinum electrodes built into the organ baths. These electrical stimuli induced a contractile response with two distinct components previously characterized: a rapid cholinergic contraction followed by a more slowly developing contraction classic for excitatory (procontractile) NANC contractions.20,21 Once consistent repetitive contractile responses were obtained, cumulatively increasing concentrations of propofol (0.5–100 μM), thiopental (0.5–200 μM), ketamine (0.5–50 μM), or etomidate (0.008–16 μM) were added to the baths.

Exogenous Acetylcholine. Rings were contracted with 1–1.5 μM acetylcholine until three consistent sustained contractions were achieved. Then, propofol (0.5–100 μM), thiopental (0.5–200 μM), ketamine (0.5–50 μM), and etomidate (0.008–16 μM) were added to the baths after a sustained contraction to exogenous acetylcholine.

Exogenous Histamine. Rings were contracted with 1–10 μM histamine and then washed as above until three consistent sustained contractions were achieved. Then, propofol (0.5–100 μM) or thiopental (0.5–200 μM) was added to the baths after a sustained contraction to exogenous histamine was established.

Exogenous Endothelin-1. Tracheal rings were contracted with a single concentration (1 μM) of endothelin-1 before cumulatively increasing concentrations of propofol (0.5–100 μM) or thiopental (0.5–200 μM) were added to the baths after attainment of a stable contraction.

Exogenous Substance P. Tracheal rings were contracted with a single concentration (1–5 μM) of substance P, and after the attainment of a stable contraction, cumulatively increasing concentrations of propofol (0.5–100 μM) or thiopental (0.5–200 μM) were added to the baths. In a separate group of experiments, tracheal rings were pretreated with compound 48/80 (815 mM) and N-vanillylnonanamide (10 μM) to deplete mast cells and NANC nerves of neurotransmitters, respectively. In preliminary studies, tracheal rings contracted with either N-vanillylnonanamide or compound...
48/80 did not contract to a second exposure, confirming the successful depletion of neurotransmitters. After the attainment of a stable contraction in response to substance P, cumulatively increasing concentrations of propofol or thiopental were added as mentioned earlier.

**Exogenous Acetyl-Substance P or Neurokinin A.** Tracheal rings were contracted with a single concentration (0.1 μM) of the neurokinin-1 receptor-selective agonist acetyl-substance P or the neurokinin-2 receptor agonist neurokinin A. After the attainment of a stable contraction for 5 min in response to acetyl-substance P or neurokinin A, cumulatively increasing concentrations of propofol (20–100 μM) were added as mentioned earlier with continuous digital recording of muscle force.

**Reagents**

Propofol (ICN Biomedicals, Costa Mesa, CA) and thiopental (Sigma–Aldrich, St. Louis, MO) were diluted in dimethyl sulfoxide (Fisher Scientific, Waltham, MA) before further dilution in water such that final dimethyl sulfoxide concentration in the bath did not exceed 0.1%. Etomidate (Hospira, Lake Forest, IL) in propylene glycol was diluted in water such that the final concentration of propylene glycol did not exceed 0.2%. Acetyl-substance P, atropine, histamine, acetylcholine, endothelin-1, compound 48/80, indomethacin, and thiorphan were purchased from Sigma–Aldrich. Ketamine and pentobarbital were purchased from Henry Schein Veterinary Co. (Indianapolis, IN). Neurokinin A, tetrodotoxin, and substance P (Calbiochem, San Diego, CA) were suspended in acetic acid and diluted with buffer with no change in the final pH.

**Statistical Analysis**

All statistical analyses were conducted using Graphpad Instat 3.01 software (GraphPad Software, Inc., San Diego, CA) using repeated-measure two-way ANOVA with a post hoc Student t test with Bonferroni correction for multiple comparisons with statistical significance set at \( P < 0.05 \). All \( P \) values were two-tailed. In all experiments, \( n \) is the number of individual tracheal rings studied. All results are presented as mean ± SEM.

**Results**

**Intravenous Anesthetic Relaxation of EFS-induced Tracheal Ring Contractions**

Guinea pig tracheal rings were contracted by EFS using electrical parameters and pretreatments to allow the distinction between cholinergic (postganglionic parasympathetic nerve...
release of acetylcholine) or NANC (C-fiber release of tachykinins) contractions. None of the intravenous anesthetics under study (propofol, thiopental, etomidate, or ketamine) in clinically relevant concentrations relaxed the cholinergic component of EFS-induced contraction (fig. 1, n = 11). Dimethyl sulfoxide (0.1%) and propylene glycol (0.2%) vehicle controls for propofol and etomidate, respectively, neither had significant effect on baseline tone nor induced contractions (data not shown).

In contrast, clinically relevant concentrations of propofol relaxed the NANC component of EFS-induced contraction mediated by tachykinins (n = 9, 20 μM, P < 0.01; 50–100 μM, P < 0.001; fig. 2A). Thiopental caused significant relaxation only at the high end of concentrations achieved clinically (n = 8, 100 μM, P < 0.01; 200 μM, P < 0.001; fig. 2B). Neither ketamine nor etomidate relaxed NANC EFS-induced contraction (n = 11, fig. 2C, and n = 10, fig. 2D, respectively). Ketamine induced a small but significant potentiation of NANC contractions in clinically relevant concentrations (0.5 μM, P < 0.05; 1–5 μM, P < 0.01; fig. 2C).

**Intravenous Anesthetic Relaxation of Exogenous Agonist-induced Contractions**

In agreement with our cholinergic EFS data, propofol was less effective than thiopental, with only the highest concentration of propofol considered clinically relevant (50 μM) attenuating acetylcholine-induced contractions (n = 10, P < 0.05, 50 μM; P < 0.001, 100 μM; fig. 3A). Thiopental concentration dependently relaxed exogenous acetylcholine-induced contractions well within clinically relevant concentrations (n = 11, 10–200 μM, P < 0.001; fig. 3B). Ketamine (n = 5; fig. 3C) and etomidate (n = 5; fig. 3D) were without significant effects on the acetylcholine-induced contraction at clinically relevant concentrations.

Consistent with our NANC EFS findings, propofol, but not thiopental, etomidate, or ketamine, relaxed an exogenous substance P-induced contraction within clinically relevant concentrations (n = 12, 20 μM, P < 0.05; 50–100 μM, P < 0.001; fig. 4A). Thiopental relaxed substance P-induced contractions only at a concentration above those considered clinically relevant (200 μM, n = 11, P < 0.001; fig. 4B). Neither ketamine nor etomidate significantly relaxed an exogenous substance P-induced contraction (figs. 4C and 4D, respectively).

After the depletion of endogenous tachykinins from NANC nerves by capsaicin and histamine from mast cells with compound 48/80, propofol was still more effective than thiopental at relaxing substance P-induced contractions, confirming that this preferential effect of propofol...
was at the level of the airway smooth muscle (n = 4, 20–100 μM; fig. 5).

Additional studies were performed in tracheal rings contracted with the NK1-selective agonist acetyl-substance P or the NK2-selective agonist neurokinin A. Propofol at concentrations considered above the clinical range (100 μM) relaxed both NK1- and NK2-induced contractions; however, propofol significantly attenuated only a NK2-induced contraction at concentrations well within the clinically achieved range (50 μM, P < 0.001 for NK2 compared with NK1 relaxation, n = 6; fig. 6).

Histamine- (1–10 μM) and endothelin-1 (1 μM)-induced contractions were sustained for shorter periods of time compared with contractions induced with acetylcholine or substance P. Spontaneous relaxation of histamine- or endothelin-1-induced contractions was not increased by clinically achieved concentrations of propofol or thiopental (n = 9 for each drug against each contractile agonist; fig. 7).

Discussion

Our primary finding is that clinically relevant concentrations of propofol selectively and uniquely inhibited isolated guinea pig airway smooth muscle contraction induced by selective neurokinin-2 receptor activation and the NANC nerve component of EFS. None of the intravenous anesthetics tested (propofol, thiopental, etomidate, or ketamine) relaxed cholinergic-mediated EFS-induced contraction of airway smooth muscle. Thiopental was more effective than propofol at relaxing exogenous acetylcholine-induced contractions. These findings conflict with the accepted assumptions that the mechanism of preferential protection from irritant-induced bronchoconstriction during intubation by propofol is attenuation of cholinergic nerve acetylcholine release and that irritant-induced vagal nerve acetylcholine release is the predominant mediator of bronchospasm by tracheal intubation.

Interpretation of these findings must account for the relative concentrations of intravenous anesthetics achieved in the airway tissue after bolus administration before irritation of the upper airway during endotracheal tube intubation. The measurement of plasma propofol concentrations during clinical administration is complex, and the concentration of intravenous anesthetics present at the tissue in airways after induction doses is unknown, but serum concentrations allow for a relative comparison. Induction doses of propofol (2–3 mg/kg intravenously) result in peak plasma concentrations of 60–80 μM, whereas maintenance infusions of propofol...
Propofol reportedly achieve approximately 30 μM concentrations.²³–²⁶ Although the concentration in individual tissue compartments is unknown, high tissue uptake of propofol by the lung (30% of bolus dose) has been reported.²⁷ A large percentage of propofol is protein-bound, although the fraction of free drug actually increases at lower concentrations. This would lessen the potential error in our concentration calculations that are performed in the absence of protein binding in buffer in organ baths.²⁸ The peak serum concentrations of etomidate, thiopentone, and ketamine after anesthetic induction are reported to be 5, 25, and 6 μM, respectively.²⁹,³⁰

Many studies in humans and animal airway tissue models demonstrate propofol attenuation of acetylcholine,²,³¹,³² histamine,³³ and endothelin-1 contractile responses² but only at propofol concentrations above those achieved clinically (100–300 μM). Muscarinic receptor-mediated signaling coupled to L-type calcium channels,⁵ intracellular calcium changes,³³,³⁴ or inositol phosphate synthesis⁷ has only been effected by high concentrations of propofol (> 100 μM). Calcium sensitivity in permeabilized canine tracheal smooth muscle cells in the absence or presence of muscarinic receptor activation was not affected by propofol even at concentrations of 270 μM.³⁵ In dogs, propofol attenuated methacholine bronchoconstriction⁶ the neural component of histamine-induced bronchoconstriction,² and vagal nerve-induced bronchoconstriction⁴ only at concentrations of 20 mg/kg (typical human induction dose is 2–3 mg/kg). Taken together, these studies have demonstrated that at clinically relevant concentrations, propofol does not have significant effects on cholinergic modulation of airway smooth muscle.

Conversely, in a sheep model, vagal nerve-induced bronchoconstriction has been shown to be more sensitive to low concentrations of propofol than cholinergic constriction mediated at the airway smooth muscle. Delivery of propofol via the bronchial artery to sheep resulted in attenuation of vagal nerve-induced bronchospasm at lower doses (300 μg/min) and attenuation of methacholine-induced bronchoconstriction only at doses (3 mg/min) believed to be above clinically relevant concentrations by these authors, perhaps suggesting relaxation of an NANC-induced constriction by their parameters.³,³⁷

To confirm that the preferential relaxation of NANC mediated EFS-induced contraction by propofol is not confounded by other C fiber, mast cell, or epithelial cell media-

![Image](https://example.com/image.png)
Exogenous Substance P Contraction after depletion of Non-Adrenergic, Non-Cholinergic Neurotransmitters

Fig. 5. Guinea pig tracheal ring muscle force generated in response to substance P expressed as percent change from peak contraction and response to intravenous anesthetics. (A) Propofol, 0.5–100 μM (n = 4), and thiopental, 10–200 μM (n = 4), versus substance P time control after C-fiber neurotransmitter depletion by capsaicin analog N-vanillylnonanamide, and (B) propofol, 10–100 μM (n = 4), and thiopental, 10–200 μM (n = 4), versus substance P time control after mast cell neurotransmitter depletion by compound 48/80. Only propofol causes significant relaxation of substance P-induced contraction after mast cell and nonadrenergic, noncholinergic nerve neurotransmitter depletion, suggesting a role for propofol-induced relaxation of substance P-induced contraction at the airway smooth muscle itself.

Exogenous Neurokinin-1 vs. Neurokinin-2 Receptor Selective Agonists

Fig. 6. Guinea pig tracheal ring muscle force generated by selective neurokinin-1 and neurokinin-2 receptor agonists acetyl-substance P (Ac sub P) and neurokinin A (NKA), respectively. Data expressed as percent change from peak contraction and response to 20–100 μM propofol (n = 10) versus acetyl-substance P and NKA. Data are expressed as mean ± SEM. Although propofol relaxed both neurokinin-1 and -2 receptor-mediated contractions, only neurokinin-2-mediated contraction was relaxed at clinically relevant concentrations. Relaxation of neurokinin-2-specific agonist NKA suggests that the protective effect of propofol against reflex-induced bronchoconstriction is, at least in part, mediated by the neurokinin-2 receptor-mediated nonadrenergic, noncholinergic contraction. *** P < 0.001.
Exogenous Histamine and Endothelin-1-Induced Contractions

**Fig. 7.** Guinea pig tracheal ring muscle force generated by addition of exogenous histamine or endothelin-1. Data expressed as percent change from peak contraction and response to intravenous anesthetics. (A) Propofol, 0.5–100 μM (n = 10), and thiopental, 0.5–200 μM (n = 10), versus histamine-induced contraction time control, (B) propofol, 0.5–100 μM (n = 10), and thiopental, 0.5–200 μM (n = 10), versus endothelin-1-induced contraction time control. * P < 0.05. Data are expressed as mean ± SEM. Neither propofol nor thiopental relaxed either histamine- or endothelin-1-induced contractions at clinically relevant doses.

More recently, interest in the role of tachykinins in asthma and chronic obstructive pulmonary disease (COPD) and the demonstration of a relationship between reactivity to methacholine and tachykinins in asthmatic airways have led to recent studies demonstrating the effectiveness of dual or triple neurokinin subtype-specific antagonists in blocking neurokinin-induced bronchoconstriction in asthmatics. Taken together, these studies support a tachykinin-mediated contractile pathway in human airway smooth muscle and lend support to the relevance of the current studies in guinea pig airway to the human airway. Despite these important documented roles of tachykinins in bronchoconstriction and more specifically in irritant-induced bronchoconstriction, nothing is known about the interaction of intravenous anesthetics with tachykinins during a routine irritant to the upper airway: an endotracheal tube, and it is possible that the magnitude of NANC contraction in humans differs from the magnitude of NANC contraction in guinea pigs.

Our findings suggest that the mechanism of the protective effect of propofol on irritant-induced bronchoconstriction may be either by decreasing NANC nerve transmission or by attenuating the contractile effect of liberated tachykinins at the neurokinin 2 receptor on the airway smooth muscle itself.

**References**


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As the founder and first president of the American Association of Anesthetists, James Tayloe Gwathmey, M.D. (1862–1944), produced what many regard as America’s first truly comprehensive textbook on anesthesia. Yes, the gilt-lettered, red-covered first edition of his Anesthesia was lavishly illustrated (above, courtesy of the Wood Library-Museum). However, it was Gwathmey’s collaboration with renowned chemist Charles Baskerville, Ph.D., that answered Gwathmey’s plea 8 yr prior “for the scientific administration of anesthetics.” Even 8 yr after its publication, the first edition of Gwathmey’s Anesthesia was saluted by Dr. F. H. McMechan as “the most comprehensive anesthesia textbook now extant. . . .” (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

George S. Bause, M.D., M.P.H., Honorary Curator, ASA’s Wood Library-Museum of Anesthesiology, Park Ridge, Illinois, and Clinical Associate Professor, Case Western Reserve University, Cleveland, Ohio. UJYC@aol.com.