

The Relaxant Effect of Propofol on Isolated Rat Intrapulmonary Arteries

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Propofol is a widely used anesthetic. Many studies have shown that propofol has direct effects on blood vessels, but the precise mechanism is not fully understood. Secondary intrapulmonary artery rings from male rats were prepared and mounted in a Multi Myograph System. The following constrictors were used to induce contractions in isolated artery rings: high K^+ solution (60 mmol/L); U46619 solution (100 nmol/L); 5-hydroxytryptamine (5-HT; 3 μ mol/L); or phenylephrine (Phe; 1 μ mol/L). The relaxation effects of propofol were tested on high K^+ or U46619 precontracted rings. Propofol also was added to induce relaxation of rings precontracted by U46619 after pretreatment with the nitric oxide synthase inhibitor N^G -nitro-L-arginine methyl ester (L-NAME). The effects of propofol on Ca^{2+} influx via the L-type Ca^{2+} channels were evaluated by examining contraction-dependent responses to $CaCl_2$ in the absence or presence of propofol (10 to 300 μ mol/L). High K^+ solution and U46619 induced remarkable contractions of the rings, whereas contractions induced by 5-HT and Phe were weak. Propofol induced dose-dependent relaxation of artery rings precontracted by the high K^+ solution. Propofol also induced relaxation of rings precontracted by U46619 in an endothelium-independent way. Propofol at different concentrations significantly inhibited the Ca^{2+} -induced contractions of pulmonary rings exposed to high K^+ -containing and Ca^{2+} -free solution in a dose-dependent manner. Propofol relaxed vessels precontracted by the high K^+ solution and U46619 in an endothelium-independent way. The mechanism for this effect may involve inhibition of calcium influx through voltage-operated calcium channels (VOCCs) and receptor-operated calcium channels (ROCCs).

Key Words: Calcium influx, Endothelium, Propofol, Pulmonary artery

INTRODUCTION

The intravenous anesthetic propofol is widely used as an anesthetic in clinics and intensive care units. Circulatory suppression occurs after administration of propofol, which may involve decreased myocardial contractility and peripheral vascular resistance. Many studies have shown that propofol has direct effects on blood vessels, but the precise mechanism for these effects is not fully understood. Vasodilation effects of propofol have been demonstrated in several *in vitro* studies of blood vessels, including porcine coronary artery [1], rat aorta [2], pulmonary artery [3], coronary artery [4], renal artery [5], and fetal placental vessels [6]. In contrast, Edanarya [7] demonstrated that propofol increased

rat pulmonary vascular resistance and attenuated acetylcholine-induced pulmonary vasodilation. Other studies claimed that propofol enhanced vasoconstriction[8]. Thus, the effect of propofol and its mechanism of action may vary with species and location of vessels. In this study, we used isolated rat secondary intrapulmonary artery rings to observe the effects of propofol on pulmonary vascular tone to deduce the possible mechanism of action and to provide laboratory data to guide clinical drug use.

METHODS

Preparation of artery rings

This study was performed after obtaining permission from the ethics committee of our hospital. Healthy adult male Sprague-Dawley rats (provided by the animal laboratory at Sun Yat-sen University) weighing 200 to 300 g were anesthetized by intraperitoneal injection of pentobarbital sodium (150 mg/kg). The cardiopulmonary tissue

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ABBREVIATIONS: L-NAME, N^G -nitro-L-arginine methyl ester; DMSO, dimethyl sulfoxide; Phe: phenylephrine; 5-HT, 5-hydroxytryptamine; EGTA, ethylene glycol tetraacetic acid; VOCCs, voltage-operated calcium channels; ROCCs, receptor-operated calcium channels; NO, nitric oxide; TXA_2 , Thromboxane- A_2 .

was removed from each rat and placed into a container filled with ice cold Krebs's solution. Second order intrapulmonary small arteries were removed and cut into several rings about 1~2 mm in length. Each ring was mounted in the chamber of a Multi Myograph System with two wires passing through the lumen. Each chamber contained 5 ml of Krebs's solution bubbled constantly with 95% O₂ plus 5% CO₂. The room temperature was maintained at 37°C throughout the duration of the experiment. After an equilibration period of 60 min, each ring was stretched to an optimal tension of 2 mN, and each ring then was contracted by administration of 60 mmol/L K⁺ at 30 min intervals until two consecutive contractions occurred. Contractile ability of each ring was confirmed by visualization of good contraction after exposure to 60 mmol/L K⁺ solution. The Krebs's solution in the chambers was changed every 15 min during the equilibration period. In some rings, the endothelial layer was mechanically disrupted by gently rubbing a tiny wire back and forth over the luminal surface several times. Functional removal of the endothelial layer was verified by lack of a relaxant response to 1 μmol/L acetylcholine. U46619 and propofol were dissolved in the solvent dimethyl sulfoxide (DMSO). To make sure that the highest concentration of DMSO (1 : 500) did not affect the U46619- or high K⁺-induced vessel tone, several rings were contracted by U46619 or high K⁺, then DMSO at 1 : 500 concentration was added to the chamber.

Effects of propofol on vessels contracted by different vasoconstrictors

Endothelium-intact rings were contracted by administration of 60 mmol/L high K⁺ solution, 100 nmol U46619, 3 μmol/L 5-hydroxytryptamine (5-HT), or 1 μmol phenylephrine (Phe), and the contractile responses were recorded. If the response was more than 3 mN, cumulative doses of propofol (1 to 300 μmol/L) were added to the chambers; if not, the vasoconstrictors were cumulatively added to the chambers to make sure the dose was high enough to cause vasoconstriction.

The role of the endothelium on the vasodilation effect of propofol

Endothelium-intact rings were contracted with 100 nmol/L U46619, then propofol (1 to 300 μmol/L) was cumulatively added in the absence or presence of 1 nmol/L N^G-nitro-L-arginine methyl ester (L-NAME). Endothelium-denuded rings were precontracted with 100 nmol/L U46619. Propofol was added as described above.

The role of Ca²⁺ on the vasodilation effects of propofol

The ability of propofol to modulate Ca²⁺ influx via the L-type Ca²⁺ channels was evaluated by examining concentration-dependent responses to CaCl₂ (0.01 to 3 mmol/L) in the absence or presence of propofol (10 to 300 μmol/L). In this set of experiments, endothelium-intact rings were rinsed three times in a Ca²⁺-free solution containing 500 μmol/L of ethylene glycol tetraacetic acid (EGTA), then incubated in a Ca²⁺-free 60 mmol/L K⁺ (without or with propofol, 20 min preincubation) before cumulative addition of CaCl₂. Other rings were precontracted with 60 mmol/L K⁺ solution to open the voltage-gated Ca²⁺ channels, followed by the addition of 1 μmol/L nifedipine to block L-type volt-

age-gated Ca²⁺ channels. After the tone returned to the basal level, which indicated that most, if not all, of the L-type voltage-gated channels were blocked, the rings were recontracted with 100 nmol/L U46619. Cumulative doses (1 to 300 μmol/L) of propofol then were added to the chamber, and the relaxation curve was determined.

Data measurements

Relaxation was calculated as the percentage of contractions induced by 60 mmol/L K⁺ or 100 nmol/L U46619. E_{max} represents the maximal response percentage. EC₅₀ refers to the concentration of a drug that reduced (or increased) the maximal contraction by 50%. The negative logarithm of the dilator (or contractor) concentration that resulted in half of the maximal relaxation or contraction (pD₂) was calculated. Curves were analyzed by non-linear curve fitting using Graphpad software (Version 3.0).

Data analysis

The software SPSS 13.0 was used to conduct statistical analyses. Results are shown as mean±S.E.M of *n* arterial rings. The paired student's t-test was used to assess the effects of propofol on precontracted rings in the absence or presence of L-NAME. The independent Student's t-test was used to analyze the effects of propofol on precontracted rings with or without endothelium. One-way ANOVA followed by the LSD test was used when more than two groups were compared. p<0.05 was considered to be statistically significant.

RESULTS

Effects of different vasoconstrictors on isolated rat intrapulmonary arteries

Administration of 60 mmol/L high K⁺ solution or 100 nmol/L U46619 caused strong contraction of isolated second order rat intrapulmonary arteries, but the effects of 5-HT or Phe were very weak, even when very high concentrations of 5-HT (10 μmol/L) or Phe (30 μmol/L) were used (Table 1).

Effects of propofol on non-receptor-dependent and receptor-dependent vasoconstrictors

Propofol relaxed rings precontracted by both the high K⁺ (non-receptor-dependent vasoconstrictor) solution and U46619 (receptor-dependent vasoconstrictor) in a concentration- de-

Table 1. Reaction of isolated rat secondary pulmonary artery to different vasoconstrictors ($\bar{x}\pm s$, n=4)

	High K ⁺ solution (60 mmol/L)	U46619 (100 nmol/L)	5-HT (3 μmol/L)	Phe (1 μmol/L)
(mN)	10.67±5.98	11.59±7.09	1.81±0.91	0.08±0.07
(%)	100	106.76±16.62	20.93±14.84	10.67±16.61

mN represented contractions every contractors induced, % represented percentage of contractions every contractors induced to 60 mmol/L high K⁺ solution.

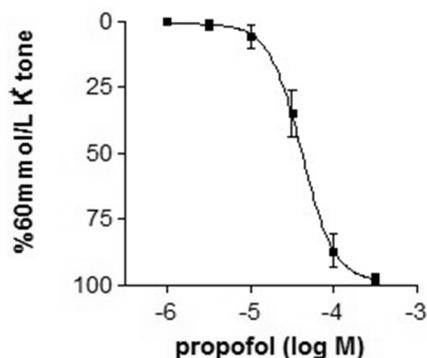


Fig. 1. Effect of propofol on 60 mmol/L K^+ precontracted secondary intrapulmonary artery rings. Responses are expressed as percentage of precontraction induced by 60 mmol/L K^+ -containing solution. Propofol induced relaxation in rings contracted by 60 mmol/L K^+ -containing solution in a concentration-dependent manner ($\bar{x} \pm s$, $n=6$).

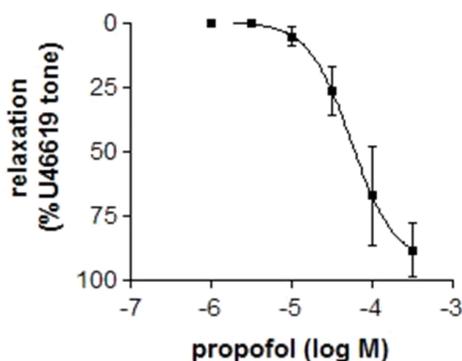


Fig. 2. Effect of propofol on 100 nmol/L U46619 precontracted secondary intrapulmonary artery rings. Responses are expressed as percentage of precontraction induced by 100 nmol/L U46619. Propofol induced relaxation in rings contracted by 100 nmol/L U46619 in a concentration-dependent manner ($\bar{x} \pm s$, $n=6$).

pendent manner (Figs. 1 and 2). The maximal relaxant effect of propofol on the high K^+ -precontracted rings was $97.57 \pm 2.05\%$ and pD_2 was 4.38 ± 0.08 ; in the U46619-precontracted rings, E_{max} was $88.18 \pm 10.33\%$ and pD_2 was 4.15 ± 0.27 (Figs. 1 and 2).

The role of endothelium on propofol-induced relaxation

Propofol induced relaxation of U46619-mediated contraction in both endothelium-intact and endothelium-denuded rings in a concentration-dependent manner (Figs. 3 and 4). Results showed that 1 μ mol/L L-NAME incubation did not affect propofol-induced maximal relaxation in endothelium-intact rings, but it did affect the value of pD_2 (relaxation: $82.60 \pm 22.15\%$ in control, $77.62 \pm 26.58\%$ in L-NAME, $n=5$, $p=0.213$; pD_2 : 4.21 ± 0.26 in control, 4.01 ± 0.28 in L-NAME, $n=5$, $p=0.012$). Propofol induced a similar degree of relaxation of both endothelium-intact and endothelium-denuded U46619-precontracted rings (relaxation: $82.60 \pm 22.15\%$ with endothelium and $86.27 \pm 18.37\%$ without endo-

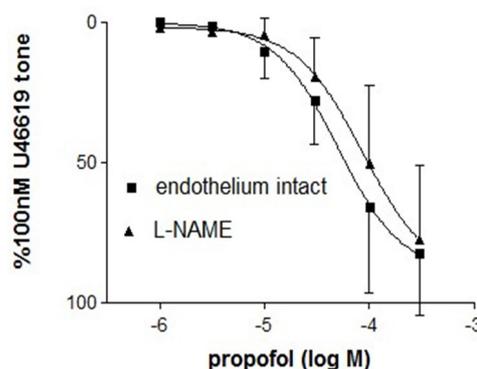


Fig. 3. The role of the endothelium on the vasodilation effect of propofol using endothelium intact rings precontracted by 100 nmol/L U46619. Responses are expressed as percentage of precontraction induced by 100 nmol/L U46619. Propofol induced relaxation in the absence or presence of L-NAME (the nitric oxide synthase inhibitor). No significant difference of E_{max} was observed in the absence or presence of L-NAME ($n=5$ for each group).

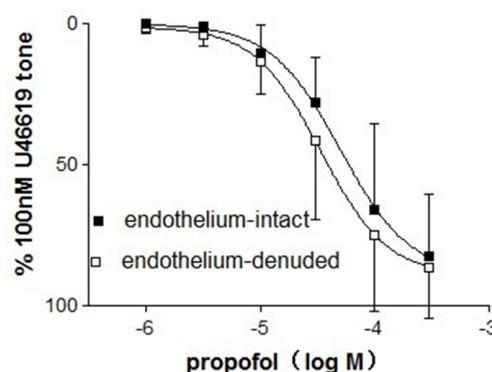


Fig. 4. The role of the endothelium on the vasodilation effect of propofol using endothelium intact rings or endothelium denuded rings precontracted by 100 nmol/L U46619. Responses are expressed as percentage of precontraction induced by 100 nmol/L U46619. No significant difference in E_{max} was observed between the endothelium-intact and endothelium-denuded groups ($n=5$ for each group).

thelium, $p=0.783$; pD_2 : 4.21 ± 0.26 with endothelium and 4.41 ± 0.36 without endothelium, $p=0.343$).

Effect of propofol on Ca^{2+} channels

Different concentrations of propofol (10 to 300 μ mol/L) were tested to evaluate their effect on $CaCl_2$ induced contractions. Cumulative addition of $CaCl_2$ induced contractions in the Ca^{2+} -free 60 mmol/L K^+ solution in the absence ($n=5$) and presence of propofol (10 to 300 μ mol/L, $n=5$). Propofol inhibited $CaCl_2$ -induced contraction with progressive reduction of maximal contraction with increasing concentrations ($p=0.000$), but the pD_2 value did not differ significantly between groups. Propofol at 100 and 300 μ mol/L totally inhibited $CaCl_2$ -induced contraction (Fig. 5).

Precontraction of rings by administration of 60 mmol/L K^+ could be fully reversed by the addition of 1 μ mol/L nifedipine, which indicates that the L-type Ca^{2+} channel was

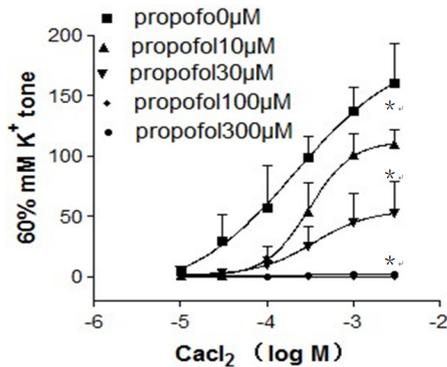


Fig. 5. CaCl₂-induced contraction in Ca²⁺-free solution containing 60 mmol/L K⁺ in the absence (n=5) and presence of propofol (10 to 300 μmol/L, n=5). A significant difference in E_{max} between control and propofol-treated groups is indicated by an asterisk (p < 0.001).

fully inhibited. A high concentration of K⁺ in the extracellular bath causes membrane depolarization, which opens voltage-gated L-type Ca²⁺ channels and results in vascular contraction. After the inhibition of L-type Ca²⁺ channels with nifedipine, subsequent addition of U46619 could still induce contraction. Cumulative addition of propofol (1 to 300 μmol/L) caused a concentration-dependent inhibition of U46619-induced contraction (E_{max}=88.97±5.60%).

DISCUSSION

The main findings of the present study were as follows: (1) Isolated rat intrapulmonary arteries exhibited a strong contractile response when they were exposed to high K⁺ solution and U46619; (2) propofol induced both non-receptor-dependent and receptor-dependent contraction; (3) propofol relaxed U46619 precontracted pulmonary rings in an endothelium-independent manner; and (4) the mechanism for these responses may involve an inhibition of influx of extracellular Ca²⁺ via voltage-operated calcium channels (VOCCs) and receptor-operated calcium channels (ROCCs). The first two findings have been reported previously and are generally accepted; although the second two findings have also been reported in the literature, they are controversial.

The advantage of the approach used in our study is that we used secondary intrapulmonary arteries, which are narrow vessels that are involved in pulmonary physiologic and pathogenic phenomena. Propofol is commonly used anesthetic, but its use is often accompanied by short-term circulatory suppression (e.g., hypotension or low heart rate) [8]. Propofol has direct effects on vessel tone, but the precise mechanism for this effect is not fully understood.

Solutions containing high K⁺ or U46619 induced contraction of isolated rat intrapulmonary arteries, but 5-HT or Phe did not, even at high concentrations. Propofol induced relaxation of both non-receptor-dependent and receptor-dependent contraction, with great potency on KCl-induced contraction when the concentration of propofol accumulated to 300 μmol/L. U46619 is a thromboxane mimic. Thromboxane-A₂ (TXA₂) is an unstable prostanoid produced by thromboxane-A synthase. It acts on the TxA₂ receptor

to induce smooth muscle contraction. Increases of TXA₂ in plasma reflect a disorder of endothelium function. Therefore, propofol may be a good anesthetic and vessel dilator in patients with endothelium function disorders.

The vascular endothelium produces many substances to modulate relaxation and contraction of vascular smooth muscles. Nitric oxide (NO) is one of the important endothelium-derived relaxing factors synthesized by NO synthase. NO activates dissoluble guanylate cyclase, which increases cAMP in vascular smooth muscle cells. cAMP downregulates intracellular Ca²⁺, which results in vascular smooth muscle relaxation. The NO synthase inhibitor L-NAME blocks NO synthase, thus reducing the production of NO. In the present study, propofol induced similar relaxation on both endothelium-intact and endothelium-denuded U46619 precontracted rings, and no significant difference was observed between endothelium-intact rings in the absence or presence of L-NAME. These results show that the effect of propofol on precontracted intrapulmonary artery rings likely does not occur through the endothelium. Wallerstedt et al. [9] found that propofol relaxed human omental arteries and veins in an endothelium-independent manner. Liu et al. [10] reported that propofol inhibited KCl-, nor-epinephrine-, and U46619-induced contractions of isolated rat renal arterioles, with greater inhibition of KCl-induced contraction, which may indicate that propofol inhibits contractions involved in inhibition of extracellular Ca²⁺ influx. Our study showed similar results.

Ca²⁺ plays a very important role in cellular function, and it also is involved in the pathogenesis of diseases such as pulmonary hypertension [11]. When the vascular smooth muscle contracts, the [Ca²⁺]_i increases mainly via VOCCs and ROCCs. VOCCs are activated by membrane depolarization in vascular smooth muscle cells when the extracellular K⁺ concentration is elevated [12]. In the present study, propofol significantly reduced CaCl₂-induced vasoconstriction in the high K⁺ solution. This is direct evidence that propofol acts as antagonist on L-type Ca²⁺ channels in vascular smooth muscle isolated from rat intrapulmonary artery. Propofol also reduced U46619-elicited contraction, which indicates that propofol may inhibit TXA₂-sensitive receptor-operated Ca²⁺ channels. Furthermore, when the artery rings were first incubated with nifedipine to block L-type Ca²⁺ channels, propofol also inhibited U46619-induced contraction in a dose-dependent manner. Thus, propofol may also act as a non-L-type Ca²⁺ channel blocker [13]. However, the exact mechanism of action of propofol on intrapulmonary arteries still requires further investigation.

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REFERENCES

1. Klockgether-Radke AP, Schulze H, Neumann P, Hellige G. Activation of the K⁺ channel BK(Ca) is involved in the relaxing effect of propofol on coronary arteries. *Eur J Anaesthesiol.* 2004;21:226-230.
2. Yu J, Kakutani T, Mizumoto K, Hasegawa A, Hatano Y. Propofol inhibits phorbol 12, 13-dibutyrate-induced, protein

- kinase C-mediated contraction of rat aortic smooth muscle. *Acta Anaesthesiol Scand*. 2006;50:1131-1138.
3. **Kondo U, Kim SO, Nakayama M, Murray PA.** Pulmonary vascular effects of propofol at baseline, during elevated vaso-motor tone, and in response to sympathetic alpha- and beta-adrenoreceptor activation. *Anesthesiology*. 2001;94:815-823.
 4. **Park KW, Dai HB, Lowenstein E, Sellke FW.** Propofol-associated dilation of rat distal coronary arteries is mediated by multiple substances, including endothelium-derived nitric oxide. *Anesth Analg*. 1995;81:1191-1196.
 5. **Liu Y, Chang H, Niu L, Xue W, Zhang X, Liang Y, Zhang M.** Effects of propofol on responses of rat isolated renal arteriole to vasoactive agents. *Vascul Pharmacol*. 2009;51:182-189.
 6. **Soares de Moura R, Silva GA, Tano T, Resende AC.** Effect of propofol on human fetal placental circulation. *Int J Obstet Anesth*. 2010;19:71-76.
 7. **Edanaga M, Nakayama M, Kanaya N, Tohse N, Namiki A.** Propofol increases pulmonary vascular resistance during alpha-adrenoreceptor activation in normal and monocrotaline-induced pulmonary hypertensive rats. *Anesth Analg*. 2007;104:112-118.
 8. **Kondo U, Kim SO, Nakayama M, Murray PA.** Pulmonary vascular effects of propofol at baseline, during elevated vaso-motor tone, and in response to sympathetic alpha- and beta-adrenoreceptor activation. *Anesthesiology*. 2001;94:815-823.
 9. **Wallerstedt SM, Törnebrandt K, Bodelsson M.** Relaxant effects of propofol on human omental arteries and veins. *Br J Anaesth*. 1998;80:655-659.
 10. **Liu Y, Chang H, Niu L, Xue W, Zhang X, Liang Y, Zhang M.** Effects of propofol on responses of rat isolated renal arteriole to vasoactive agents. *Vascul Pharmacol*. 2009;51:182-189.
 11. **Firth AL, Won JY, Park WS.** Regulation of Ca^{2+} signaling in pulmonary hypertension. *Korean J Physiol Pharmacol*. 2013;17:1-8.
 12. **Akata T.** Cellular and molecular mechanisms regulating vascular tone. Part 1: basic mechanisms controlling cytosolic Ca^{2+} concentration and the Ca^{2+} -dependent regulation of vascular tone. *J Anesth*. 2007;21:220-231.
 13. **Leung YK, Du J, Huang Y, Yao X.** Cyclic nucleotide-gated channels contribute to thromboxane A2-induced contraction of rat small mesenteric arteries. *PLoS One*. 2010;5:e11098.