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Propofol Relaxes Extrapulmonary Artery but not Intrapulmonary Artery through Nitric Oxide Pathway

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ABSTRACT

The object of this study was to compare vasorelaxing responses to propofol by the intrapulmonary artery (IPA) and the extrapulmonary artery (EPA), and to identify the mechanisms of action. Rat pulmonary arterial rings were isolated and suspended in organ chambers where isometric tension development was measured under optimal resting tension. All pulmonary arterial rings were pre-contracted with phenylephrine. Propofol (Diprivan™) and the vehicle (10% Intralipid™) were administered cumulatively in the presence or absence of N^{ω}-nitro-L-arginine methyl ester (L-NAME). Sodium nitroprusside (SNP), a nitric oxide donor, was administered cumulatively. Propofol relaxed both EPA and IPA in a dose dependent manner ($p<0.05$), while the vehicle alone showed no effect. The vasorelaxing responses to propofol were significantly higher in EPA than IPA at higher concentrations (10^{-4} M and $10^{-4.5}$M) ($p<0.05$), and were decreased by L-NAME in EPA ($p<0.05$), though it had no effect in IPA. The concentration for SNP causing 50% relaxation was not significantly different between the two arteries. We concluded that the response of smooth muscle to nitric oxide was the same between EPA and IPA; however, the vasorelaxing mechanisms of propofol seemed to be different between them at higher doses, suggesting that a mechanism exists and operates through the nitric oxide pathway.

Key words: *Propofol, Pulmonary circulation, Endothelium, Nitric oxide*

Propofol is a new intravenous anesthetic commonly used in clinical anesthesia because of its rapid onset and short duration of action. One of the mechanisms of action in the central nervous system is direct activation of the γ-aminobutyric acid receptor-chloride ionophore complex and an increase in chloride conductance[5]. Hypotension after propofol administration is not infrequent in clinical anesthesia, and one of the mechanisms is apparently associated with a decrease in systemic vascular resistance[2]. However, the effect of propofol on pulmonary circulation is still controversial, as differences have been demonstrated among animal species and experimental methods [1,3,4,7,10,14,15,19].

Systemic vascular tone is modulated partly by a continuous release of nitric oxide (NO)[11], and the active release of NO also maintains pulmonary vascular tone[17]. Petro et al found that propofol stimulates NO release from cultured porcine endothelial cells[15]. This may be one of the mechanisms assumed to induce hypotension and vasodilation after propofol administration.

The pulmonary artery develops embryologically from two originating parts: one from the sixth aortic arch and the other from the lung bud. Adrenergic nerve fibers are found in the extrapulmonary artery (EPA) arising from the sixth aortic arch, while no portion of the intrapulmonary artery (IPA), arising from the lung bud, has any adrenergic innervation[12].

We hypothesized that vasorelaxing responses to propofol may depend on the pulmonary arterial regions, i.e. EPA and IPA. Therefore, we investigated vasorelaxing responses to propofol in EPA and IPA, as well as the role of NO in the mechanisms of propofol-induced vasorelaxation of the pulmonary artery.

MATERIALS AND METHODS

The study protocol was approved by the Animal Research Committee of the Research Facilities for Laboratory Animal Science at our institute. Male Wistar rats (Charles River Japan Inc., Yokohama, Japan), 10–12 weeks old, weighing 330–450 g,

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were used. They were anesthetized with intraperitoneal pentobarbital (50 mg/kg), and the lungs and heart were excised *en bloc* and placed in a modified Krebs-Henseleit solution [mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, dextrose 11, and ethylenediamine tetraacetic acid (EDTA) 0.026] at room temperature (approximately 25°C). Paired pulmonary arterial rings, from EPA and IPA, were isolated from the left lung and, after the connective tissue was cleaned off, were sliced into ring segments 3 mm in length. The rings were suspended vertically for isometric tension recordings between two triangular hooks in organ chambers containing 20 ml of Krebs-Henseleit solution, and bubbled with a mixed gas containing 95% O_2/5% CO_2 at 37°C. One hook was fixed at the bottom of the chamber and the other was connected to a force-displacement transducer (TB-611T, NihonKohden, Tokyo, Japan) through an amplifier (AP-601G, NihonKohden, Tokyo, Japan) and then to a digital recorder (Omniace RT3200, NEC San-ei, Tokyo, Japan).

Active and resting tension relationships were obtained by increasing the resting tension, using a range of force from 0.25 to 2.0 g, according to the methods of Toda[18]. After equilibration of the resting tension, the rings were contracted with 60 mM of KCl to measure active tension. The optimal resting tension was then determined with 1.5 g for EPA and 1.0 g for IPA. A cumulative concentration-response curve to phenylephrine (10^{-9}–$10^{-5.5}$ M) was obtained under optimal resting tension. The effective concentration that produced a 50% response (EC_{50}) was determined to be $10^{-7.29}$ M for EPA and $10^{-7.36}$ M for IPA. Therefore, we used 10^{-7} M of phenylephrine for the pre-contraction of EPA and IPA. The rings were pre-contracted with phenylephrine, and cumulative concentration-response curves to propofol (10^{-7}–10^{-4} M) were obtained by taking 10^{-4} M of papaverine-induced relaxation as 100%. Since commercially available propofol (Diprivan™) is provided in a 10% Intralipid™ emulsion, we also investigated the effects of 10% Intralipid™ as a vehicle.

In order to measure the effects of sodium nitroprusside (SNP), other rings were pre-contracted with phenylephrine, and cumulative concentration-response curves to SNP were determined. The inhibitory concentration causing 50% relaxation of the contraction to phenylephrine (IC_{50}) for SNP was calculated. Other rings were pretreated with 300 μM of N^{ω}-nitro-L-arginine methyl ester (L-NAME) 20 min before pre-contraction with phenylephrine, and then the cumulative concentration-response curve to propofol was obtained. Finally, 10^{-6} M of acetylcholine chloride (ACh) was added, and the endothelium was confirmed to be intact.

Krebs-Henseleit buffer, phenylephrine, L-NAME, papaverine, ACh, and EDTA were purchased from Sigma Chemical Co., St. Louis, USA; $CaCl_2$ and $NaHCO_3$ were from Katayama Chemical, Osaka, Japan, and Diprivan™ was from AstraZeneca Pharmaceuticals Japan, Osaka, Japan. Intralipid™ was from Ohtsuka Pharmaceuticals, Tokushima, Japan.

For statistical analysis, the effects of propofol and the vehicle were compared using ANOVA, followed by Scheffé's test. The vasorelaxing responses to propofol between EPA and IPA were compared by a paired t-test, while the inhibitory effects of L-NAME with EPA and IPA were compared by an unpaired *t*-test. The IC_{50} for SNP was calculated by regression analysis and compared by an unpaired *t*-test using StatView 4.0, Abacus Concept, Inc., Berkeley, USA. Statistical significance was considered when p was less than 0.05. Values are expressed as mean ± SEM. N is the number of rats from which the pulmonary arterial rings were isolated.

RESULTS

Propofol caused vasorelaxing responses in both EPA and IPA in a dose dependent manner ($p<0.05$), while the vehicle solution showed none (Fig. 1). The responses were significantly higher in EPA than IPA at high concentrations (10^{-4} M and $10^{-4.5}$M) ($p<0.05$) (Fig. 1). The IC_{50} to SNP was determined to be $10^{-8.28\pm0.25}$ M for EPA and $10^{-8.21\pm0.24}$ M for IPA. SNP relaxed both EPA and IPA dose-dependently after pre-contraction with

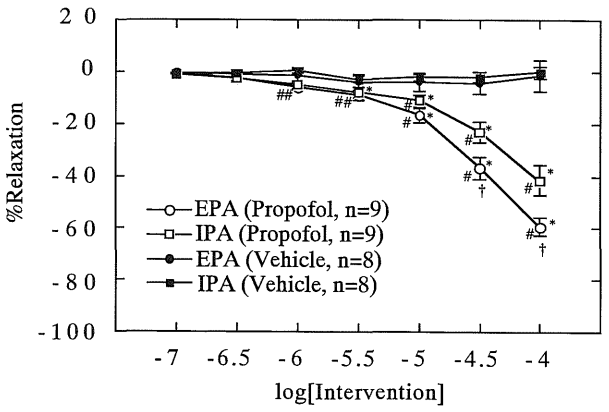

Fig. 1. Rings were pre-contracted with 10^{-7} M of phenylephrine, and cumulative concentration-response curves to propofol (10^{-7}–10^{-4} M) (n = 9) and the vehicle solution (10% intralipid™) (n = 8) were obtained. Propofol relaxed both EPA and IPA in a dose dependent manner, however, the vehicle solution had no effect on either. At higher concentrations of propofol ($10^{-4.5}$ M or more), the degree of relaxation was higher in EPA than IPA. Data are given as mean ± SEM. EPA = extrapulmonary artery, IPA = intrapulmonary artery, % Relaxation = percentage obtained after taking 10^{-4} M of papaverine-induced relaxation as 100%. *$p<0.05$ *versus* % relaxation at 10^{-7} M of propofol. #$p<0.05$ *versus* vehicle. †$p<0.05$ *versus* IPA.

phenylephrine, while the IC_{50} was not significantly different between EPA and IPA. After pretreatment with L-NAME, propofol relaxed EPA and IPA in the same manner as in Fig. 1. Vasorelaxing responses to propofol were decreased by L-NAME in EPA ($p<0.05$), but not in IPA (Fig. 2).

DISCUSSION

We demonstrated that propofol relaxed pulmonary arteries dose-dependently, and that the vasorelaxing response to propofol was dependent on the region of the pulmonary artery. L-NAME, NO synthase (NOS) inhibitor, blunted the vasorelaxing responses to propofol in EPA, but not in IPA. There were no differences in the vasorelaxing responses to SNP, a NO donor. As a result, we considered that the response of smooth muscle to

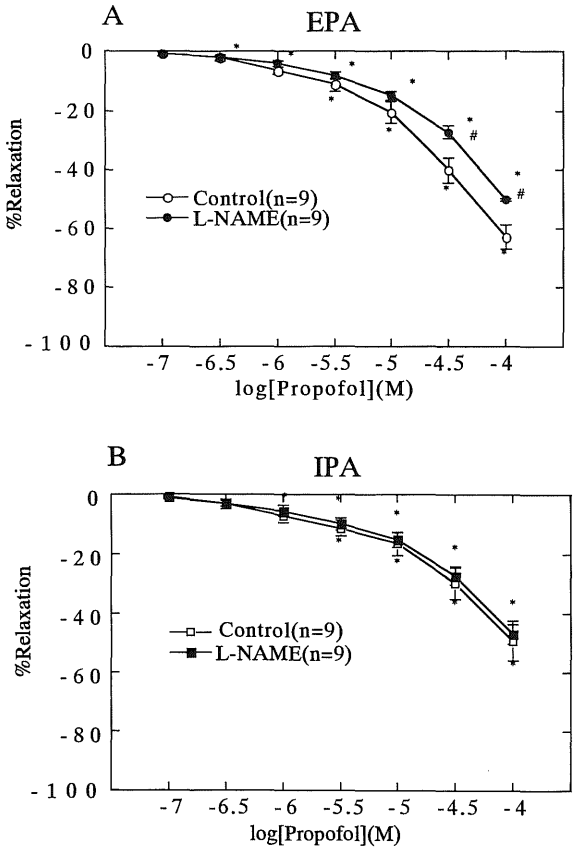

Fig. 2. Rings were pre-contracted with 10^{-7} M of phenylephrine, and cumulative concentration-response curves to propofol ($10^{-7} - 10^{-4}$ M) were obtained in the presence or absence of 300 μM of L-NAME. Propofol relaxed both EPA and IPA in a dose dependent manner after pretreatment with L-NAME. The vasorelaxing response to propofol in EPA was decreased by L-NAME (A); however, there was no effect in IPA (B). Data are presented as mean ± SEM. EPA = extrapulmonary artery, IPA = intrapulmonary artery, L-NAME = N^{ω}-nitro-L-arginine methyl ester, % Relaxation = percentage obtained after taking 10^{-4} M of papaverine-induced relaxation as 100%. *$p<0.05$ *versus* % relaxation at 10^{-7} M of propofol. #$p<0.05$ *versus* control.

NO was the same between EPA and IPA. However, the vasorelaxation mechanisms of propofol seem to be different between them, suggesting a mechanism through the NO pathway.

The regulation of vascular constriction and dilation is classified into three major mechanisms: a neural mechanism, a humoral mechanism, and a local mechanism, which includes endothelial intervention[12]. The endothelial intervention was mainly investigated in the present experimental model.

Nerve terminals exist in the isolated arteries. Some adrenergic nerve fibers are found in EPA; however, IPA has none of this type of innervation[6]. Geiger et al reported differential NO production in intrapulmonary arterial and aortic endothelial cells[4], which mechanisms depend on inducible NOS. It seems that both sympathetic innervation and endothelin-mediated vasodilation are different between EPA and IPA[7]. The pulmonary artery develops embryologically from two originating parts, one from the sixth aortic arch and the other from the lung bud. The sixth aortic arch develops into EPA and the lung bud becomes IPA. EPA has sympathetic innervation, while IPA does not[6]. Vasodilation mediated by endothelin B receptor is more potent in EPA than IPA[7]. From an embryological point of view, the vasodilation mechanism of propofol seems to be different between EPA and IPA.

It is considered that the primary mechanism of vasodilation of volatile anesthetics is a decrease in the intracellular calcium concentration, while other mechanisms, including inhibition of protein kinase C and endothelium dependency, are presumed. The same mechanisms are presumed in vasodilation caused by propofol. It was previously demonstrated that propofol caused marked vasodilation in pulmonary arteries, though the propofol vehicle (Intralipid™) had no effect[14]. Thiopental and etomidate are direct pulmonary vasoconstrictors, and ketamine and propofol are direct pulmonary vasodilators, while midazolam had no direct effects on isolated rat lung specimens[16]. These effects on pulmonary vasculature do not vary with baseline pulmonary vascular tone, and only propofol has endothelium-dependent effects[16]. Propofol causes a release of NO from cultured porcine aortic endothelial cells[15], which may account for the hypotension associated with propofol. However, the duration of exposure to propofol would have been too short to activate inducible NOS activity in the present experiment.

Our results suggested that NOS inhibitor blunted the vasorelaxing responses to propofol in EPA. However, NOS inhibitor did not completely reverse the vasorelaxation by propofol in EPA, and produced no effect on IPA. Other mechanisms, which are endothelium-dependent or endothelium-independent may exist. Other endothelium-derived relaxing factors, i.e. prostacyclin[14] or

endothelium-derived hyperpolarizing factor[8], were not investigated in this study.

Recent studies have revealed that propofol might inhibit the vasodilation through endothelium. Hypoxic pulmonary vasoconstriction is preserved during ketamine anesthesia, but is potentiated during propofol anesthesia. This potentiated response during propofol anesthesia appears to be caused by an inhibition of adenosine triphosphate-sensitive potassium channel-mediated pulmonary vasodilation[13]. Propofol impairs the signal transduction pathway for acetylcholine-induced pulmonary vasodilation, which involves the endothelial, but not the vascular smooth muscle, component of the response[9]. The vasodilation is mediated by two components, NO and a cytochrome P450 metabolite, and propofol selectively attenuates the vasodilation by inhibiting both of these endothelium-derived mediators[8]. There were some differences in experimental methods in these studies. Further experiment might reveal the nature of these mechanisms.

In conclusion, vasorelaxing responses to propofol were different depending on the region of the pulmonary artery, and one of the mechanisms is likely to be through the NO pathway in EPA.

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