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Effects of propofol and ketamine on ATP-induced contraction of the rat trachea

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Abstract
Purpose. ATP causes airway smooth-muscle contraction in patients with asthma and chronic obstructive pulmonary disease. Propofol and ketamine attenuate the airway smooth-muscle contraction induced by histamine and acetylcholine. However, it is not clear whether propofol and ketamine affect the ATP-induced airway smooth-muscle contraction.
Methods. We examined the effects of propofol and ketamine on the ATP-induced contraction and ATP-P_2-purinoceptor binding.
Results. Propofol attenuated the ATP-induced contraction in a dose-dependent manner, with a 50% inhibitory concentration of 54 ± 22 μM. Ketamine at 300 μM attenuated the ATP-induced contraction. In the binding study, propofol attenuated the binding of the P_2-purinoceptor with [^{3}H]-ATP in a dose-dependent manner, while ketamine did not attenuate this binding.
Conclusion. Propofol attenuates ATP-induced contraction through the inhibition of ATP-P_2-purinoceptor binding.

Key words ATP · Propofol · Ketamine · Tracheal smooth muscle

Introduction

Intravenous administration of ATP occasionally causes bronchospasm in patients with asthma and chronic obstructive pulmonary disease (COPD) [1,2] and may even cause bronchospasm in normal subjects [3]. The mechanism involves ATP directly activating P_2-purinoceptors on the airway smooth-muscle cell membrane [4], and ATP activating mast cells, eosinophils, and neurons, which subsequently release chemical mediators [5]. When the receptors on the airway smooth-muscle cell membrane are stimulated by ATP or the released chemical mediators, a phosphatidylinositol (PI) response is evoked, resulting in airway smooth-muscle contraction.

Propofol and ketamine attenuate the tracheal smooth-muscle contraction induced by histamine, acetylcholine (ACh), and vagal nerve stimulation [6–8]. Propofol and ketamine inhibit the increase in intracellular Ca^{2+} induced by various stimulants [9–11]. However, it is not clear whether propofol and ketamine affect the ATP-induced airway smooth-muscle contraction. The present study was designed to investigate the effects of propofol and ketamine on ATP-induced contraction and ATP-P_2-purinoceptor binding in the rat trachea.

Materials and methods

The studies were conducted under the guidelines approved by the Animal Care Committee of Nagasaki University School of Medicine. Thirty-six male Wistar rats, weighing 350–450 g, were used for the experiments. The rats were anesthetized with pentobarbital (50 mg·kg^{-1}; i.p.), and the trachea was rapidly isolated.

Contractile response

Each trachea was cut into 3-mm-wide segments with a McIlwain tissue chopper (Mickle Laboratory Engineering, Gomshall, UK). Each tracheal ring segment was suspended between two stainless steel hooks and placed in a 5-ml water-jacketed organ chamber (Kishimotoika, Kyoto, Japan) containing Krebs-Henseleit solution (composition in mM, 118 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11 glucose, 0.05 Na_2-ethyldiamine tetraacetic acid [EDTA]). The solution was continuously aerated with O_2 95% / CO_2 5% at a temperature of 37°C. Isometric tensions were measured using an isometric transducer (Kishimotoika), and the changes in isometric force were recorded using a

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MacLab system (Milford, MA, USA). The resting tension was adjusted periodically to 1 g during the equilibration period. The ring was washed every 15 min for 60 min.

At first, ATP was added stepwise, cumulatively, to induce active contraction at 1–1000 μM. The values for tension were expressed as the changes from baseline tension. Next, The effects of propofol and ketamine on the ATP-induced contraction of the rat tracheal ring were also observed. Ten minutes after the addition of 1, 3, 10, 30, 100, or 300 μM propofol or ketamine, contraction was induced by the addition of ATP at 300 μM. We used different rats for each anesthetic and each concentration to avoid the possibility of impaired reproducibility of smooth-muscle contraction even though the rings exposed to high concentrations of anesthetics were washed. The values for tension were expressed as the changes from the tension just before ATP was added, and the results were expressed as a percentage of the response to 300 μM ATP in the absence of the anesthetics.

Receptor-binding study

To detect the effects of propofol and ketamine on the affinity of ATP with the P_2-purinoceptor in tracheal smooth muscle, [^{3}H]-ATP was used as a radioligand. The rat trachea was immediately removed and placed in isopentane at –30°C. Frozen 10-μm-thick sections of the rat trachea were cut on a cryostat at –20°C, thaw-mounted onto gelatin-coated slides, and stored overnight under vacuum at 4°C. Related tissue sections were labeled in vitro with [^{3}H] -ATP in 2 ml of incubation buffer, according to the method of Shibata et al. [12]. Briefly, after preincubation at room temperature (23°C) for 45 min in the incubation buffer, the trachea was incubated with increasing concentrations of [^{3}H]-ATP, or 50 nM [^{3}H]-ATP in the presence of 3–3000 μM of propofol or ketamine, for 45 min, in 50 mM Tris-HCl buffer containing 100 mM NaCl, 10 mM Na_2-EDTA, and 100 mM phenylmethylsulfonyl fluoride. After incubation, the slides were washed three times (1 min each time) at 4°C in Tris-HCl buffer, and rinsed quickly in ice-cold distilled water. Tissue sections were dried under a stream of cold air. Quantitation of radioligand binding in tracheal smooth muscle was done using a highly sensitive computerized radioluminographic system with imaging plates coated with fine photostimulable phosphor crystals (Bioimaging analyzer BAS 2000; Fuji Photo Film, Tokyo, Japan). The dried sections were exposed to a radioluminographic imaging plate (BAS-TR2025; Fuji Photo Film). The values for photostimulated luminescence obtained directly from the imaging plates by the computerized scanning system were converted to the bound radioactivity of the section. The results were expressed as a percentage of the value in the absence of the anesthetics.

Statistical analysis

Data values are expressed as means ± SD. To determine the dissociation constant (Kd), and the inhibition constant (Ki), radioligand binding data were analyzed using the GraphPad Prism (San Diego, CA, USA). Dose-effect curves were fitted by nonlinear regression (GraphPad Prism). The results of repeated measures and groups were analyzed by two-way analysis of variance. Differences were considered significant at $P < 0.05$.

Results

The recording of the effects of ATP on the resting tension of rat tracheal rings is shown in Fig. 1. ATP caused significant tracheal smooth-muscle contraction at a dose of 300 μM or greater (Fig. 2). Propofol significantly attenuated the ATP-induced tracheal smooth muscle contraction in a dose-dependent manner (Fig. 3). The 50% inhibitory concentration (IC_{50}) of propofol on ATP-induced contraction was 54 ± 22 μM. Ketamine also attenuated the ATP-induced tracheal smooth muscle contraction at 300 μM, and this attenuation was less than that produced by propofol (Fig. 3). In the binding study, the Kd value of [^{3}H]-ATP in the rat trachea was 34.3 ± 9.8 nM. Propofol attenuated the binding of the P_2-purinoceptor with [^{3}H]-ATP in a dose-dependent manner (Fig. 4), and the Ki value was 126 μM. Ketamine did not attenuate the binding of the P_2-purinoceptor with [^{3}H]-ATP (Fig. 4).

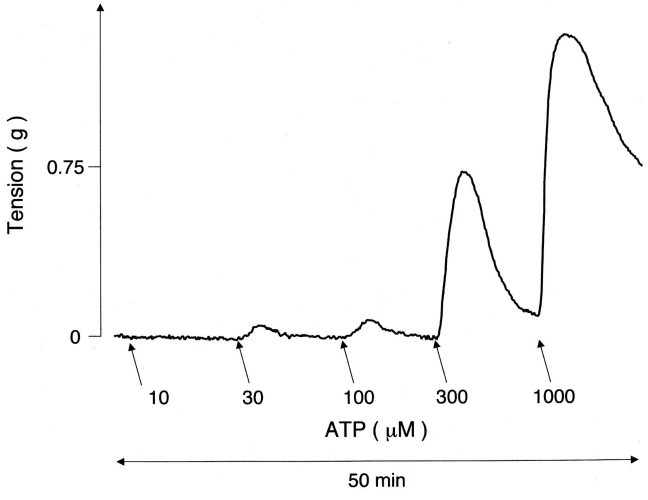

Fig. 1. Typical recording of the effects of ATP on resting tension of rat tracheal rings

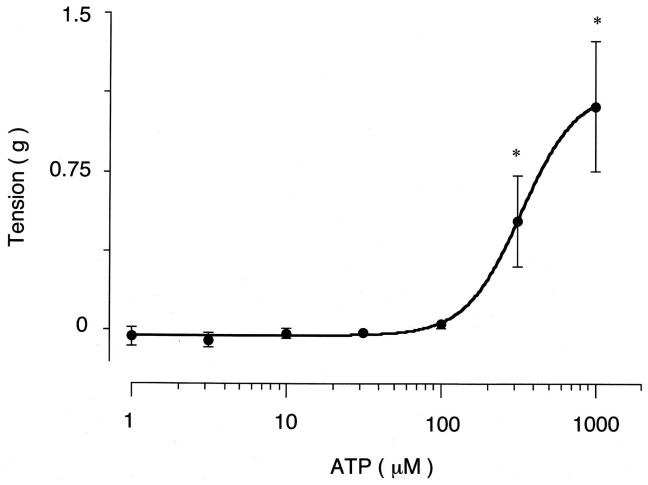

Fig. 2. The effect of ATP on smooth-muscle contraction of the rat trachea (mean ± SD; $n = 5$–6). $^{*}P < 0.05$ versus ATP 0 μM

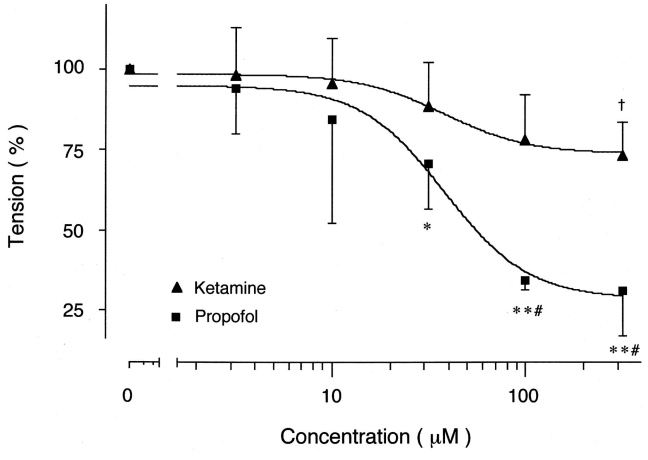

Fig. 3. The effects of propofol and ketamine on 300-μM ATP-induced contraction of the rat trachea (mean ± SD; $n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$ versus propofol 0 μM; $^{\dagger}P < 0.05$ versus ketamine 0 μM; $^{\#}P < 0.05$ versus ketamine

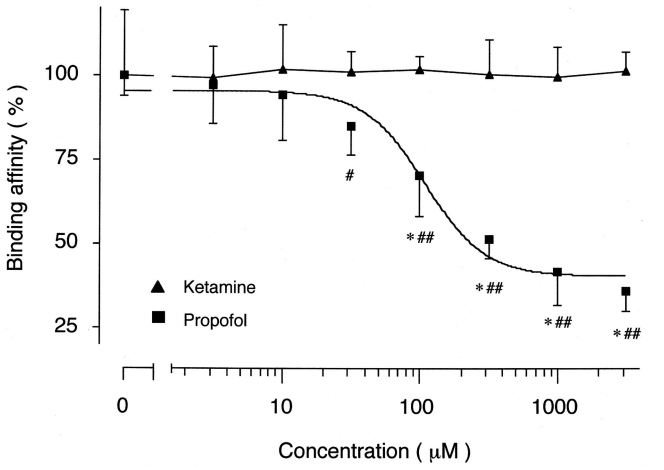

Fig. 4. The effects of propofol and ketamine on [^{3}H]-ATP binding to the P_2-purinoceptor in the rat trachea (mean ± SD; $n = 6$). $^{*}P < 0.05$ versus propofol 0 μM. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ versus ketamine

Discussion

The main findings in the present study were that the ATP-induced tracheal smooth-muscle contraction and the affinity of P_2-purinoceptors for [^{3}H]-ATP binding were inhibited by propofol dose-dependently.

Although the mechanism involved in the attenuation of airway smooth-muscle contraction by propofol has not been fully clarified, three plausible explanations can be advanced.

(1) Propofol may act directly on the P_2-purinoceptors of the airway smooth-muscle cell membrane. We examined the effects of propofol on the ATP-induced contractile properties and the binding affinity of ATP for P_2-purinoceptors. Although the intracellular mechanisms involved in bronchodilation are not simple, one of the important factors is Ca^{2+}. ATP activates the phosphatidylinositol (PI) response through G-protein-coupled phospholipase C (PLC) [4], and subsequently increases Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx from the extracellular space. Propofol is lipid-soluble, and it attenuates the airway smooth-muscle contraction induced by ACh [13], shifting the dose-response curve of carbachol (CCh)-induced airway smooth-muscle contraction to the right. Lin et al. [14] reported that propofol also decreased the release of Ca^{2+} from internal stores and decreased Ca^{2+} influx, and that it attenuated CCh-induced inositol phosphate accumulation in canine airway smooth muscle. Thus, it is likely that propofol could inhibit the activation by ATP of the PI response through G-protein-coupled PLC, resulting in the relaxation of ATP-induced contraction of the airway smooth muscle.

(2) Propofol may inhibit ACh release from vagal nerve terminals. ATP potentiates the spontaneous secretion of ACh at neuromuscular synapses in *Xenopus* cell cultures [15]. ATP interacts with the parasympathetic nervous system, which can be involved in neurogenic bronchoconstriction. Propofol may attenuate ACh release from postganglionic parasympathetic nerve terminals in the rat trachea, resulting in attenuation of the contractile response. Because parasympathetic postganglionic neurons are considered to be close to the targeted end-organ, the tracheal rings used in the present study would have contained parasympathetic postganglionic neurons. Thus, the inhibition by propofol of the ATP-induced contraction of the rat trachea may have been due to the inhibition of ACh release.

(3) Propofol may inhibit the ATP-induced contraction through the inhibition of histamine release from mast cells and eosinophils. ATP can play a mechanistic role in asthma and COPD through its action on multiple cell types relevant to these disorders, including mast cells and eosinophils [5]. Mast cells and eosinophils release histamine, which activates histamine receptors in airway smooth-muscle cell membranes, resulting in bronchoconstriction in some animals. We used the tracheal rings of Wistar rats, and found that ATP induced tracheal smooth-muscle contraction. However, histamine did not induce tracheal smooth-muscle contraction in this species [16]. Thus, it is unlikely that the propofol-induced decrease in the ATP-induced contraction of the rat trachea in our study was due to the inhibition of histamine release.

In the present study, although ketamine at 300 μM attenuated the ATP-induced contraction, ketamine did not affect the affinity of P_2-purinoceptors for [^{3}H]-ATP binding. In smooth-muscle strips stimulated with ACh, ketamine causes a dose-dependent decrease in force, and the ketamine-induced relaxation is associated with a decrease in the intracellular Ca^{2+} concentration [10]. Ketamine inhibited CCh- [17] and histamine- [18] induced contractions of the tracheal smooth muscle. Ketamine relaxes rabbit femoral arteries by reducing Ca^{2+} and PLC activity [19]. Thus, ketamine at a high dose could have attenuated the ATP-induced contraction through intracellular mechanisms of Ca^{2+} handling, because it did not affect the P_2-purinoceptors of airway smooth muscle.

The peak plasma concentrations of propofol [20] and ketamine [21] in clinical settings are 50 and 60 μM, respectively. Because 97%–99% of propofol and 20% of ketamine in plasma are protein-bound, the free propofol and ketamine concentrations are about 0.5–1.5 μM, and 50 μM, respectively. The dose required for tracheal smooth-muscle relaxation in the present study was out of the clinical range. Lin et al. [14] reported that, although propofol at 10 μM significantly attenuated the increased intracellular Ca^{2+} concentration induced by CCh in cultured canine tracheal smooth muscle cells, it was necessary to have a dose of at least 100 μM of propofol to shift the dose-contraction curves of CCh in isolated trachea to the right. They concluded that the need for this higher dose may have been caused by the more complete distribution of propofol in cultured cells than in intact tissues, and that higher doses of propofol may be needed to cross the connective tissues to reach smooth muscle.

There have been no reports on the effects of intravenous and volatile anesthetics on ATP-induced tracheal smooth-muscle contraction. Volatile anesthetics such as sevoflurane and isoflurane have potent relaxant effects on airway smooth muscle [22,23], and have the possibility to attenuate ATP-induced contractions.

In conclusion, propofol attenuates ATP-induced contractions through the inhibition of ATP-P_2-purinoceptor binding. Propofol is considered to be a suitable anesthetic in patients who receive ATP during endovascular stenting surgery.

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