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Propofol Stimulates Ciliary Motility via *the Nitric Oxide–Cyclic GMP Pathway in Cultured Rat Tracheal Epithelial Cells*

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Background: **Airway ciliary motility is impaired by inhaled anesthetics. Recent reports show that nitric oxide (NO) induces upregulation in ciliary beat frequency (CBF), and others report that propofol, an intravenous anesthetic, stimulates NO release; this raises the possibility that propofol increases CBF by stimulating the NO–cyclic guanosine monophosphate (cGMP) signal pathway. In this study, the authors investigated the effects of propofol on CBF and its relation with the NO–cGMP pathway using the pharmacologic blockers N^G-monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor; 1*H*-[1,2,4]oxidazole[4,3-a]quinoxalin-1-one (ODQ), a soluble guanylyl cyclase inhibitor; and KT5823, a cGMP-dependent protein kinase inhibitor, in cultured rat tracheal epithelial cells.**

Methods: **Rat tracheal tissues were explanted and cultured for 3–5 days. Images of ciliated cells were videotaped using a phase-contrast microscope. Baseline CBF and CBF 25 min after exposure to propofol or blocker were measured using video analysis.**

Results: **Vehicle (0.1% dimethyl sulfoxide; n = 11) increased CBF by 0.2 ± 1.7% (mean ± SD) from baseline. Propofol stimulated CBF significantly ($P < 0.01$) and dose dependently (1 μM, 2.0 ± 1.9%, n = 6; 10 μM, 8.2 ± 6.7%, n = 9; 100 μM, 14.0 ± 4.7%, n = 10). Intralipid (0.05%), the clinical vehicle of propofol, did not affect CBF (−0.2 ± 2.2%; n = 5). The enhancement of CBF with use of 100 μM propofol was abolished ($P < 0.01$) by coadministration of 10 mμM L-NMMA (2.4 ± 3.6%; n = 5), 100 μM ODQ (−0.3 ± 2.2%; n = 6) or 30 μM KT5823 (−0.1 ± 4.1%; n = 8). L-NMMA, ODQ, or KT5823 alone did not change CBF.**

Conclusions: **These results show that propofol stimulates CBF *via* the NO–cGMP pathway in rat tracheal epithelial cells, suggesting a possible advantage of propofol in decreasing respiratory risk. (Key words: Airway epithelium; ciliary movement; soluble guanylyl cyclase.)**

CLEARANCE of foreign particles, such as dust and bacteria and debris, from the respiratory tract by airway cilia is an important host defense mechanism.[1–3] It is thought that patients with conditions associated with impaired ciliary function, such as Kartagener syndrome, chronic bronchitis, and asthma or airway injury (including smoking), are predisposed to respiratory infection or atelectasis. Postoperative pulmonary complications often occur in patients with respiratory risks, especially after prolonged anesthesia. Inhaled anesthetics may be one of the risk factors because halothane and isoflurane potently decrease ciliary beat frequency (CBF).[2–5] The use of an anesthetic that does not affect or that promotes ciliary motility may benefit surgical patients with respiratory risk.

Nitric oxide (NO) is a cell-signaling molecule that has broad physiologic activities.[6] NO is produced by NO synthase (NOS) from L-arginine. NO activates soluble guanylyl cyclase (sGC) and produces cyclic guanosine monophosphate (cGMP) in target cells. cGMP activates cGMP-dependent protein kinase (PKG), which causes protein phosphorylation and biologic effects. In addition to NO release and cGMP production in the airway,[7,8] NOS, sGC, and PKG have been found in airway epithelial cells.[8–10] Accumulating evidence has shown that NO and the NO–cGMP signaling pathway play a pivotal role in regulating ciliary motility in airway epithelium.[11–19]

Propofol (2,6 diisopropyl-phenol), an intravenous anesthetic, is reported to stimulate NO release from vascular endothelial cells,[20,21] which raises the possibility that propofol stimulates NO release and increases CBF *via*

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the NO–cGMP pathway in the airway. In this study, we investigated the effects of propofol on CBF and its relation with the NO–cGMP pathway using pharmacologic blockers such as N^G-monomethyl-L-arginine (L-NMMA), an NOS inhibitor; 1*H*-[1,2,4]oxidazole[4,3-a]quinoxalin-1-one (ODQ),[22] an sGC inhibitor; and KT5823,[23] a PKG inhibitor, in cultured rat tracheal epithelial cells.

Materials and Methods

Preparation of Cultured Rat Tracheal Epithelial Cells

Preparation of cultured rat tracheal epithelial cells was performed according to the methods previously described by Dirksen *et al.*[24] The study protocol was approved by the animal research committee of The Johns Hopkins University School of Medicine. Twelve adult, male Sprague-Dawley rats (body weight, 300–350 g; Hilltop, Scottdale, PA) were anesthetized with use of halothane, and the thoracic cage was rapidly opened. The trachea was immediately removed and washed with Hank balanced salt solution (GibcoBRL, Grand Island, NY) with 25 mM N-(2-hydroxy-ethyl)-1-piperazineethanesulfonic acid (HEPES; GibcoBRL), pH 7.4. The excised tissues were cut into small pieces (0.5–1 mm^2) and placed onto glass plates coated with rat tail collagen. The plates were incubated at 37°C in a humidified carbon dioxide incubator (95% air–5% carbon dioxide). Culture medium was a Dulbecco modified Eagle medium (DMEM; GibcoBRL) supplemented with 0.37% (wt/vol) $NaHCO_3$, 10% fetal calf serum (GibcoBRL), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B (Sigma, St. Louis, MI), and 25 mM HEPES.

Protocol and Measurement of Ciliary Beat Frequency

After 3–5 days of incubation, the culture plate was mounted on a phase-contrast microscope (IMT-2; Olympus, Tokyo, Japan). The observation medium was DMEM with 0.037% (wt/vol) $NaHCO_3$ supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml amphotericin B, and 25 mM HEPES. The medium was adjusted to and maintained at pH 7.4 ± 0.1 at a constant temperature (24 ± 1°C) during the experimental period. If any bacterial–fungal contamination was found or if there were no more than three cells with actively beating cilia (defined as CBF greater than 6.0 beats/s) within a 400×-power observation field, the plate was discarded.

Propofol (RBI, Natick, MA), L-NMMA (Alexis, San Diego, CA), ODQ (Alexis) and KT5823 (Carbiochem, San Diego, CA) were dissolved in dimethyl sulfoxide (DMSO; Sigma) in phosphate-buffered saline and diluted appropriately; final DMSO concentration in the medium was 0.1%. Intralipid (KabiVitrum, Wilmington, DE), the propofol vehicle in clinical use, was diluted in the observation medium to a final concentration 0.05%.

The cells were viewed at 400× magnification. All observations were monitored and recorded for analysis using a 3CCD color videocamera (IK-TU40A; Toshiba, Tokyo, Japan), an S-VHS video cassette recorder (HR-S5400U; JVC, Tokyo, Japan), and a Trinitron color monitor (CVM-1271; Sony, Tokyo, Japan). After a 5-min control recording period, propofol, vehicle, or a blocker were added at random to the medium, and the image was recorded for the subsequent 25 min. To determine CBF, video images were later captured at 30 frames/s and digitized using a Macintosh computer (Apple Computer Inc., Santa Clara, CA) and Adobe Premiere software (Adobe, San Jose, CA). The images of cilia were viewed frame by frame by a researcher who was blind to the drug preparation administered. CBF was counted manually at least 3 times/cell and averaged. The average was regarded as the CBF value of the single cell. The CBF value of one plate was the average of values for at least three independent cells.

Statistical Analysis

All values were expressed as mean ± SD. Values of *n* represent the number of the plates. Comparisons of trends over time of two groups were performed with use of two-factor repeated-measures analysis of variance. Time-matched values in the groups were compared using the Bonferroni test after one-way analysis of variance. A *P* value less than 0.05 was considered to be statistically significant.

Results

Propofol at a dose of 100 μM (n = 10) increased CBF significantly ($P < 0.0001$), from 7.3 ± 0.7 beats/s at time 0 (baseline) to 8.4 ± 0.8 beats/s 25 min after the administration, compared with vehicle administration (0.1% DMSO, n = 11; 7.4 ± 0.6 beats/s at baseline to 7.4 ± 0.6 beats/s at 25 min; fig. 1A). The effect of propofol plateaued at 15–25 min postadministration.

Exposure to 0.05% intralipid (n = 5), the clinical vehicle of propofol, did not affect CBF (−0.2 ± 2.2%

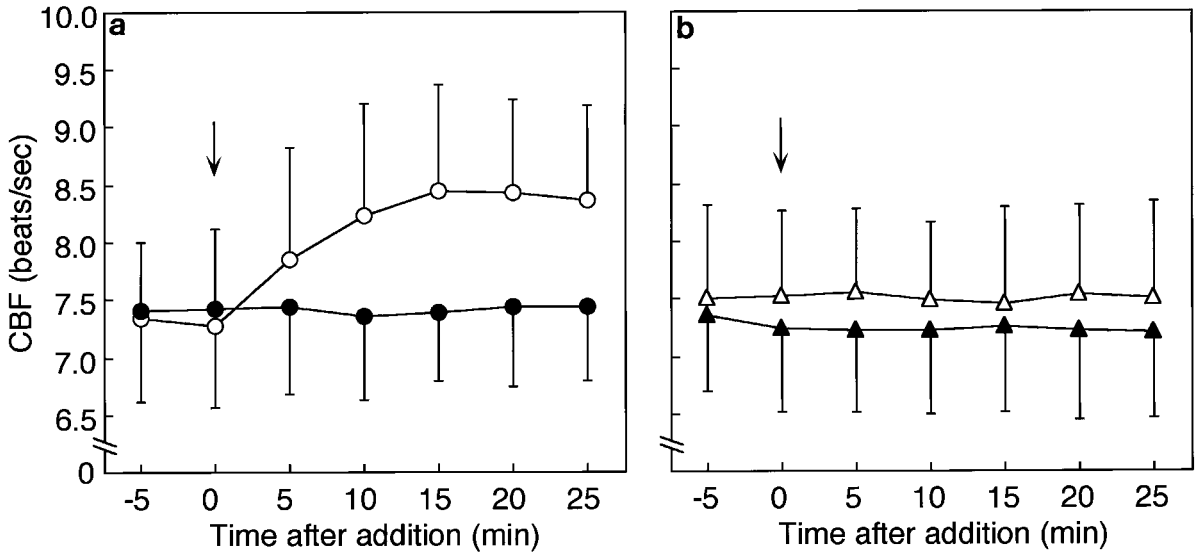

Fig. 1. Time course of the effect of (*A*) propofol and (*B*) intralipid (the clinical vehicle of propofol) on ciliary beat frequency (CBF) in cultured rat tracheal epithelial cells. After the control period (−5 min–0 min), dimethyl sulfoxide (DMSO; final concentration, 0.1%; n = 11; closed circles), propofol (final concentration, 100 μM; n = 10; open circles), Dulbecco modified Eagle medium (DMEM; n = 11; closed triangles) or intralipid (final concentration, 0.05%; n = 5; open triangles) was added to the medium at time zero (arrow), and CBF was observed during the subsequent 25 min. Values are mean ± SD. There is a significant difference over time between propofol and DMSO ($P < 0.0001$), but not between intralipid and DMEM ($P = 0.7336$).

increase from baseline; baseline = 100%; fig. 1B). Observation medium (DMEM) alone also did not change CBF (−0.3 ± 3.3%).

Propofol increased CBF in a dose-dependent manner (0 mM or vehicle, 0.2 ± 1.7% increase from baseline, n = 11; 1 μM, 2.0 ± 1.9%, n = 6; 10 μM, 8.2 ± 6.7%, n = 9; 30 μM, 13.0 ± 7.1%, n = 8; 100 μM, 14.0 ± 4.7%, n = 10; 300 μM, 13.8 ± 6.4%, n = 9; fig. 2). The effect of propofol plateaued at doses of 30–300 μM.

Enhancement of CBF by 100 μM propofol was significantly and dose-dependently inhibited by coadministration of L-NMMA (0.1 mM, 11.0 ± 2.9%, n = 4; 1 mM, 5.6 ± 7.7%, n = 10; 10 mM, 2.4 ± 3.6%, n = 5; fig. 3). Ten millimoles of L-NMMA alone did not affect CBF (−0.3 ± 3.3%; n = 6).

The enhancement of CBF by 100 μM propofol was significantly and dose-dependently inhibited by coadministration of ODQ (1 μM, 14.0 ± 5.9%, n = 4; 10 μM, 8.0 ± 4.8%, n = 4; 100 μM, −0.3 ± 2.2%, n = 6; fig. 4). One hundred micromoles of ODQ completely abolished the propofol-induced CBF enhancement. ODQ alone did not affect CBF (−0.5 ± 2.2%; n = 6).

Enhancement of CBF by 100 μM propofol was significantly and dose-dependently inhibited by coadministration of KT5823 (0.03 μM, 13.8 ± 3.9%, n = 5; 0.3 μM, 9.0 ± 4.2%, n = 5; 3 μM, 5.0 ± 3.5%, n = 5; 30 μM, −0.1 ± 4.1%, n = 8; fig. 5). Thirty micromoles of KT5823 totally inhibited the propofol-induced CBF enhancement. KT5823 alone did not affect CBF (−1.3 ± 4.1%; n = 7).

Discussion

In this study, we demonstrated that propofol stimulates CBF in a dose-dependent fashion and suggest that the NO–cGMP signaling pathway is involved in its stimulation in cultured rat tracheal epithelial cells (fig. 6).

The plasma concentrations of propofol reported in humans and rats during sedation or anesthesia are approximately 2–10 μg/ml, or 10–50 μM.[25–27] These doses of propofol stimulate CBF in our *in vitro* setting. Because propofol in blood is more than 95% protein-bound in rats and humans,[28–30] actual concentrations of the drug in tissues are uncertain, but probably are much less than the apparent plasma concentrations. Although we performed our experiment using the serum protein–contained medium, we cannot eliminate the possibility that the concentrations that stimulate CBF *in vitro* are different from those that can stimulate CBF *in vivo*.

Raphael and Butt[31] demonstrated that CBF of nasal tissues in patients anesthetized using propofol and alfentanil did not change, whereas isoflurane anesthesia de-

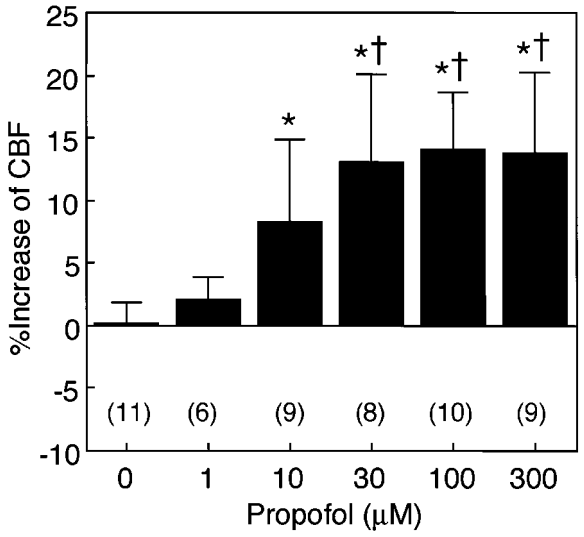

Fig. 2. Effect of propofol (0–300 μM) on ciliary beat frequency (CBF) in cultured rat tracheal epithelial cells. Immediately after the baseline CBF was determined, vehicle (0.1% dimethyl sulfoxide) or various concentrations of propofol were added at time 0, and CBF was measured 25 min thereafter. Values are expressed as a percentage change from baseline CBF (baseline = 100%) and represent the mean ± SD. There is a significant difference among the groups ($P < 0.0001$). *$P < 0.05$, significantly different from vehicle; †$P < 0.05$, significantly different from 1 μM propofol.

creased CBF.[31] His group also reported that propofol at a dose of 70 μM did not change CBF in human nasal turbinate explants.[32] The discrepancy between their results and ours may be, at least in part, the result of different experimental conditions and use of tissues from different species. We used rat tracheal tissues after 3–5 days of culture, whereas they used human nasal tissues on the same day of harvest.

Inhalation anesthetics such as halothane and isoflurane have been shown to depress ciliary function,[2–5,31] although short exposure (< 2 min) stimulates CBF.[33,34] Other anesthesia-related drugs, such as local anesthetics, barbiturates, and benzodiazepines, also inhibit CBF.[35–38] Patients with airway diseases who have impaired ciliary function are at greater risk of postoperative pulmonary complications. Anesthetic or sedative drugs that have an accelerating effect on CBF are advantageous in patients with respiratory risk. Considering the limitations of our model, additional studies, including a series of experiments for *in vivo* measurements, are needed to verify our observations.

We and others have reported that endothelial and inducible NOS (eNOS and iNOS), sGC, and PKG-I are localized in ciliated airway epithelia.[8–10] Nasal NO concentration correlates with ciliary functions.[7] NO or cGMP production from airway epithelium has also been reported.[8,12] NOS inhibitors decrease CBF after prestimulation with isoproterenol, substance P, bradykinin, tumor necrosis factor α, interleukin-1β, L-arginine, and ethanol.[11–18] 8-Bromo-cGMP activates PKG and increases CBF.[8,18] PKG inhibitors significantly inhibit L-arginine–, nitroprusside-, or isoproterenol-induced enhancement of CBF.[8,18] Thus, these studies indicate that ciliary motility is regulated by the NO–cGMP pathway in an autocrine–paracrine fashion. Petros *et al.*[20] reported that propofol stimulated cGMP formation in cocultured porcine endothelial and smooth muscle cells, and this stimulation was inhibited by a NOS inhibitor. Liu *et al.*[21] demonstrated that propofol increased cGMP content in cultured bovine vascular smooth muscle cells, and this increase was inhibited by sGC inhibitors. These results raise the possibility that propofol stimulates NO and cGMP production through activation of NOS and sGC in the airway. Presently, we cannot say which type of cell produces NO by propofol stimulation because, in addition to ciliated epithelial cells, there are other kinds of cells, in-

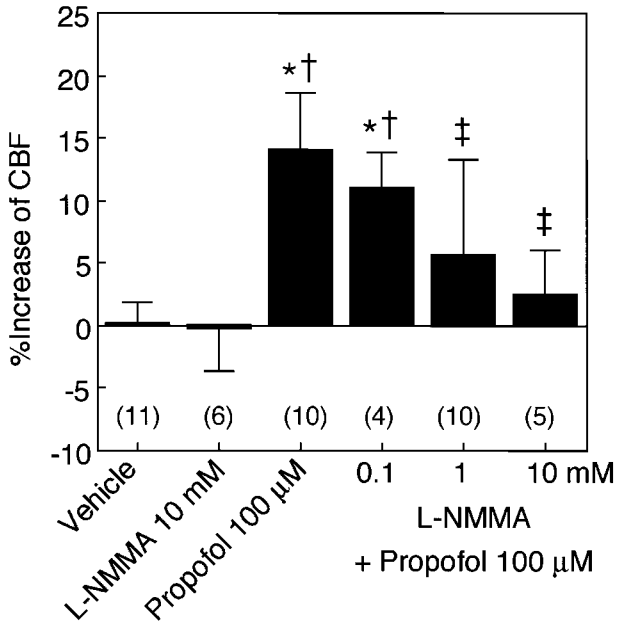

Fig. 3. Effect of N^G-monomethyl-L-arginine (L-NMMA) on propofol-induced enhancement of ciliary beat frequency (CBF). CBF was measured at baseline and 25 min after the addition of vehicle (0.1% dimethyl sulfoxide), propofol (100 μM), or various concentrations of L-NMMA (0.1–10 mM). Values are expressed as a percentage change from baseline CBF and represent the mean ± SD. There is a significant difference among the groups ($P < 0.0001$). *$P < 0.05$, significantly different from vehicle; †$P < 0.05$, significantly different from 10 mM L-NMMA; ‡$P < 0.05$, significantly different from 100 μM propofol.

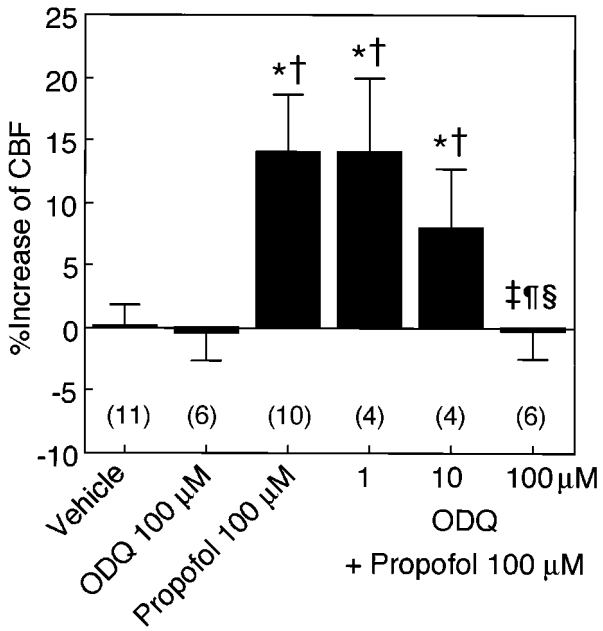

Fig. 4. Effect of 1-*H*-[1,2,4]oxidazole[4,3-a]quinoxalin-1-one (ODQ, Ki = 0.3 μM)[22] on propofol-induced enhancement of ciliary beat frequency (CBF). CBF was measured at baseline and 25 min after the addition of vehicle (0.1% dimethyl sulfoxide), propofol (100 μM), or various concentrations of ODQ (1–100 μM). Values are expressed as a percentage change from baseline CBF and represent the mean ± SD. There is a significant difference among the groups ($P < 0.0001$). *$P < 0.05$, significantly different from vehicle; †$P < 0.05$, significantly different from 100 μM ODQ; ‡$P < 0.05$, significantly different from 100 μM propofol; ¶$P < 0.05$, significantly different from 1 μM ODQ + 100 μM propofol; §$P < 0.05$, significantly different from 10 μM ODQ + 100 μM propofol.

cluding nonciliated epithelial cells, smooth muscle cells, and fibroblasts, in our cultured tracheal tissues.[24] Miyawaki *et al.*[39] reported that propofol inhibited acetylcholine-stimulated cGMP formation in rat aortic strips, indicating a need for further studies to clarify the propofol actions on NO–cGMP formation not only in airways, but also in the vasculature.

The intracellular free-calcium concentration ($[Ca^{2+}]i$) is crucially involved in the regulation of CBF.[2] Elevation of $[Ca^{2+}]i$ increases CBF, whereas the decrease of $[Ca^{2+}]i$ causes CBF to slow. Recently, Uzlaner and Priel[17] demonstrated that dibutylyl–cGMP alone did not stimulate CBF in cultured rabbit tracheal epithelial cells when $[Ca^{2+}]i$ was not increased, whereas it could elevate CBF strongly when $[Ca^{2+}]i$ was increased by ionomycin or adenosine triphosphate (ATP), suggesting that elevated $[Ca^{2+}]i$ is necessitated for the NO–cGMP system-induced enhancement of CBF. Elevated $[Ca^{2+}]i$ also activates endothelial NOS and the NO–cGMP pathway.[6,40] There is a possibility that propofol increases $[Ca^{2+}]i$ in airway epithelial cells. Propofol is reported to increase $[Ca^{2+}]i$ in cultured rat embryonic brain cells and human glial cells,[41] whereas it reduced $[Ca^{2+}]i$ in cardiomyocytes[42] and vascular smooth muscle cells.[43] The discrepancy between results obtained by Raphael and Butt[31] and Hann *et al.*[32] and ours are possibly attributable to a difference in $[Ca^{2+}]i$ condition. Further studies are needed to investigate the effect of propofol on $[Ca^{2+}]i$ in airway epithelial cells.

The current study clearly shows that propofol stimulates CBF *via* the NO–cGMP pathway in cultured rat tracheal ciliated epithelial cells. Although this study suggests a possible advantage for using propofol in patients with respiratory risk, further studies, including clinical trials, are necessary.

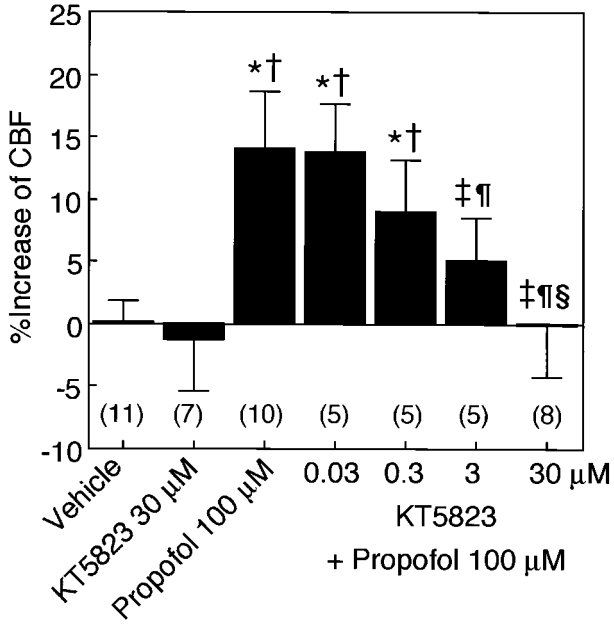

Fig. 5. Effect of KT5823 (Ki = 0.2 μM)[23] on propofol-induced enhancement of ciliary beat frequency (CBF). CBF was measured at baseline and 25 min after addition of vehicle (0.1% dimethyl sulfoxide), propofol (100 μM), or various concentrations of KT5823 (0.03–30 μM). Values are expressed as a percentage change from baseline CBF and represent the mean ± SD. There is a significant difference among the groups ($P < 0.0001$). *$P < 0.05$, significantly different from vehicle; †$P < 0.05$, significantly different from 30 μM KT5823; ‡$P < 0.05$, significantly different from 100 μM propofol; ¶$P < 0.05$, significantly different from 0.03 μM KT5823 + 100 μM propofol; §$P < 0.05$, significantly different from 0.3 μM KT5823 + 100 μM propofol.

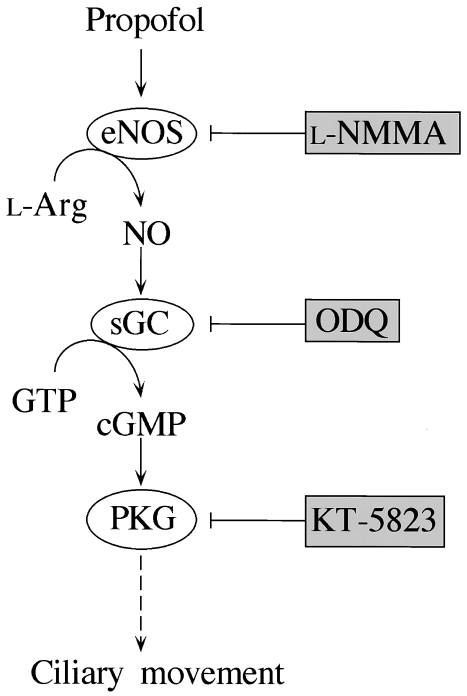

Fig. 6. A hypothetical scheme. Propofol may stimulate the nitric oxide–cyclic guanosine monophosphate (NO–cGMP) signaling pathway that activates ciliary movement in the airway. eNOS = endothelial type of NO synthase; L-Arg = L-arginine; sGC = soluble guanylyl cyclase; GTP = guanosine triphosphate; PKG = cGMP-dependent protein kinase; L-NMMA = N^G-monomethyl-L-arginine; ODQ = 1-*H*-[1,2,4]oxidazole[4,3-a]quinoxalin-1-one.

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