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Mechanisms Underlying the Inhibitory Effect of Propofol on the Contraction of Canine Airway Smooth Muscle

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Background: **Propofol has been shown to produce relaxation of preconstricted airway smooth muscle. Although the inhibition of calcium mobilization is supposed to be the major mechanism of action, the whole picture of the mechanisms is not completely clear.**

Methods: **Contractile response was performed using canine tracheal rings. The effects of propofol on carbachol-induced mobilization of intracellular Ca^{2+} and phosphoinositide hydrolysis were measured using cultured canine tracheal smooth muscle cells by monitoring fura-2 signal and assessing the accumulation of [^{3}H]-inositol phosphates. To detect the effect of propofol on muscarinic receptor density and affinity, [^{3}H]*N*-methyl-scopolamine was used as a radioligand for receptor binding assay.**

Results: **Pretreatment with propofol shifts the concentration–response curves of carbachol-induced smooth muscle contraction to the right in a concentration-dependent manner without changing the maximal response. Propofol not only decreased the release of Ca^{2+} from internal stores but also inhibited the calcium influx induced by carbachol. In addition, carbachol-induced inositol phosphate accumulation was attenuated by propofol; the inhibitory pattern was similar to the contractile response. Moreover, propofol did not alter the density of muscarinic receptors. The dissociation constant value was not altered by pretreatment with 100 μM propofol but was significantly increased by 300 μM (propofol, 952 ± 229 pM; control, 588 ± 98 pM; $P < 0.05$).**

Conclusions: **Propofol attenuates the muscarinic receptor–mediated airway muscle contraction. The mechanism underlying these effects was attenuation of inositol phosphate generation and inhibition of Ca^{2+} mobilization through the inhibition of the receptor-coupled signal-transduction pathway. (Key words: Anesthetics; Ca^{2+}; contraction; fura-2; inositol phosphates; propofol.)**

PROPOFOL (2,6-di-isopropylphenol), a widely used intravenous anesthetic with the characteristics of rapid onset, short duration of action, and rapid elimination,[1] is reported to antagonize fentanyl-induced bronchoconstriction during surgery,[2] and to inhibit postoperative bronchospasm in patients with hyperreactive airway disease.[3] In addition, *in vitro*, propofol has been shown to produce relaxation of tracheal smooth muscle (TSM) with spontaneous tone or contraction induced by acetylcholine, carbachol, histamine, prostaglandin $F_2\alpha$, and potassium.[4–6] It is well known that Ca^{2+} plays an important role in the contraction of smooth muscle. The regulation of intracellular Ca^{2+} ($[Ca^{2+}]_i$) is integrated by several mechanisms located on plasma membrane and intracellular organelles.[7] These include the Ca^{2+} release from the internal store and influx of extracellular Ca^{2+}. In vascular smooth muscle cells, propofol produced a relaxing effect through the inhibition of Ca^{2+} mobilization.[8] Furthermore, propofol has been shown to inhibit voltage-dependent Ca^{2+} channels in porcine TSM cells.[9]

In mammalian airway, the parasympathetic nervous system, a predominant neural pathway, plays an important role in the regulation of airway diameter and resistance to airflow.[10] Acetylcholine released from parasympathetic nerve endings stimulates the smooth muscle contraction *via* its binding to muscarinic cholinergic receptors (mAChRs).[11] Following the stimulation of M_3 receptors on airway smooth muscle,[12–14] phospholipase C is activated *via* a guanosine 5′-triphosphate–binding protein, which hydrolyzes phosphoinositide, leading to the formation of inositol-1,4,5-trisphosphate (IP_3). In rat

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aortic smooth muscle, propofol has been shown to inhibit the IP_3 production induced by endothelin-1 and arginine vasopressin.[8] Thus, propofol might attenuate the IP_3-induced Ca^{2+} mobilization.

The present study therefore was undertaken to investigate how propofol might modulate the pharmacological properties of mAChRs, inositol phosphate (IP) accumulation, Ca^{2+} mobilization, and subsequently the contraction of airway smooth muscle.

Materials and Methods

Animals

Mongrel dogs of either sex, 20–30 kg, purchased from a local supplier, were used throughout this study. Dogs were housed indoors in the Laboratory of Animal Resource Center at Chang Gung University under automatically controlled temperature and light cycle and fed with standard laboratory chow and tap water *ad libitum*. The procedures used in this study have been reviewed and approved by the University's animal use committee, indicating that the procedures are in accord with the institutional guidelines for animal use. Dogs were anesthetized with ketamine (6 mg/kg intramuscular), and the lungs were ventilated mechanically *via* an endotracheal tube. The tracheae were surgically removed.

Contractile Response

The removed trachea, about 20 cm long from larynx to bifurcation, was quickly transferred to oxygenated (95% O_2 plus 5% CO_2) Krebs-Henseleit solution of the following composition (in mM): NaCl 117, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.2, $NaHCO_3$ 20, $CaCl_2$ 2.4, glucose 1, HEPES 20. The trachea was opened by cutting the cartilage rings opposite to the smooth muscle. The muscle was cleaned in two steps. The initial step in each dissection involved removal of the epithelium and submucosal tissue with forceps. The connective tissue on the serosal surface was carefully cleaned. In order to hook the muscle strips to the transducer, the section of cartilage up to the point of insertion of the muscle was maintained. Then the trachea was cut into separate individual rings. The TSM strips were mounted in an organ bath containing physiologic solution with the composition (in mM): NaCl 125, KCl 5, $MgCl_2$ 2, NaH_2PO_4 0.5, $NaHCO_3$ 5, $CaCl_2$ 1.8, glucose 10, HEPES 10, and gases with 95% O_2 plus 5% CO_2 at 37°C. The TSM strips were equilibrated in physiologic solution for 60 min with three changes of physiologic solution and maintained under an optimal tension of 1.0 g. Contraction was recorded isometrically *via* a force-displacement transducer connected to a recorder (BIOPAC System, Goleta, CA). To determine the effects of propofol on contraction induced by carbachol, TSM strips were initially stimulated by 50 mM KCl, and the tensions were defined as the maximal contraction. Following washing out and equilibration, the TSM strips were divided into three groups, incubated 30 min with dimethylsulfoxide (DMSO) or 100 μM or 300 μM propofol (dissolved in DMSO), respectively. Cumulative concentration response curves for carbachol (10 nM to 1 mM) were constructed. Results are expressed as a percentage of maximal response induced by 50 mM KCl.

Isolation of Tracheal Smooth Muscle Cells

Tracheal smooth muscle cells (TSMCs) were isolated according to the method described previously.[15,16] In brief, the smooth muscle was dissected, minced, and transferred to the dissociation medium containing 0.1% collagenase IV, 0.025% deoxyribonuclease I, and 0.025% elastase IV and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin and 2.5 μg/ml fungizone) in a solution composed of (in mM): NaCl 137, KCl 5, $CaCl_2$ 1.1, $NaHCO_3$ 20, NaH_2PO_4 1, glucose 11, HEPES 25, *p*H 7.4. The tissue pieces were gently agitated at 37°C in a rotary shaker for 1 h; the released cells were collected and the residue was digested in the same fresh enzyme solution for an additional hour at 37°C. All released cells were collected and washed twice with Dulbecco's modified Eagle's medium (DMEM) and Ham's nutrient mixture F-12 medium (1:1, v/v). The cells, suspended in DMEM/F-12 containing fetal bovine serum (FBS) 10%, were preplated onto petri dishes (60 mM) and incubated at 37°C for 1 h to remove fibroblasts. The cells then were counted and diluted with DMEM/F-12 to 2×10^5 cells/ml. The cells, suspended in DMEM/F-12 containing 10% FBS, were plated onto 24-well (0.5 ml per well), 12-well (1 ml per well), or six-well (2 ml per well) culture plates containing glass coverslips coated with collagen for receptor binding assay, IP accumulation, and Ca^{2+} measurement, respectively. The culture medium was changed after 24 h and then changed every 3 days. After 5 days in culture, the cells were changed to DMEM/F-12 containing FBS (1%) for 24 h at 37°C. Then the cells were cultured in DMEM/F-12 containing FBS (1%) supplemented with insulin-like growth factor (10 ng/ml) and insulin (1 μg/ml) for 12–14 days.

To characterize the isolated and cultured TSMCs and to

exclude the possibility of contamination by epithelial cells and fibroblasts, the cells were identified by an indirect immunofluorescent staining method using a monoclonal antibody of light chain myosin.[17] Over 95% of the cells were smooth muscle cells.

Accumulation of Inositol Phosphates

The effect of propofol on the hydrolysis of phosphoinositol was assayed by monitoring the accumulation of [^{3}H]-labeled IPs as described by Berridge *et al.*[18] Cultured TSMCs were incubated with 5 μCi/ml of myo-[2-^{3}H]-inositol at 37°C for 2 days. TSMCs were washed two times with phosphate-buffered saline and incubated in Krebs-Henseleit buffer (Krebs-Henseleit solution, *p*H 7.4) containing (in mM): NaCl 117, $MgSO_4$ 1.1, KH_2PO_4 1.2, $NaHCO_3$ 20, $CaCl_2$ 2.4, glucose 1, HEPES 20, and LiCl 10 at 37°C for 30 min. Propofol was added 30 min before the addition of the agonist. Control group was treated with the same concentration of DMSO. After the addition of carbachol, incubation was continued for another 60 min. Reactions were terminated by addition of 5% perchloric acid followed by sonication and centrifugation at 3,000*g* for 15 min.

The perchloric acid soluble supernatants were extracted four times with ether, neutralized with potassium hydroxide, and applied to a column of AG1-X8, formate form, 100–200 mesh (Bio-Rad, Hercules, CA). The resin was washed successively with 5 ml of 60 mM ammonium formate and 5 mM sodium tetraborate to eliminate free myo-[^{3}H]-inositol and glycerophosphoinositol, respectively. The fraction of total IPs was eluted with 5 ml of 1 M ammonium formate and 0.1 M formic acid. The amount of [^{3}H]-IPs was determined in a radiospectrometer (Beckman LS5000TA, Fullerton, CA).

[^{3}H]N-Methyl Scopolamine Binding Assay

For detection of the effect of propofol on muscarinic receptor density or affinity on TSMCs, [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) was used as a radioligand. Total number of cell-surface [^{3}H]NMS binding sites per well was determined by incubating cells in phosphate-buffered saline containing various concentrations of [^{3}H]NMS ranging from 20 to 1,000 pM. nonspecific binding of [^{3}H]NMS was determined in the presence of 1 μM atropine sulfate. Binding reactions were incubated at 37°C for 45 min and terminated by removing the reaction mixture and washing the cells three times with phosphate-buffered saline. Cells were solubilized in 0.1 N NaOH and scintillation fluid and counted in a radiospectrometer. The dissociation constant (K_D) and total receptor density (B_{max}) were calculated by GraphPad Prism (GraphPad Software, San Diego, CA).

Measurement of Intracellular Ca^{2+} Level

$[Ca^{2+}]_i$ was measured in confluent monolayers with the calcium-sensitive dye fura-2/AM as described by Grynkiewicz *et al.*[19] Upon confluence, the cells were cultured in DMEM/F-12 with 1% FBS 1 day before measurements were taken. The monolayers were covered with 1 ml of DMEM/F-12 with 1% FBS containing 5 μM fura-2/AM and incubated at 37°C for 45 min. At the end of the loading period, the coverslips were washed twice with physiologic buffer as described in Contractile Response. The cells were incubated in the same buffer with or without propofol for another 30 min to complete dye deesterification. The coverslip was inserted into a quartz cuvette at an angle of approximately 45 degrees to the excitation beam and placed in the thermostatted holder of an SLM 8000C spectrofluorometer (SLM, Urbana, IL). Continuous stirring was achieved with a magnetic stirrer.

Fluorescence of Ca^{2+}-bound and unbound fura-2 was measured by rapidly alternating the dual excitation wavelengths between 340 and 380 nm and electronically separating the resultant fluorescence signals at an emission wavelength of 510 nm. The autofluorescence of each monolayer was subtracted from the fluorescence data. The ratios of the fluorescence at the two wavelengths were computed and used to calculate changes in $[Ca^{2+}]_i$. The ratios of maximum and minimum fluorescence of fura-2 were determined by the addition of ionomycin (10 μM) in the presence of Phocal buffer containing 5 mM Ca^{2+} and by adding 5 mM EGTA at *p*H 8 in Ca^{2+}-free Phocal buffer, respectively. The K_D of fura-2 for Ca^{2+} was assumed to be 224 nM.[19]

Materials

Propofol was purchased from Aldrich (Milwaukee, WI). DMEM and FBS were purchased from J.R. Scientific (Woodland, CA). Myo-[2-^{3}H]inositol (18 Ci/mmol) and [^{3}H]*N*-methyl scopolamine (84 Ci/mmol) were obtained from Amersham (Buckinghamshire, UK), and DMEM-inositol free medium was from Life Technologies (Gaithersburg, MD). Fura-2/AM was ordered from Molecular Probes (Eugene, OR) and enzyme and other chemicals from Sigma (St. Louis, MO).

Statistical Analysis

Concentration–effect curves were fitted by GraphPad Prism. EC_{50} values were estimated by the same program

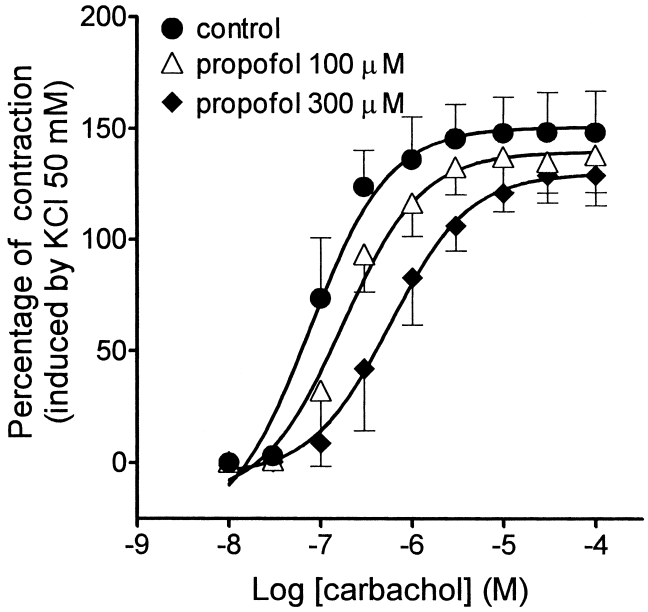

Fig. 1. Inhibition of carbachol-induced tracheal smooth muscle (TSM) strips contraction by propofol. The TSM strips were divided into three groups, incubated 30 min with dimethylsulfoxide (control, *circles*), 100 μM propofol (*triangles*), and 300 μM propofol (*diamonds*), respectively. Cumulative concentration–response curves for carbachol (10 nM to 1 mM) were obtained. Data are normalized to maximal response induced by 50 mM KCl and expressed as mean ± SD of six separate experiments.

and expressed as the mean pEC_{50} ($-\log EC_{50}$, expressed in M) ± SD. Data were expressed as the mean ± SD and analyzed with the Student *t* test or one-way analysis of variance as indicated at a 0.05 level of significance. The Bonferroni post-test was performed if one-way analysis of variance was significant.

Results

Effect of Propofol on the Contraction of TSM Strips

To assess the effect of propofol on modulation of the constrictor response to cholinergic stimulation, concentration–response relationships to carbachol were compared among TSM strips pretreated with DMSO (control), 100 μM propofol, and 300 μM propofol. The pEC_{50} values were 7.12 ± 0.26, 6.75 ± 0.21, and 6.24 ± 0.10, respectively (fig. 1). Compared with the controls, propofol of either dose significantly decreased the pEC_{50} ($P < 0.05$) and shifted the concentration–response curves of carbachol to the right in a dose-dependent manner. However, the maximal responses to carbachol were slightly but not significantly attenuated by 100 μM or 300 μM propofol with the values of 137.9 ± 16.8% and 128.8 ± 13.9%, respectively, compared with the value of 148.1 ± 18.4% obtained in controls.

Effects of Propofol on Carbachol-induced Ca^{2+} Mobilization

Calcium ions play a key role in the contraction of TSMCs. The activation of mAChRs results in the release of Ca^{2+} from sarcoplasmic reticulum and the entry of extracellular Ca^{2+}. In our previous study, carbachol produced a biphasic $[Ca^{2+}]_i$ change in the presence of extracellular Ca^{2+}, which displayed an initial transient peak and a sustained plateau phase.[13]

To check whether propofol attenuates the increase of $[Ca^{2+}]_i$ induced by carbachol, the effect of propofol on the concentration–response curve of carbachol was examined. The confluent coverslips with TSMC monolayers were pretreated with DMSO (control) or propofol (100 μM or 300 μM) for 30 min, and the initial peak of $[Ca^{2+}]_i$ induced by carbachol was measured. Concentration–response curves for carbachol (10 nM to 0.1 mM) were constructed. Figure 2A–C shows the traces of carbachol-induced $[Ca^{2+}]_i$ change in various concentrations. As displayed in figure 2D, the pEC_{50} values for carbachol were 6.85 ± 0.58, 5.93 ± 0.18, 4.55 ± 1.33 in the control and propofol (100 μM and 300 μM)–treated TSMCs, respectively, with corresponding maximal increase in $[Ca^{2+}]_i$ of 132.4 ± 22.7, 91.0 ± 24.5, and 26.1 ± 20.4 nM, respectively. Compared with the controls, maximal response and pEC_{50} to carbachol were significantly attenuated ($P < 0.05$) by either dose of propofol. Accordingly, propofol shifted the dose—response curve for carbachol to the right in a dose-dependent manner.

To further elucidate the mechanism of the attenuation of $[Ca^{2+}]_i$ increase, the effect of propofol on Ca^{2+} release from internal stores and the influx of extracellular Ca^{2+} was examined. The confluent coverslips with TSMC monolayers (different cells from different dogs) were pretreated with DMSO (control) or propofol (1 μM to 300 μM) for 30 min. The initial peak of $[Ca^{2+}]_i$ induced by carbachol (100 μM) was measured in the absence of extracellular Ca^{+}. Then, Ca^{2+} was added with the final extracellular Ca^{2+} concentration at 1.8 mM, and $[Ca^{2+}]_i$ was measured. Figure 3A shows the trace of carbachol-induced $[Ca^{2+}]_i$ increase in the absence of extracellular Ca^{2+} before 300 s, and the presence of Ca^{2+} when it was added at 300 s. The maximal increased $[Ca^{2+}]_i$ measured in the absence of extracellular Ca^{2+} represents its release from internal

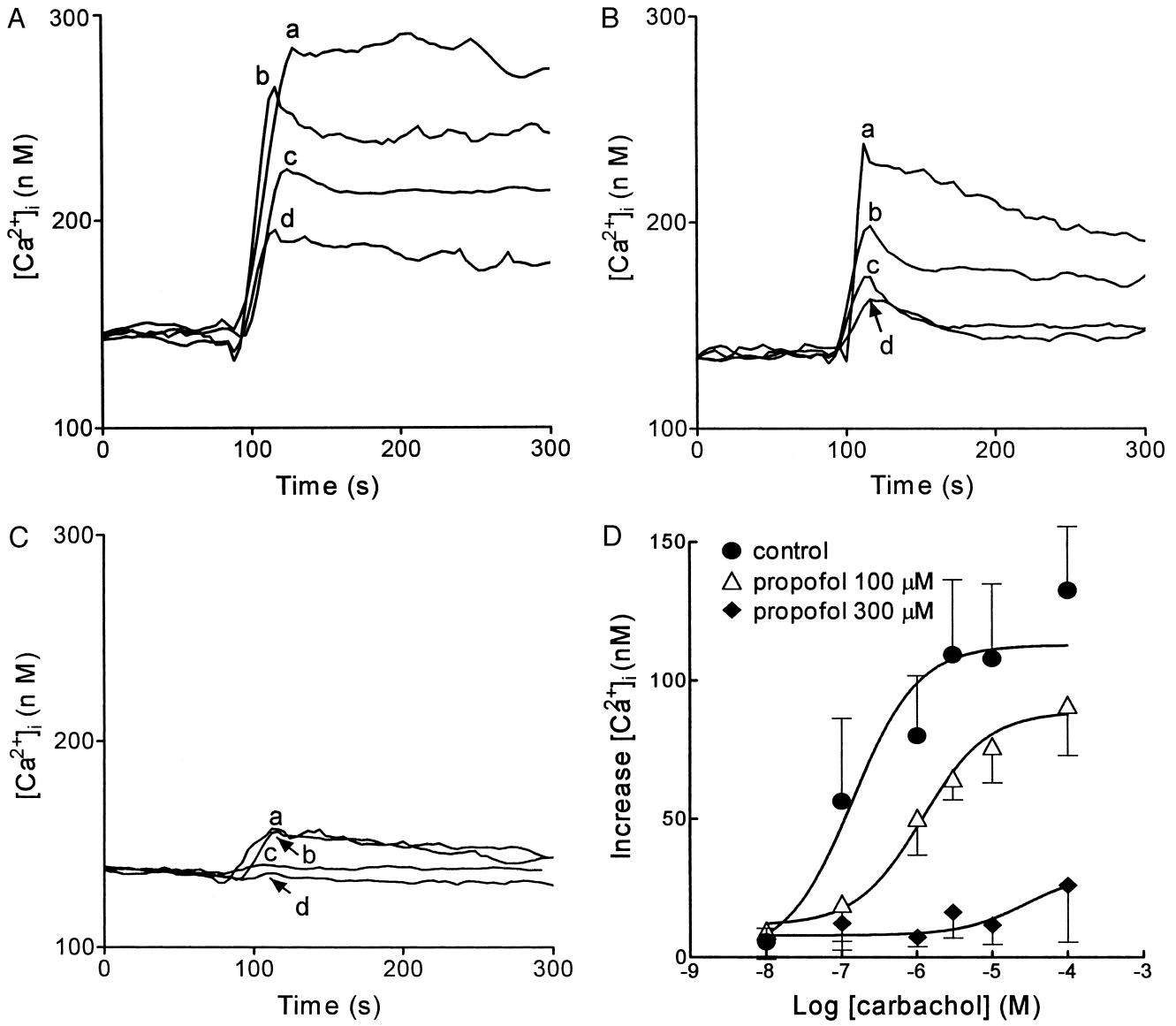

Fig. 2. Inhibition of the carbachol-induced $[Ca^{2+}]_i$ increase by propofol. Tracheal smooth muscle cells were incubated in the absence (*A*) or presence of 100 μM (*B*) or 300 μM (*C*) propofol for 30 min, $[Ca^{2+}]_i$ was measured with various concentrations of carbachol (a, 100 μM; b, 10 μM; c, 1 μM; d, 100 nM) added at 100 s. (*D*) The concentration–response curves for carbachol (10 nM to 0.1 mM; *circles,* control; *triangles,* 100 μM propofol; *diamonds,* 300 μM propofol). Data represent mean ± SD derived from six separate experiments as the increase above the resting level.

store, that is, sarcoplasmic reticulum, and $[Ca^{2+}]_i$ measured in the readdition of Ca^{2+} represents Ca^{2+} influx from extracellular source. Pretreatment of TSMCs with propofol at concentrations of 10, 100, and 300 μM significantly attenuated carbachol-induced Ca^{2+} release from internal stores, with maximal responses at 51.1 ± 8.5, 45.3 ± 6.9, 30.3 ± 7.6 nM, respectively, as compared with control cells at 73.7 ± 11.5, $P <$ 0.05 (fig. 3B). Following the measurement of transient $[Ca^{2+}]_i$, Ca^{2+} (1.8 mM) was readded to measure its influx into TSMCs. Propofol alone had no effect on $[Ca^{2+}]_i$ (data not shown). These results showed that pretreatment of theses cells with 10, 100, and 300 μM propofol significantly blocked carbachol-induced Ca^{2+} influx, with maximal responses at 71.5 ± 20.0, 67.6 ± 17.8, and 56.4 ± 19.5 nM, respectively, as

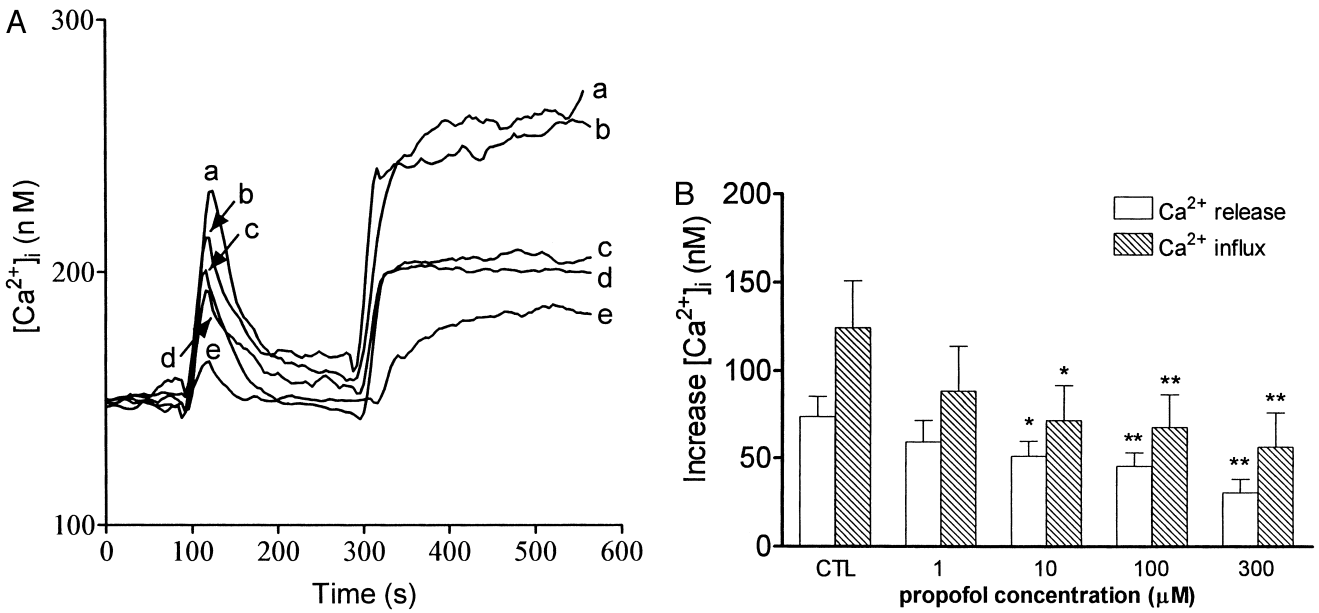

Fig. 3. Effect of propofol on the carbachol-induced Ca^{2+} release and influx. (*A*) Tracheal smooth muscle cells were incubated in the absence (a, control) or presence of propofol (b, 1 μM; c, 10 μM; d, 100 μM; e, 300 μM) for 30 min. The initial peaks of $[Ca^{2+}]_i$ induced by carbachol (100 μM added at 100 s) were measured (*B*, *open column*) in the absence of extracellular Ca^{2+}. Then, Ca^{2+} was added at 300 s, with a final extracellular Ca^{2+} concentration of 1.8 mM, and $[Ca^{2+}]_i$ was measured (*B*, *hatched column*). Data represent mean ± SD derived from six separate experiments as the increase above the resting level. *$P < 0.05$ *vs.* control; **$P < 0.01$ *vs.* control.

compared with control cells at 124.4 ± 26.4 nM, $P < 0.05$ (fig. 3B). However, at the lower concentration 1 μM of propofol did not affect carbachol-induced Ca^{2+} response in TSMCs.

Effect of Propofol on Carbachol-induced IP Accumulation

Propofol attenuates the Ca^{2+} release induced by carbachol, whereas Ca^{2+} release is determined by the binding of IP_3 to its receptors on the sarcoplasmic reticulum.[20] To determine whether propofol interferes with the production of IP_3, the IP accumulation induced by carbachol was measured in the absence or presence of propofol (100 μM and 300 μM). As shown in figure 4, propofol significantly shifted the concentration response curves of carbachol to the right in a dose-dependent manner. The maximal response for carbachol was also inhibited by propofol. The pEC_{50} values, 4.20 ± 0.26 and 4.00 ± 0.22, for carbachol-induced IP accumulation were significantly attenuated in the presence of 100 and 300 μM propofol, respectively, as compared with control (4.90 ± 0.12, $P < 0.05$).

Effect of Propofol on [^{3}H]NMS Binding

The finding that propofol inhibited the production of IP indicated that it may interfere with the receptor-coupled signal transduction. The action site may be located at the muscarinic receptors. To examine whether propofol alters B_{max} or K_D for the muscarinic receptor, a receptor binding assay was performed in TSMCs treated with propofol for 30 min, using [^{3}H]NMS as a radioligand. Figure 5A shows the saturation binding isotherm derived from one of five experiments. Scatchard plot analysis of the specific bound [^{3}H]NMS is displayed in figure 5B. The x-intercept of the least-squares fit to Scatchard plot is a measure of B_{max}, and the negative reciprocal of the slope is the K_D. The B_{max} and K_D values are summarized in table 1. These results demonstrate that pretreatment of TSMCs with both concentrations of propofol does not significantly change the B_{max} of mAChRs. Similarly, the K_D value was not significantly altered by pretreatment with 100 μM propofol. However, propofol concentration at 300 μM significantly attenuated the affinity of mAChR for [^{3}H]NMS binding (propofol, 952 ± 229 pM; control, 588 ± 98 pM; $P < 0.05$).

Discussion

It has been well known that parasympathetic nervous system forms the predominant neural pathway and plays

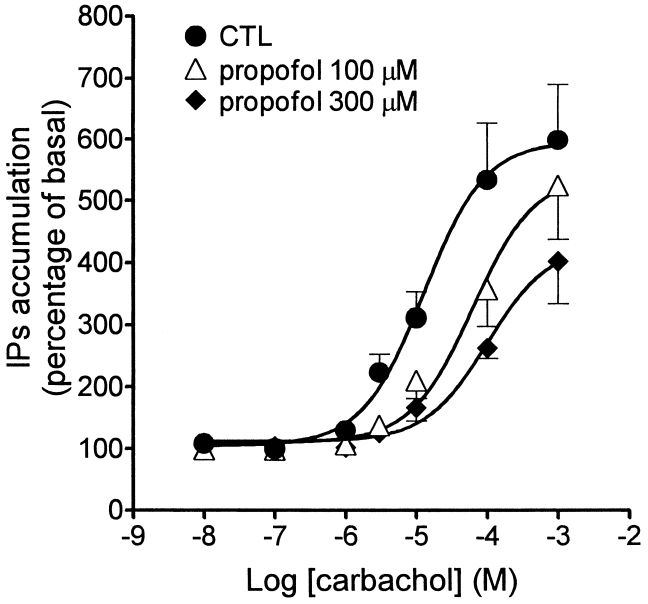

Fig. 4. Inhibition of carbachol-induced inositol phosphate (IP) accumulation by propofol. IP accumulation was measured in the abscence (control, *circles*) and presence of 100 μM (*triangles*) or 300 μM (*diamonds*) propofol. Concentration–response curves for carbachol-induced inositol phosphate accumulation were obtained. Results were normalized to the basal levels of inositol phosphates and expressed as mean ± SD of five separate experiments determined in triplicate.

an important role in the regulation of airway diameter and resistance to airflow in mammalian airway.[10] Neurotransmitter acetylcholine released from parasympathetic nerve endings stimulates the contraction of airway smooth muscle *via* the activation of guanosine 5′-triphosphate–binding protein-coupled signal-transduction pathway.[15,21] The activation of G-protein further activates phospholipase C, which hydrolyzes phosphoinositide, leading to the formation of IP_3 and diacylglycerol.[22] IP_3 in turn stimulates the release of calcium ions from its intracellular stores.[23] Diacylglycerol translocates the cytosolic protein kinase C to the cell membrane, and the activation of protein kinase C further phosphorylates substrates on cell membrane, resulting in the opening of calcium channel.[24,25] In addition, the activation of receptors also results in the opening of calcium channels *via* the guanosine 5′-triphosphate–binding protein or membrane depolarization.[26,27] The increased $[Ca^{2+}]_i$ activates myosin light chain kinase through a calmodulin-dependent mechanism.[28] The active kinase phosphorylates myosin light chain, leading to initiation of contraction. Thus, alteration in the components of the receptor-coupled signal-transduction pathway may be associated with the change in the tension of airway smooth muscle. In the present study, these results demonstrated that propofol attenuated the contraction mediated by mAChRs in this tissue. The mechanism underlying the relaxant effects might be caused inhibition of Ca^{2+} mobilization and attenuation of the generation of IPs *via* the inhibition of the receptor-coupled signal-trasduction pathway.

In the current study, our results regarding the relaxant effect of propofol on airway smooth muscle (fig. 1) are consistent with other studies either *in vivo*[2,3] or *in vitro*.[4–6] Because Ca^{2+} plays an important role in the contraction of smooth muscle, we examine the effect of propofol on the Ca^{2+} mobilization and IP accumulation in cultured TSMCs induced by carbachol. In our previous study, a cell-culture model of TSMCs expressing functional mAChRs was shown to be a useful method for studying the physiologic function of airway smooth muscle.[29] The density of mAChRs expressed in primary culture of TSCMs was approximately 75% of that in freshly isolated TSMCs. Furthermore, carbachol produced a biphasic $[Ca^{2+}]_i$ change in the presence of extracellular Ca^{2+}, which displayed an initial transient peak and a sustained plateau phase.[13] In contrast, in the absence of extracellular Ca^{2+}, carbachol induced a smaller transient peak, and the sustained plateau phase was not observed. This represents the Ca^{2+} release from the internal store in the absence of extracellular Ca^{2+}. Readdition of Ca^{2+} to TSMCs after stimulation with carbachol in the absence of extracellular Ca^{2+} caused a sustained increase in $[Ca^{2+}]_i$, which was defined as the Ca^{2+} influx from extracellular source. In the present study, we demonstrated that propofol not only attenuated the Ca^{2+} mobilization but also decreased the pEC_{50} in the concentration–response curves for carbachol (fig. 2), consistent with the results obtained from freshly isolated rat TSMCs.[6] We further examined the effect of propofol on Ca^{2+} release from internal store and the influx of extracellular Ca^{2+}. Propofol was shown to attenuate not only the Ca^{2+} release from SR but also the influx of extracellular Ca^{2+} (fig. 3). The inhibition of Ca^{2+} influx may be caused by blockade of voltage-dependent Ca^{2+} channels as shown in porcine TSM.[9] In addition, in rat TSM, Quedraogo et al.[6] also demonstrated that propofol inhibits its KCl-induced $[Ca^{2+}]_i$ increase, which indicates the inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. It is also the case in other cell types that propofol inhibits the voltage-dependent Ca^{2+} channels.[8,30–32] Taken together, our results are consistent with these studies that propofol might inhibit voltage-dependent Ca^{2+} channels and attenuate Ca^{2+} in-

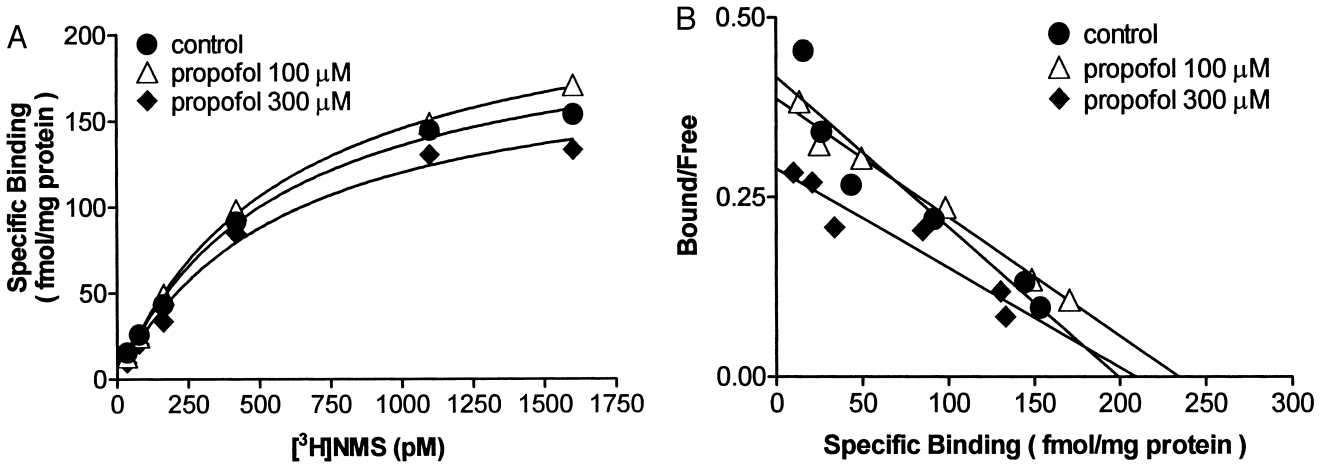

Fig. 5. Comparison of muscarinic cholinergic receptor density and affinity in propofol-treated tracheal smooth muscle cells. The cells were incubated in the absence (control, *circles*) or presence of 100 μM (*triangles*) or 300 μM (*diamonds*) propofol for 30 min. (*A*) Binding assays were performed in triplicate with concentration of [^{3}H]NMS ranging from 20 to 1,600 pM. Each curve represents one experiment. (*B*) Scachard plot of specific [^{3}H]NMS binding data in (*A*). X-intercept of least-squares fit to Scachard plot is a measure of maximal receptor density (B_{max}) and negative reciprocal of slope is the dissociation constant (K_D). Data are from one of five experiments.

flux.[8,13,30–32] In addition, Ca^{2+} homeostasis is integrated by several mechanisms located on the plasma membrane and inside of cells. Propofol may also influence the Ca^{2+}–adenosine triphosphatase pumps that move Ca^{2+} both to the internal stores and outside of the cells. Because of the limitations of experimental techniques, we did not make an attempt to differentiate the effect of propofol on these components in this study. The effect of propofol on these pump activities needs to be further investigated.

Ca^{2+} release from intracellular stores is regulated by two mechanisms: IP_3-induced Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release.[20,33] We further examined the effects of propofol on the production of IPs and demonstrated that propofol attenuates the IP production induced by carbachol (fig. 4), which is associated with inhibition of Ca^{2+} release (fig. 3). It seems unlikely that propofol depletes the Ca^{2+} content of SR, because it does not alter the basal level of $[Ca^{2+}]_i$. In rat aortic smooth muscle, propofol has been shown to inhibit the IP_3 production induced by endothelin-1 and arginine vasopressin,[8] which further supports our observation. As mentioned previously, IP_3 is a second messenger in the M_3 receptor-coupled signaling pathway. This finding also indicates that propofol may interfere with the receptor-coupled signal-transduction pathway.

Table 1. Comparison of B_{max} and K_D in Control and Propofol-treated TSMCs

	B_{max} (fmol/mg protein)	K_D (pM)
Control	185 ± 17	588 ± 98
Propofol 100 μM	192 ± 26	720 ± 259
Propofol 300 μM	183 ± 23	952 ± 229*

Comparison of mAChR density and affinity in propofol-treated tracheal smooth muscle cells. The cells were incubated in the absence (control) or presence of 100 μM or 300 μM propofol for 30 min. Binding assays were performed with concentration of [^{3}H]NMS ranging from 20 to 1,600 pM. B_{max} and K_D were derived from Scatchard plot of specific [^{3}H]NMS binding data. Data represent mean ± SD of five separate experiments determined in triplicate.

* $P < 0.05$ *versus* control.

To locate the site of action, the effect of propofol on the B_{max} and K_D of muscarinic receptors was examined. It was shown that propofol did not alter the B_{max}, which was consistent with the results that propofol had no effect on endothelin-1 binding to its receptor.[34] In addition, propofol has been shown to attenuate histamine-induced contraction of human TSM and acetylcholine-induced Ca^{2+} mobilization in rat TSMCs.[6] These results suggest that the effect of propofol is not restricted to mAChR activation. However, at the concentration of 300 μM, propofol significantly decreased the binding affinity of mAChRs. These data indicate that at the concentration of 100 μM, the action site is downstream to agonist–receptor binding, however, at higher concentration, the receptor-binding dynamic was also altered. Propofol may interfere with the components of receptor–G-protein–phospholipase C pathway; however, the precise site of action is not known. These findings demonstrated that

the inhibition of receptor–G-protein–phospholipase C pathway by propofol was involved in the inhibition of smooth muscle contraction.

In an *in vivo* study of a bolus intravenous dose of ^{14}C-propofol (7-10 mg/kg) to dogs, propofol concentrations were 4–16 μg/ml (22–88 μM) at 2 min. Duration of sleep ranged from 5 to 8 min.[35] However, the free plasma concentration of propofol was less than 8.8 μM, because of its strong protein-binding properties (>90%). At anesthetic level, the concentration of propofol in whole brain (225 μM) was 7.8 times that in plasma (29 μM) 30 min after intravenous infusion of propofol at 60 $mg \cdot kg^{-1} \cdot h^{-1}$.[36] The effective concentration in plasma is usually less than 1 μM, because propofol is a highly protein-binding agent.[37] Thus, the concentrations used in this study are one or two orders of magnitude higher than achieved *in vivo* under clinical conditions. However, there are no data related to the tissue:plasma or tissue:blood ratio in canine TSM. The results in the contractile-response experiment revealed that propofol did not reduce the maximal tension induced by carbachol (fig. 1). However, propofol significantly attenuated the maximal IP accumulation and $[Ca^{2+}]_i$ change induced by carbachol (fig. 2D and fig. 4). This discrepancy may result from the difference between freshly isolated TSM tissues and cultured smooth muscle cells. In addition, although less likely, it may be caused by the more complete distribution of propofol in cultured cells than in intact tissues. Moreover, higher concentrations of propofol may be needed to across the connective tissues served as a permeability barrier to reach smooth muscle. This may be the reason why 10 μM propofol attenuates carbachol-induced increase in $[Ca^{2+}]_i$ but has no effect on contractile response. However, all the inhibitory effects of propofol are obtained at high concentrations, and the clinical relevance is not clear.

In conclusion, this study examined the relaxant effect of propofol on canine airway smooth muscle and elucidated the mechanisms of its action. These results demonstrated that (1) propofol attenuated the contraction induced by carbachol in a concentration-dependent manner; (2) propofol attenuated not only the IP_3-induced Ca^{2+} release from internal store but also the influx of external Ca^{2+}; (3) carbachol-induced IP_3 production was also attenuated by propofol, indicating that propofol interfered with the signal transduction pathway; and (4) propofol at a higher concentration (300 μM) decreased the binding affinity of muscarinic receptor but did not alter the receptor density. Taken together, propofol inhibited the carbachol-induced contraction of canine TSM. The mechanisms underlying this inhibition may involve the inhibition of the muscarinic receptor–mediated signal transduction pathway.

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