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The influence of propofol, remifentanil and lidocaine on the tone of human bronchial smooth muscle

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ABSTRACT

Background: Bronchoscopy is generally a safe procedure, but the induction of anaesthesia can induce bronchospasm. Consequently we investigated the influence of propofol, remifentanil and lidocaine on the tone of the human bronchial smooth muscle.
Materials and methods: The influence of propofol, remifentanil and lidocaine on the contractile response of human bronchial smooth muscle to electrical field stimulation (EFS) has been evaluated. The role of capsaicin-sensitive sensory nerves and of inducible nitric oxide synthase has also been assessed. Furthermore, the interaction between these three dugs has been measured by Bliss Independence (BI) theory. Statistical significance ($P < 0.05$) was assessed by Student's t test or ANOVA.
Results: Propofol (1.3 μg ml^{-1}) and lidocaine (1 mg ml^{-1}) reduced the baseline tone of bronchial rings ($-14.45 \pm 4.53\%$ and $-33.40 \pm 1.07\%$, respectively, $P < 0.05$), whereas remifentanil had not such effect. Aminoguanidine prevented the relaxant effect of propofol. Propofol did not alter the bronchial contractile response to EFS following 30 min of treatment, whereas remifentanil enhanced the bronchial tension ($133.83 \pm 9.38\%$, control $101.93 \pm 6.82\%$, $P < 0.05$ $P < 0.05$) and lidocaine completely abolished the contractility at 1 mg ml^{-1} ($P < 0.05$). The desensitization of capsaicin-sensitive sensory nerves normalized the hyperresponsiveness induced by remifentanil ($-26.77 \pm 1.68\%$, $P < 0.05$). Significant BI antagonism ($P < 0.001$) was detected for propofol and lidocaine on the bronchial hyperresponsiveness induced by remifentanil.
Conclusion: Propofol and remifentanil may be used safely for bronchoscopy, although remifentanil should be associated with propofol or lidocaine to prevent the potential opioid-mediated bronchospasm.

1. Introduction

The recent American College of Chest Physicians (ACCP) consensus statement on the use of topical anaesthesia, analgesia, and sedation during flexible bronchoscopy in adult patients proposes that all physicians performing bronchoscopy should consider analgesic and sedative agents, when feasible [1]. In particular, sedation is suggested in all patients undergoing bronchoscopy unless contraindications exist [1]. Nowadays, on the basis of published evidence, bronchoscopy without sedation could possibly be considered unethical, especially in cases where complex and time-consuming procedures are planned [2].

On the other hand, although bronchoscopy is generally considered to be a safe procedure, bronchospasm is a possible adverse effect [3]. The induction of anaesthesia and intubation of the trachea may cause airway constriction, and preoperative bronchospasm may occur in patients with normal or pathological airways [4].

Opioids and propofol, which is a sedative–hypnotic agent frequently used in the induction and maintenance of anaesthesia [5], are central for inducing deep sedation in the setting of bronchoscopy, along with lidocaine, the preferred topical anaesthetic for this procedure [1,6,7]. Unfortunately, data on the use of opioids as single agents for bronchoscopy are limited because they are used in combination with benzodiazepines [1]. Also data evaluating propofol, for flexible bronchoscopy are limited, although Stolz and

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colleagues [8] reported that it is as effective and safe as combined sedation in patients undergoing such a type of procedure. Moreover, as yet, data evaluating the direct impact of these agents on human airways are scarce.

Therefore, the purpose of this study was to investigate the effect of propofol, lidocaine and remifentanil, a novel synthetic ultra-short acting μ-opioid agonist, on contraction of human airway smooth muscle (ASM).

2. Materials and methods

2.1. Tissue preparation

Macroscopically normal airways were obtained from 13 patients (6 male, 7 female, 56.4 ± 4.3years old) undergoing surgery for lung cancer, without a history of chronic airway disease or diabetes mellitus. There were no differences among patients concerning treatments, age and diagnosis ($P > 0.05$).

Samples were taken from an area as far as possible from the malignancy. Histology confirmed the absence of microscopic alteration of bronchial tissues. Samples were placed into Krebs-Henseleit buffer solution (KH) (NaCl, 119.0 mmol; KCl, 5.4 mmol; $CaCl_2$, 2.5 mmol; KH_2PO_4 mmol, 1.2 mmol; $MgSO_4$, 1.2 mmol; $NaHCO_3$, 25.0 mmol; glucose, 5.5 mmol; pH 7.4) containing indomethacin (5 μM) and transported to the laboratory. None of the patients were receiving treatment with xanthines, β_2-adrenoceptor agonists, glucocorticosteroids or muscarinic antagonists. Preoperative lung function parameters were generally normal and there were no signs of respiratory infections.

Patients signed an informed consent consistent with document concerning the collection of biological samples for research purposes (National Committee of Bioethics, National Committee of Biosafety, Biotechnology and Sciences: collection of biological samples for research purposes, February 16, 2009, Italy).

Airways were cut into rings ($n \simeq 70$; thickness: 1–2 mm; diameter: 5–7 mm) and transferred into a 4 channel 10 ml Isolated Organ Bath system (Ugo Basile Italy) containing KH-buffer (37 °C) plus indomethacin (5 μM) and aerated with O_2/CO_2 (95:5%). Bronchial tissues were mounted into the Isolated Organ Bath system at the least 24 h after resection from the lung in order to avoid any interference with anaesthetics used in the surgery.

Tissues were allowed to equilibrate for 90 min during which time the KH-buffer was changed every 10 min. Samples were mounted on hooks where one hook was attached with thread to a stationary rod and the other hook tied with thread to an isometric force displacement transducer. During equilibration, passive tension (0.5–1.0 g) was determined. The isometric change in tension was measured using a force transducer (Fort10WPI Ugo Basile Italy) and the responsiveness was assessed by acetylcholine (100 μM). On reaching a plateau response, rings were washed three times and allowed to equilibrate for a further 30 min as described elsewhere [9,10].

2.2. Preparation of drugs

The following drugs and compounds were used in this study: acetylcholine, aminoguanidine, capsaicin, indomethacin and lidocaine (Sigma–Aldrich, Italy); propofol (AstraZeneca, Italy); remifentanil (GlaxoSmithKline, Italy).

All drug stock solutions were prepared in distilled water, excluding capsaicin and indomethacin, which were dissolved in ethanol and indomethacin was after added to KH-buffer. Other drugs were then diluted in KH-buffer to be used at the final concentration in the isolated organ bath system. The maximal amount of ethanol added to the bath (<0.01%) did not alter the reactivity of the preparation to acetylcholine or EFS [11,12].

Compounds were stored in small aliquots under refrigeration until used. Fresh aliquots were used for each experiment after dilution in KH-buffer.

2.3. Transmural stimulation

Each organ bath was fitted with two platinum plate electrodes (1 cm^2) placed alongside the tissue (10 mm apart) for electrical field stimulation (EFS). In order to simulate *ex vivo* the vagus firing normally observed at the mean physiological frequency *in vivo* in human, the experimental studies were carried out by contracting bronchial rings with EFS (biphasic pulse with a constant current of 10 V, 0.5 ms, 10 s) at 10 Hz, one pulse every 5 min, as described elsewhere [13,14].

2.4. Capsaicin-sensitive sensory nerves desensitization protocol

In some experiments, the influence of capsaicin-sensitive sensory nerves on the bronchial contraction mediated by EFS was assessed. Bronchial rings were initially desensitized by five consecutive administrations of capsaicin (10 μM, 1 h apart from each other) as described elsewhere [15,16] and followed by transmural stimulation [17,18].

2.5. Study design

At the beginning of the study, reference bronchial contractions induced by EFS were recorded. After that, isolated airways were stimulated by EFS and treated for 30 min with propofol (1.3 μg ml^{-1}) and remifentanil (1 ng ml^{-1}) in order to mimic *in vitro* the conditions (timing and drug concentrations) typical for a sedation carried out via plasma-site concentrations by target-controlled infusion (TCI) in patients undergoing bronchoscopy [19–22]. The drug concentrations in the isolated organ bath were equivalent to the plasma-site concentrations set up for bronchoscopies performed at the Division of Thoracic Surgery, Sant'Andrea Hospital (Rome, Italy) by using the open-source software StanPump (Stanford, CA, USA) for TCI [19–22].

Since the topical instillation/nebulisation of a local anaesthetic along the respiratory tree is a usual practice during bronchoscopy, we also evaluated the influence of lidocaine, ranging from 1 μg ml^{-1} to 1 mg ml^{-1}, on the responsiveness of human bronchi to EFS [6,7].

In further experiments, the isolated bronchi were pre-treated for 45 min with the selective inhibitor of inducible nitric oxide (NO) synthase (iNOS), aminoguanidine (100 μmol) and then treated with propofol [23]. Moreover, the EFS responsiveness in the presence of remifentanil was tested in desensitized tissues [15,16].

2.6. Interaction analysis

The analysis of the potential synergism/antagonism between propofol, lidocaine and remifentanil was measured by applying the Bliss Independence (BI) theory. The main assumption of the BI theory is that two or more agents act independently from one another. In particular, if fulfilling the criterion, the mode, and possibly also the site of action of the compounds in the mixture, always differ. The BI theory for two agents is expressed by the following equation: $E(x,y) = Ex + Ey - (Ex^{*}Ey)$, where E is the fractional effect, and x and y are the doses of two compounds in a combination experiment. If the combination effect is higher than the expected value from the above equation, the interaction is synergistic, while if this effect is lower, the interaction is antagonistic. Otherwise, the effect is additive and there is no interaction [24–29]. In this protocol, the BI equation was characterized by X = remifentanil and Y = propofol or Y = lidocaine.

2.7. Analysis of results

Bronchial contractile tension induced by EFS was measured as percentage of control bronchi and polynomial curves were constructed by fitting models of biological data using nonlinear regression [30]. *E*max was identified as the highest contractile force induced by EFS stimulation and the offset ($t_{1/2}$, min) indicates the time to evoke a half of maximal contraction. For every three bronchial rings mounted in the isolated organ bath system, one was used as a time control [31].

When necessary, appropriate curve-fitting to a sigmoidal model was used to calculate the *E*max and the EC_{50} for dose response curves. The equation used was log (agonist) *vs.* response, Variable slope, expressed as Y = Bottom + (Top−Bottom)/{1+10-[(Log EC50-X)*HillSlope]} [30,32].

Statistical significance was assessed by Student's *t* test or ANOVA with Bonferroni post-tests if necessary. All data analyses were performed using computer software (GraphPad Prism version 5.00 for Mac, GraphPad Software, San Diego California USA). Values are presented as mean ± SEM of *n* bronchial rings from different subjects and the level of statistical significance was defined as $P < 0.05$ [33].

3. Results

3.1. Baseline characteristics of bronchial rings

There were no significant differences ($P > 0.05$) between the baseline characteristics of the human isolated bronchial rings employed in the study concerning the wet weight (245.3 ± 20.9 mg), the contraction induced by acetylcholine 100 μM (469 ± 124 mg) and the contraction induced by EFS 10 Hz before treatments with drugs (441 ± 65 mg).

3.2. Influence of propofol, remifentanil and lidocaine on the baseline tension of bronchial rings

In preliminary studies, we evaluated the influence of propofol, remifentanil and lidocaine on the baseline tension of human bronchial rings after the equilibration time.

Propofol and lidocaine at 100 μg ml^{-1} weakly reduced the baseline tone of bronchial rings (−14.45 ± 4.53% $P < 0.01$ and −11.13 ± 0.36% $P < 0.05$, respectively) whereas lidocaine at the highest concentration (1 mg ml^{-1}) induced a significant relaxation of the baseline tone (−33.40 ± 1.07%, $P < 0.001$) compared to control bronchi. On the other hand, remifentanil did not modify the baseline tone in human isolated bronchi and the pre-treatment with aminoguanidine, which did not alter the baseline tension (0.53 ± 4.08%) *per se*, prevented the relaxant effect of propofol ($P > 0.05$) (Fig. 1).

3.3. Influence of propofol, remifentanil and lidocaine on the tone of human bronchial smooth muscle

Propofol did not significantly alter the bronchial response induced by EFS through 30 min of treatment (propofol 111.26 ± 6.89%, control 100.23 ± 6.10%, $P > 0.05$), whereas remifentanil significantly enhanced the bronchial tension elicited by EFS in 30 min of treatment, compared to control bronchi (remifentanil 133.83 ± 9.38%, control 101.93 ± 6.82%, $P < 0.05$). Although remifentanil induced a weak signal for bronchial relaxation at 10 min and 15 min (−16.76 ± 1.17%, $P = 0.138$), it significantly ($P < 0.05$) increased the bronchial responsiveness starting from the 20th min of treatment, inducing a positive trendline contraction of +3.41% min^{-1} (+31.98 ± 2.00% at 30th min) and a $t_{1/2}$ value of 26 ± 1 min (Fig. 2).

Lidocaine administrated at increasing concentration significantly ($P < 0.001$) reduced the bronchial *E*max tension by EFS (*E*max −9.22 ± 9.89%; EC50 68.80 μg ml^{-1}, CI95% 31.31–151.30 μg ml^{-1}) compared to control bronchi (*E*max 97.33 ± 6.82%, EC50 not detectable). Lidocaine completely abolished the bronchial contraction at the concentration of 1 mg ml^{-1}, whereas did not modify the bronchial tone at 10 μg ml^{-1}, compared to control bronchi (Fig. 3).

The EFS response of time-control bronchi did not evidence any significant modification through the study (data not shown).

3.4. The effect of capsaicin-sensitive sensory nerves and remifentanil

The desensitization of capsaicin-sensitive sensory nerves, by five consecutive capsaicin administrations, significantly reduced (−26.77 ± 1.68%, $P < 0.05$) the contraction induced by EFS in tissues treated with remifentanil, normalizing the bronchial response to

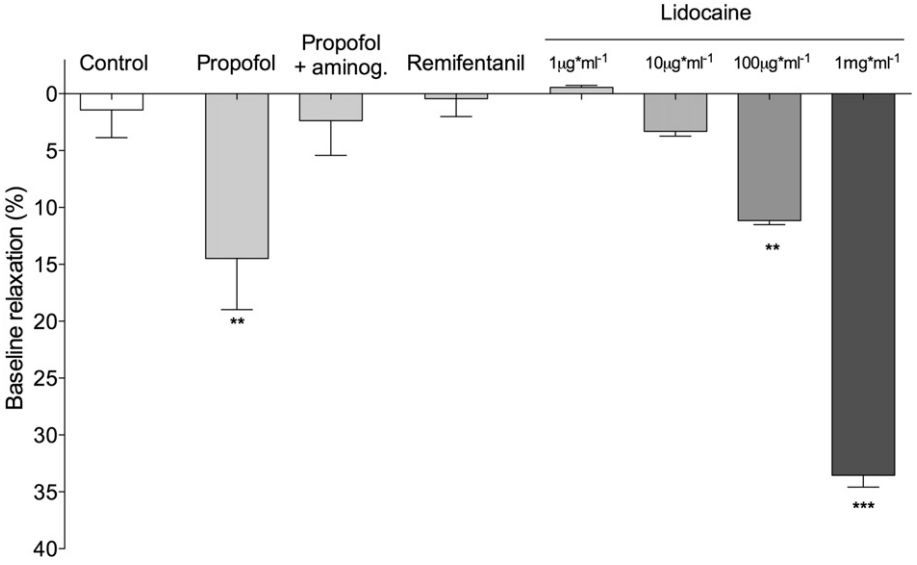

Fig. 1. Baseline relaxation of human isolated bronchi treated with propofol ($n = 5$), propofol and aminoguanidine ($n = 3$), remifentanil ($n = 4$) and lidocaine ($n = 3$). Results shown are represented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* control bronchi.

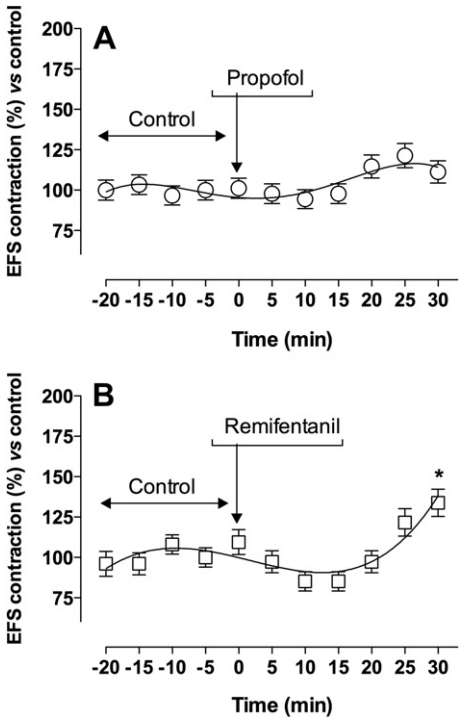

Fig. 2. Line graph representing trendline of contraction of human isolated bronchial preparations to EFS through 30 min treatment with propofol (1.3 μg ml^{-1}) and remifentanil (1 ng ml^{-1}). Points shown are from experiments performed with samples of $n = 3$ different subjects and they are represented as mean ± SEM; $^{*}P < 0.05$ *vs.* control.

EFS (remifentanil and desensitization 107.06 ± 6.71%, control bronchi 100.00 ± 5.38%, $P > 0.05$) (Fig. 4). Desensitizing bronchi with capsaicin did not significantly alter the bronchial response to EFS (93.56 ± 7.90%, $P > 0.05$) compared to control bronchi.

3.5. The protective effect of propofol and lidocaine on human bronchial hyperresponsiveness induced by remifentanil in human bronchial smooth muscle

Propofol significantly reduced (−25.03 ± 1.32%, $P < 0.05$) the contracltile effect of remifentanil (remifentanil plus propofol 108.80 ± 6.10, control 100.00 ± 6.48, $P > 0.05$). Also lidocaine (10 μg ml^{-1}) prevented (−20.28 ± 1.42%, $P < 0.05$) the bronchial hyperresponsiveness mediated by remifentanil (lidocaine plus

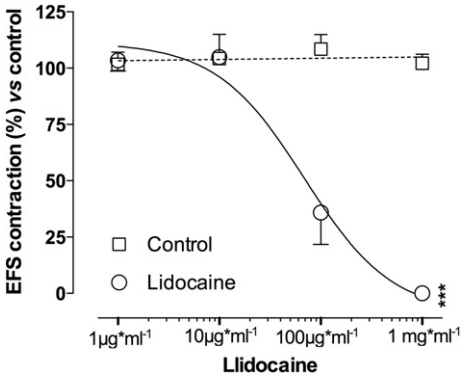

Fig. 3. Human bronchial relaxation of lidocaine on the contractile response induced by EFS. Points shown are from experiments performed with samples of $n = 3$ different subjects and they are represented as mean ± SEM; $^{***}P < 0.001$ *vs.* control bronchi.

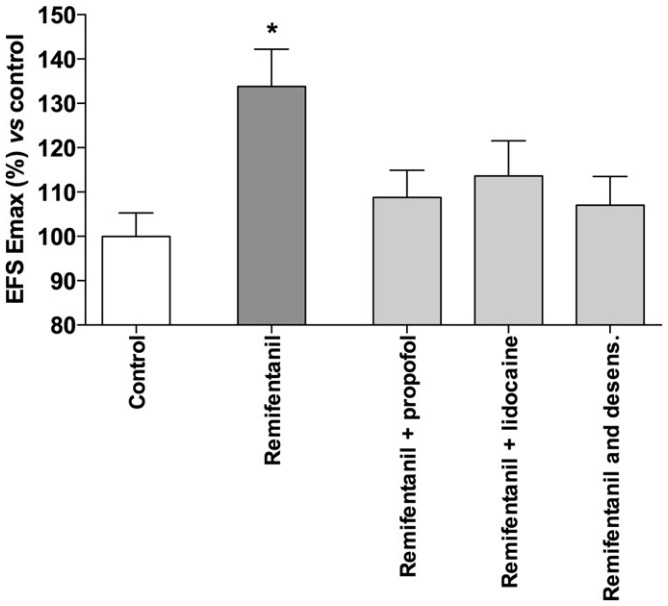

Fig. 4. Influence of propofol (1.3 μg ml^{-1}), lidocaine (1 μg ml^{-1}) and desensitization of capsaicin-sensory nerves on the hyperresponsiveness induced by remifentanil (1 ng ml^{-1}) in human bronchi stimulated with EFS. Data shown are from experiments performed with samples of $n = 3$ different subjects and they are represented as mean ± SEM. $^{*}P < 0.05$ *vs.* control bronchi.

remifentanil 113.55 ± 7.95%, control 101.93 ± 6.82%, $P > 0.05$) (Fig. 4).

The results of the BI analysis for the pharmacodynamic interaction of propofol and lidocaine on the bronchial hyperresponsiveness induced by remifentanil are summarized in Table 1. Statistically significant BI antagonism ($P < 0.001$) was detected for propofol and lidocaine on the bronchial hyperresponsiveness induced by remifentanil (Fig. 5). Since the observed drug effects were significantly lower ($P < 0.001$) than the expected effects under the BI zero-interaction hypothesis, the ΔE produced negative values for propofol plus remifentanil (−24.62 ± 1.07%) and for lidocaine plus remifentanil (−17.61 ± 1.04%).

4. Discussion

Our study on a bronchoscopy sedation model in human isolated bronchi demonstrated that the bronchial hyperresponsiveness induced by remifentanil, caused by the stimulation of capsaicin-sensitive sensory nerves, has been effectively prevented by the concomitant administration of lidocaine or propofol.

Animal models of bronchial relaxation have documented that propofol attenuates tracheal smooth muscle contraction induced by carbachol [34], histamine [35,36], and other mediators [37,38]. The mechanisms involved in this effect include a propofol-induced decrease in the release of Ca^{2+} from internal stores and also inhibition of Ca^{2+} influx [34]. In addition, propofol attenuates inositol phosphate accumulation [34] and inhibits voltage-dependent Ca^{2+} channels of tracheal smooth muscle cells [39]. Furthermore, it has been also demonstrated that propofol stimulates NO releasing from cultured endothelial and epithelial cells in animals [40,41].

Our data, which have been obtained from a model in human bronchi that closely mimics vagal firing *in vivo*, showed that propofol did not modify the bronchial responsiveness in human isolated tissues stimulated by EFS and document the safety of low concentrations of propofol on the human bronchial tone. We have

Table 1
Antagonistic effect of propofol (1.3 μg ml^{-1}) and lidocaine (1 μg ml^{-1}) on the hyperresponsiveness induced by remifentanil (1 ng ml^{-1}) on human bronchi contracted by EFS. Data shown are from experiments performed with samples of $n = 3$ different subjects and they are represented as mean ± SEM.

	Remifentanil + propofol		Remifentanil + lidocaine	
	Zero-interaction hypothesis	Observed antagonistic effect	Zero-interaction hypothesis	Observed antagonistic effect
BI interaction	0.31 ± 0.02	0.06 ± 0.01***	0.28 ± 0.01	0.10 ± 0.01***
ΔE	−0.25 ± 0.01		−0.18 ± 0.01	

***$P < 0.001$ *vs.* zero-interaction hypothesis.

also demonstrated that propofol induced relaxation on the resting tension of human bronchi via NO releasing, as the selective iNOS aminoguanidine prevented this effect.

Remifentanil, a new synthetic ultra-short acting μ-opioid agonist, has recently become a popular sedative agent for fibreoptic bronchoscopy because of its safety profiles and effective sedation [22,42,43]. However, although nowadays there are some suggestions for bronchial hyperresponsiveness mediated by opioids [44–46], there are no studies that have specifically investigated the influence of remifentanil on the human bronchial tone.

The cause of bronchial hyperresponsiveness induced by opioids has been only marginally investigated, and there are discrepancies among the few data presented in literature. In effect, previous studies have not completely clarified whether the contracturant effect of remifentanil in human is related to an increased release of histamine, to the stimulation of capsaicin-sensitive sensory nerves or to an enhanced vagus tone. Furthermore, it is also unclear whether remifentanil modulates the bronchial contraction by acting peripherally on the bronchial tree, or centrally at the level of autonomic nervous system centres [44–48].

Intriguingly, our model proved that treating human bronchi with remifentanil for 30 min enhances the bronchial responsiveness to 10 Hz EFS, and that this phenomenon is related with the activation of capsaicin-sensitive sensory nerves, as desensitizing bronchial rings prevented the hyperresponsiveness mediated by μ-opioid receptor activation.

These data confirm the findings reported by Conti and colleagues, concerning the increasing of cholinergic stimulus mediated by opioids administration [46]. Our model does not permit to exclude an interference of remifentanil on the autonomic nervous system centres, which in turn may directly or indirectly enhance the bronchial tone, as previously speculated in human and confirmed in animal studies [46,48]. However, our results suggest an increasing in cholinergic tone peripherally at the level of bronchial parasympathetic ganglia. On the other hand, we can rule out the suggested influence of histamine in the bronchial hyperresponsiveness induced by remifentanil [45,46], since the baseline tone of human isolated bronchi was not modified by the treatment with the opioid.

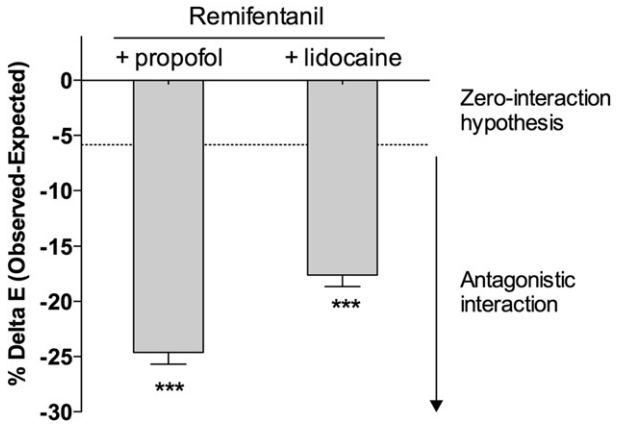

Fig. 5. Bar graph representation of the BI drug interaction model for remifentanil (1 ng ml^{-1}) plus propofol (1.3 μg ml^{-1}) and remifentanil (1 ng ml^{-1}) plus lidocaine (1 μg ml^{-1}). The *Y*-axis represents the ΔE (observed-expected response, %). The 0-values (±6.0% for remifentanil plus propofol and ±5.7% for remifentanil plus lidocaine) indicates the zero-interaction hypothesis for BI interaction, whereas the negative values represent an antagonistic (negative ΔE) interaction. The magnitude of interactions is directly related to ΔE. Points shown are from experiments performed with samples of $n = 3$ different subjects and they are represented as mean ± SEM. ***$P < 0.001$ *vs.* zero-interaction hypothesis.

Furthermore, our results only partially confirm previous data obtained by Belvisi and colleagues [49], that demonstrated that a 10 min treatment with μ-opioid agonists {[D-Ala^2-NMePhe^4-Gly-ol^5]-enkephalin (DAMGO) and H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2 (BW443C)} reduced the bronchial response to EFS. In their study, the Authors did not evaluate the effect of DAMGO and BW443C for more than 10 min, and they stimulated tissues at very low (0.5–4 Hz) or very high (32–64 Hz) EFS frequency but not at 10 Hz, which may be considered an average frequency for physiological vagus firing on parasympathetic bronchial ganglia [13,40,50]. Furthermore, the concentrations of DAMGO and BW443C were established from previous studies in guinea pig airways *in vitro*, whereas we employed drug concentrations that are effectively available at the plasma-site by target-controlled infusion (TCI) in human patients undergoing bronchoscopy [19–22,49].

In addition to the demonstration that remifentanil enhances the bronchial responsiveness to EFS, we further proved an antagonistic interaction, and therefore the protective role, of propofol on the bronchial hyperresponsiveness induced by remifentanil, likely mediated by the NO releasing induced by propofol in human isolated bronchi.

Flexible bronchoscopy is usually performed by topical administration of lidocaine along the bronchial tree, through instillation or spry [6,7]. This study provides evidences that lidocaine is effective in abolishing the human bronchial hyperresponsiveness mediated by remifentanil. In effect, we have provided evidence of an antagonistic interaction of lidocaine on the bronchial hyperresponsiveness induced by remifentanil. In effect, the ability of lidocaine to inhibit the bronchospasm induced by remifentanil is likely due to the inhibition of remifentanil-evoked discharges in capsaicin-sensitive sensory nerves originating from bronchial C-fibre endings, rather than functional antagonism on ASM shortening [51,52]. These findings suggest that the lidocaine administration through flexible bronchoscopy might antagonize the pro-contracturant side effect demonstrated for remifentanil. Therefore, we are in agreement with the suggestion of Kim and colleagues concerning the intravenous administration of low concentration of lidocaine (0.5 mg kg^{-1}) for suppressing the remifentanil-induced cough [53]. In effect, this practice might also prevent the bronchial hyperresponsiveness mediated by remifentanil, without possible systemic lidocaine toxicity.

To the best of our knowledge, this is the first study that has thoroughly investigated the interaction between propofol, lidocaine and remifentanil on the tone of human bronchi in a model of sedation that reproduces the conditions typical for flexible bronchoscopy.

Even though this study has been carried out in human isolated bronchi, our experimental approach by using *ex vivo* model has been widely validated [14,15,23,54,55] and allows evaluating the influence of contracturant/relaxant agents specifically on the bronchial wall, including the role of the nerve pathways. Furthermore, our pharmacological model allowed performing a BI interaction analysis under controlled conditions, indeed avoiding biases related to the stress induced by fibreoptic bronchoscopy *in vivo* in human [26,56,57].

Concluding, our results suggest that both propofol and remifentanil may be used safely as sedation agents through fibreoptic bronchoscopy, even though remifentanil should be associated with the administration of propofol or lidocaine, in order to prevent the potential bronchospasm.

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