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 μmol/L); or phenylephrine (Phe; 1 μmol/L). The relaxation effects of propofol were tested on high K⁺ or U46619 precontracted rings. Propofol also was added to induce relaxation of rings preconstricted by U46619 after pretreatment with the nitric oxide synthase inhibitor N³-nitro-L-arginine methyl ester (L-NAME). The effects of propofol on Ca²⁺ influx via the L-type Ca²⁺ channels were evaluated by examining contraction-dependent responses to CaCl₂ in the absence or presence of propofol (10 to 300 μmol/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 preconstricted by U46619 in an endothelium-independent way. Propofol at different concentrations significantly inhibited the Ca²⁺-induced contractions of pulmonary rings exposed to high K⁺-containing and Ca²⁺-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. Vasodilatation 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, Edanaga [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

ABBREVIATIONS: L-NAME, N³-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; TXA₂, Thromboxane-A₂.
was removed from each rat and placed into a container filled with ice cold Kreb’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 Kreb’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 was then 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 Kreb’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 L-NAME, or 1 μmol phenylephrine (Phe), and the contractile responses were recorded. If the response was more than 3 mN, cumulative doses of propofol (10 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 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 voltage-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 contracted 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. Emax represents the maximal response percentage. Emax 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 preconstricted rings in the absence or presence of L-NAME. The independent Student’s t-test was used to analyze the effects of propofol on preconstricted 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 (x±s, n=4)

| Vasoconstrictor | U46619 | 5-HT | Phe |
|-----------------|--------|------|-----|
| (60 mmol/L)     | (100 nmol/L) | (3 μmol/L) | (1 μmol/L) |
| (mN)            | 10.67±5.98 | 12.59±7.09 | 1.81±0.91 |
| (%)             | 100     | 100.76±16.62 | 20.93±14.84 |
| pD2             | 10.67±16.61 | 10.67±16.61 | 0.08±0.07 |

mN represents contraction. % represents percentage of contractions every contractile induced to 60 mmol/L high K+ solution.
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Fig. 1. Effect of propofol on 60 mmol K+ preconstricted 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 (x±s, n=6).

Fig. 2. Effect of propofol on 100 mmol/L U46619 preconstricted secondary intrapulmonary artery rings. Responses are expressed as percentage of precontraction induced by 100 mmol/L U46619. Propofol induced relaxation in rings contracted by 100 mmol/L U46619 in a concentration-dependent manner (x±s, n=6).

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

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**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 μmol/L L-NAME incubation did not affect propofol-induced maximal relaxation in endothelium-intact rings, but it did affect the value of pD2 (relaxation: 82.60±22.15% in control, 77.62±26.58% in L-NAME, n=5, p=0.213; pD2: 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-preconstricted rings (relaxation: 82.60±22.15% with endothelium and 86.27±18.37% without endothelium, p=0.783; pD2: 4.21±0.26 with endothelium and 4.41±0.36 without endothelium, p=0.343).

**Effect of propofol on Ca2+ channels**

Different concentrations of propofol (10 to 300 μmol/L) were tested to evaluate their effect on CaCl2 induced contractions. Cumulative addition of CaCl2 induced contractions in the Ca2+-free 60 mmol/L K+ solution in the absence (n=5) and presence of propofol (10 to 300 μmol/L, n=5). Propofol inhibited CaCl2-induced contraction with progressive reduction of maximal contraction with increasing concentrations (p=0.000), but the pD2 value did not differ significantly between groups. Propofol at 100 and 300 μmol/L totally inhibited CaCl2-induced contraction (Fig. 5). Preconstriction of rings by administration of 60 mmol/L K+ could be fully reversed by the addition of 1 μmol/L nifedipine, which indicates that the L-type Ca2+ channel was
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 soluble 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 preconstricted 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 preconstricted 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-, noradrenaline-, and U46619-induced contractions of isolated rat renal arterioles, with greater inhibition of KCI-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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