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Propofol Potentiates Phenylephrine-induced Contraction via *Cyclooxygenase Inhibition in Pulmonary Artery Smooth Muscle*

Koji Ogawa, M.D., Satoru Tanaka, M.D.,* Paul A. Murray, Ph.D.†*

Background: **The authors previously demonstrated *in vivo* that the pulmonary vasoconstrictor response to the α agonist phenylephrine is potentiated during propofol anesthesia compared with the conscious state. The current *in vitro* study tested the hypothesis that propofol potentiates phenylephrine-induced contraction by inhibiting the synthesis and/or activity of vasodilator metabolites of the cyclooxygenase pathway.**

Methods: **Canine pulmonary arterial rings were suspended for isometric tension recording. Intracellular calcium concentration ($[Ca^{2+}]_i$) was measured in pulmonary arterial strips loaded with acetoxylmethyl ester of fura-2. After phenylephrine-induced contraction, propofol (10^{-7} to 10^{-4} M) was administered in the presence or absence of the cyclooxygenase inhibitor ibuprofen (10^{-5} M). The effects of propofol on the arachidonic acid and prostacyclin relaxation–response curves were assessed. The amount of 6-keto prostaglandin $F_{1\alpha}$ (stable metabolite of prostacyclin) released from pulmonary vascular smooth muscle in response to phenylephrine was measured with enzyme immunoassay in the presence or absence of propofol and ibuprofen.**

Results: **Propofol potentiated phenylephrine-induced contraction in pulmonary arterial rings in a concentration-dependent and endothelium-independent manner. In endothelium-denuded strips, propofol (10^{-4} M) increased tension by 53 ± 11%, and increased $[Ca^{2+}]_i$ by 56 ± 9%. Ibuprofen also potentiated phenylephrine-induced contraction but abolished the propofol-induced increases in tension and $[Ca^{2+}]_i$. Propofol had no effect on the relaxation response to prostacyclin, whereas propofol and ibuprofen attenuated the relaxation response to arachidonic acid to a similar extent. Phenylephrine markedly increased 6-keto prostaglandin $F_{1\alpha}$ production, and this effect was virtually abolished by propofol and ibuprofen.**

Conclusion: **These results suggest that propofol potentiates α-adrenoreceptor–mediated pulmonary vasoconstriction by inhibiting the concomitant production of prostacyclin by cyclooxygenase.**

THE intravenous anesthetic propofol (2,6-di-isopropylphenol) causes vasorelaxation in a number of systemic vascular beds, including the coronary,[1,2] cerebral,[1,3] and mesenteric[1,4] circulations. This propofol-induced systemic vasorelaxation is thought to be mediated *via* inhibition of Ca^{2+} channels.[2,3,5] In comparison to the systemic circulation, relatively little is known about the effects and mechanism of action of propofol in the pulmonary circulation.

We have observed in chronically instrumented dogs that the pulmonary vasoconstrictor response to the α-adrenoreceptor agonist phenylephrine is potentiated during propofol anesthesia compared with the conscious state.[6] Moreover, in that same *in vivo* study, propofol caused pulmonary vasoconstriction when vasomotor tone was increased *via* α-adrenoreceptor activation.[6] The goal of the present *in vitro* study was to investigate the cellular mechanism responsible for these pulmonary vascular effects of propofol. Specifically, we tested the hypothesis that propofol anesthesia exerts an inhibitory influence on the cyclooxygenase pathway, which normally acts to modulate the pulmonary vasoconstrictor response to α-adrenoreceptor activation.

Materials and Methods

All experimental procedures and protocols were approved by the Cleveland Clinic Institutional Animal Care and Use Committee, Cleveland, OH.

Preparation of Pulmonary Arterial Rings and Strips

Twenty-five healthy male mongrel dogs weighing 20–25 kg were anesthetized with 30 mg/kg intravenous pentobarbital sodium and 15 μg/kg intravenous fentanyl citrate and were placed on positive-pressure ventilation. A catheter was placed in the right femoral artery, and the dogs were exsanguinated by controlled hemorrhage. A left lateral thoracotomy was performed through the fifth intercostal space, and the heart was arrested with electrically induced ventricular fibrillation. The heart and lungs were removed from the thorax *en bloc*, and the right and left lower lung lobes were dissected free. Intralobar pulmonary arteries (2–4-mm ID) were carefully dissected and immersed in cold modified Krebs-Ringer bicarbonate (KRB) solution composed of 118.3 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 0.016 mM Ca-EDTA, and 11.1 mM glucose. For protocols using pulmonary arterial rings, the arteries were cleaned of connective tissue and cut into ring segments 5 mm in length. For protocols using pulmonary arterial strips, the arteries were cut into strips (2 × 8 mm). In most rings and all strips, the endothelium was denuded by gently rubbing the intimal surface with a cotton swab. The presence and absence

* Research Fellow, † Carl E. Wasmuth Endowed Chair and Director.

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Address reprint requests to Dr. Murray: Center for Anesthesiology Research, FF40, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195. Address electronic mail to: murrayp@ccf.org.

of an intact endothelium was later verified by assessing the vasorelaxant response to acetylcholine (10^{-6} M).

Isometric Tension Experiments

Approximately 1 h was required to prepare the pulmonary arterial rings for isometric tension experiments. Rings were mounted between two stainless steel hooks in an organ bath filled with 25 ml KRB solution (37°C, pH 7.4) gassed with 95% air and 5% CO_2. One hook was anchored and the other was connected to a strain gauge (Grass Model FTO3, Quincy, MA) to measure isometric force. The rings were stretched at 10-min intervals in increments of 0.5 g to achieve optimal resting tension. Optimal resting tension was defined as the minimum amount of stretch required to achieve the largest contractile response to 20 mM KCl and was determined in preliminary experiments to be 5.0 g for the size of arteries used in these experiments. After the arterial rings had been stretched to their optimal resting tension, the contractile response to 60 mM KCl was measured. After washing out the KCl from the organ bath and the return of isometric tension to prestimulation values, a concentration–response curve to the α-adrenoreceptor agonist phenylephrine was performed in each ring. This was achieved by increasing the concentration of phenylephrine in half-log increments (10^{-8} to 3×10^{-5} M) after the response to each preceding concentration had reached a steady state. The rings were pretreated with propranolol (5×10^{-6} M, incubated for 30 min) before phenylephrine administration in all protocols to inhibit the β-agonist effect of phenylephrine. After washout of phenylephrine from the organ bath and the return of isometric tension to baseline values, the rings were again pretreated with propranolol and contracted to 50% of the maximal response to phenylephrine (ED_{50} level of tension). At steady state tension, propofol (10^{-7} to 10^{-4} M) was added to the organ chamber. Pure propofol was used to avoid any possible effects of the lipid emulsion diluent on vessel tension[7] and to avoid interference with measurements of intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration. To assess the contribution of cyclooxygenase metabolites to the propofol-induced changes in tension, similar experiments were performed in the presence of ibuprofen (10^{-5} M), a potent cyclooxygenase inhibitor. To elucidate the putative site of action of propofol on the cyclooxygenase pathway, the effects of propofol on the pulmonary vasorelaxant responses to arachidonic acid and prostacyclin were assessed. After the rings had been treated with propranolol and ibuprofen and contracted to the phenylephrine ED_{50}, arachidonic acid (10^{-8} to 3×10^{-5} M) or prostacyclin (10^{-9} to 10^{-6} M) was added to the organ chamber in a cumulative fashion in the presence or absence of propofol (10^{-4} M).

Intracellular Ca^{2+} Measurements

Pulmonary arterial strips without endothelium were loaded with 5×10^{-6} M acetoxylmethyl ester of fura-2 (fura-2–AM) solution for 15 h at room temperature (22–24°C). A noncytotoxic detergent, 0.05% Cremophor EL, was added to solubilize the fura-2–AM in the solution. After fura-2 loading, the arterial strips were washed with KRB buffer and mounted between two stainless steel hooks in a temperature-controlled (37°C) cuvette (volume = 3 ml) that was continuously perfused (12 ml/min) with KRB solution bubbled with 95% air and 5% CO_2 (pH 7.4). One hook was anchored and the other was connected to a strain gauge transducer (Grass Model FTO3) to measure isometric force. The resting tension was adjusted to 4.0 g, which was determined in preliminary studies to be optimal for achieving a maximum contractile response to 20 mM KCl. Fluorescence measurements were performed using a dual-wavelength spectrofluorometer (Deltascan RFK6002; Photon Technology International, South Brunswick, NJ) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The 340 to 380 fluorescence ratio was used as an indicator of $[Ca^{2+}]_i$. The temperature of all solutions was maintained at 37°C in a water bath. Just before data acquisition, background fluorescence was measured and subtracted automatically from the subsequent experimental measurement. Fura 2 fluorescence signals (340, 380, and 340 to 380 nm ratio) were continuously monitored at a sampling frequency of 25 Hz and collected with a software package (Felix) from Photon Technology International. After measuring changes in tension and $[Ca^{2+}]_i$ in response to 60 mM KCl, the strips were washed with fresh KRB for 30 min. The strips were pretreated with propranolol (5×10^{-6} M, incubated for 30 min) and then precontracted with phenylephrine (10^{-5} M). After tension and $[Ca^{2+}]_i$ reached new steady state values, propofol (10^{-4} M) was added to the perfusate. This procedure was repeated in strips that were pretreated with ibuprofen (10^{-5} M). Changes in tension and $[Ca^{2+}]_i$ are expressed as a percentage of the response to phenylephrine.

Measurement of 6-keto Prostaglandin $F_{1\alpha}$

The amount of 6-keto prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin, released from pulmonary arterial rings was measured with an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Rings without endothelium were incubated in the organ bath filled with KRB solution as described above. During the equilibration period, the rings were washed every 12 min to remove accumulated eicosanoid metabolites. The rings were washed immediately before addition of phenylephrine (10^{-5} M) and again 12 min after phenylephrine administration in the presence and absence of propofol (10^{-4} M) or ibuprofen (10^{-5} M). Aliquots of the organ bath fluid were frozen for later analysis. Measurements

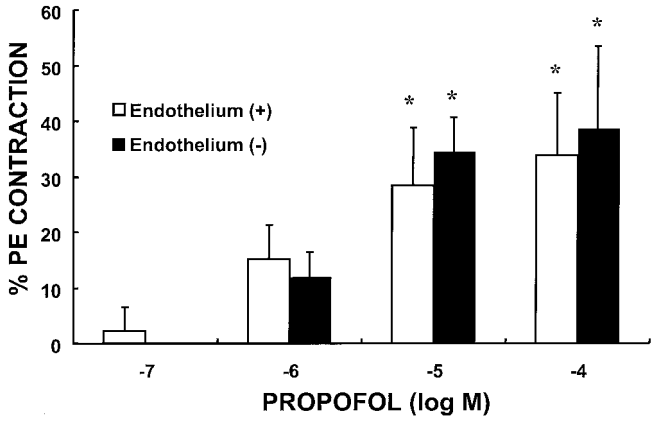

Fig. 1. Effects of propofol on phenylephrine-induced contraction of canine pulmonary arterial rings with or without endothelium (n = 7 each). Tension changes are expressed as a percentage of the contraction to phenylephrine (PE). Propofol potentiated (*$P < 0.05$) phenylephrine-induced contraction. Removal of endothelium did not affect the contractile response to propofol.

of 6-keto prostaglandin $F_{1\alpha}$ production are expressed as picograms per milligram wet weight per 12 min.

Drugs and Solutions

All drugs were of the highest purity commercially available: propofol (RBI, Natick, MA), fura-2–AM (TEF Labs, Austin, TX), ibuprofen, arachidonic acid, prostacyclin (Cayman Chemical), acetylcholine chloride, phenylephrine HCl, propranolol HCl, and Cremophor EL (Sigma Chemical, St. Louis, MO). Propofol and ibuprofen were dissolved in dimethylsulfoxide and diluted with distilled water. Arachidonic acid was dissolved in 99.5% ethanol. The final concentrations of dimethylsulfoxide and ethanol in the organ bath were less than 0.1% (vol/vol). The vehicles had no effect on tension or $[Ca^{2+}]_i$ at the concentrations used in these studies.

Data Analysis

All values are presented as mean ± SD. The ED_{50} and IC_{50} values were calculated from the linear portion of the concentration–effect curve using regression analysis. Statistical analysis used one-way analysis of variance, the Scheffé F test, and the Student *t* test for paired comparisons. A *P* value < 0.05 was considered statistically significant. In all experiments, n equals the number of dogs from which pulmonary arterial rings or strips were obtained.

Results

Effects of Propofol on Tension and Intracellular Ca^{2+} Concentration in Phenylephrine Precontracted Pulmonary Vascular Smooth Muscle

Propofol had no effect on resting tension but increased tension in pulmonary arterial rings precontracted to the phenylephrine ED_{50} (fig. 1). The propofol-induced increases in tension were similar in endothelium-intact and -denuded rings, which indicates that the contractile response to propofol is endothelium-independent (fig. 1). Thus, all additional experiments used endothelium-denuded pulmonary arteries. Pretreatment with propofol potentiated the contractile response to phenylephrine, causing a leftward shift in the concentration–effect curve and increasing the maximum response to phenylephrine (table 1). In pulmonary arterial strips, phenylephrine (10^{-5} M) increased tension and $[Ca^{2+}]_i$ (fig. 2). After the phenylephrine-induced increases in tension and $[Ca^{2+}]_i$ had stabilized, propofol further increased tension (53 ± 11%) and $[Ca^{2+}]_i$ (56 ± 9%) (fig. 2).

Table 1. Effects of Pretreatment with Propofol on Contractile Response to Phenylephrine in Endothelium-denuded Pulmonary Arterial Rings

Propofol	n	Log ED_{50}	Maximum Contraction (%)
(−)	11	−6.05 ± 0.28	81.5 ± 6.9
10^{-7} M	8	−6.03 ± 0.33	87.5 ± 11.1
10^{-6} M	8	−6.25 ± 0.26	94.2 ± 12.6
10^{-5} M	8	−6.47 ± 0.31*	109.4 ± 15.5*
10^{-4} M	8	−6.39 ± 0.18	106.7 ± 11.0*

Maximum response is expressed as a percentage contraction relative to that induced by 60 mM KCl in each ring.

* $P < 0.05$ *versus* propofol (−).

Effect of Cyclooxygenase Inhibition on Phenylephrine-induced Increases in Tension and Intracellular Ca^{2+} Concentration

We tested the hypothesis that a vasodilator metabolite of the cyclooxygenase pathway (*e.g.*, prostacyclin) modulates the pulmonary vascular response to phenylephrine. Consistent with the hypothesis, pretreatment with the cyclooxygenase inhibitor ibuprofen (10^{-5} M), potentiated ($P < 0.05$) the contractile response to phenylephrine by 73 ± 28% and increased ($P < 0.05$) the sustained $[Ca^{2+}]_i$ response by 88 ± 44% (fig. 2). Ibuprofen also caused a leftward shift in the phenylephrine concentration–effect curve (fig. 3, top) and increased the maximum contractile response to phenylephrine (fig. 3, bottom). The log ED_{50} for phenylephrine was decreased ($P < 0.05$) from −6.28 ± 0.08 in control rings to −6.65 ± 0.28 in ibuprofen-pretreated rings. The maximum contractile response to phenylephrine was increased ($P < 0.05$) from 3.9 ± 1.5 to 5.6 ± 1.6 g in ibuprofen-pretreated rings.

Effect of Cyclooxygenase Inhibition on Propofol-induced Increases in Tension and Intracellular Ca^{2+} Concentration

We tested the hypothesis that propofol potentiates phenylephrine-induced increases in tension and $[Ca^{2+}]_i$

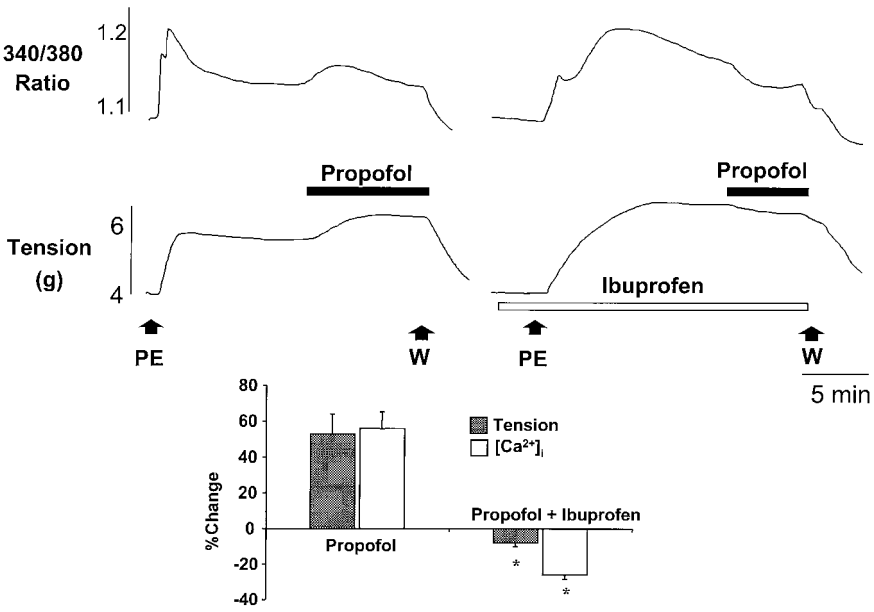

Fig. 2. Original tracing demonstrating effects of propofol on pulmonary arterial strips without endothelium after phenylephrine (PE; 10^{-5} M) precontraction in the absence (*left*) and presence (*right*) of ibuprofen (10^{-5} M). Propofol (10^{-4} M) potentiated phenylephrine-induced increases in intracellular Ca^{2+} concentration (340/380 ratio) and tension. Pretreatment with ibuprofen abolished the propofol-induced potentiation (*bottom*, n = 6 each). *$P < 0.05$ compared with strips without ibuprofen treatment. W = washout of strips with fresh medium.

by inhibiting either the synthesis or activity of a vasodilator metabolite of the cyclooxygenase pathway. Consistent with this hypothesis, pretreatment with ibuprofen abolished the propofol-induced increases in tension and $[Ca^{2+}]_i$ in phenylephrine-contracted pulmonary vascular smooth muscle (fig. 2).

Effect of Propofol on Arachidonic Acid–induced Pulmonary Vasorelaxation

We next investigated the effects of propofol on cyclooxygenase-mediated pulmonary vasorelaxation. We tested the hypothesis that propofol potentiates the response to phenylephrine by inhibiting the pulmonary vasorelaxant response to the cyclooxygenase substrate, arachidonic acid. Consistent with this hypothesis, propofol caused a rightward shift in the arachidonic acid concentration–effect curve (fig. 4). The logarithm of the inhibitory concentration of arachidonic acid causing 50% of maximum relaxation (IC_{50}) was increased ($P < 0.01$) from -5.96 ± 0.77 in control rings to -4.84 ± 0.33 in propofol-treated rings. Ibuprofen had virtually the same effect as propofol in inhibiting the pulmonary vasorelaxant response to arachidonic acid (fig. 4).

Effect of Propofol on Prostacyclin-induced Pulmonary Vasorelaxation

Our next goal was to determine whether the propofol-induced attenuated response to arachidonic acid was caused by a decrease in the vasorelaxant activity of prostacyclin. We tested the hypothesis that propofol potentiates the response to phenylephrine by specifically inhibiting the vasorelaxant response to prostacyclin. However, propofol had no effect on the pulmonary vasorelaxant response to prostacyclin in phenylephrine-contracted, ibuprofen-pretreated rings (fig. 5). The IC_{50} values for prostacyclin were -7.05 ± 0.33 and -7.07 ± 0.34 in propofol-treated and control rings, respectively.

Effect of Propofol on Phenylephrine-induced Prostacyclin Synthesis

The fact that propofol attenuated the pulmonary vasorelaxant response to arachidonic acid, but not prostacyclin, led us to test the hypothesis that propofol attenuates phenylephrine-induced prostacyclin synthesis. We measured 6-keto prostaglandin $F_{1\alpha}$ production (stable metabolite of prostacyclin) in the organ bath before and after administration of phenylephrine (10^{-5} M). Phenylephrine induced a sixfold increase in prostacyclin production in control rings (fig. 6). In contrast, propofol (10^{-4} M) virtually abolished the phenylephrine-induced increase in prostacyclin production (fig. 6). As expected, ibuprofen also abolished the phenylephrine-induced increase in prostacyclin production (fig. 6).

Discussion

The main findings of our study were as follows. Propofol caused dose-dependent, endothelium-independent pulmonary artery contraction and potentiated increases in tension and $[Ca^{2+}]_i$ in response to α-adrenoreceptor activation. Cyclooxygenase inhibition also potentiated increases in tension and $[Ca^{2+}]_i$ in response to phenylephrine but abolished propofol-induced increases in tension and $[Ca^{2+}]_i$. Propofol inhibited the pulmonary vasorelaxant response to exogenous arachidonic acid but had no effect on the vasorelaxant response to prostacyclin. Finally, propofol abolished prostacyclin synthesis in response to α-adrenoreceptor activation. Taken together, our results suggest that propofol enhances phenylephrine-induced contraction by inhibiting the ability of

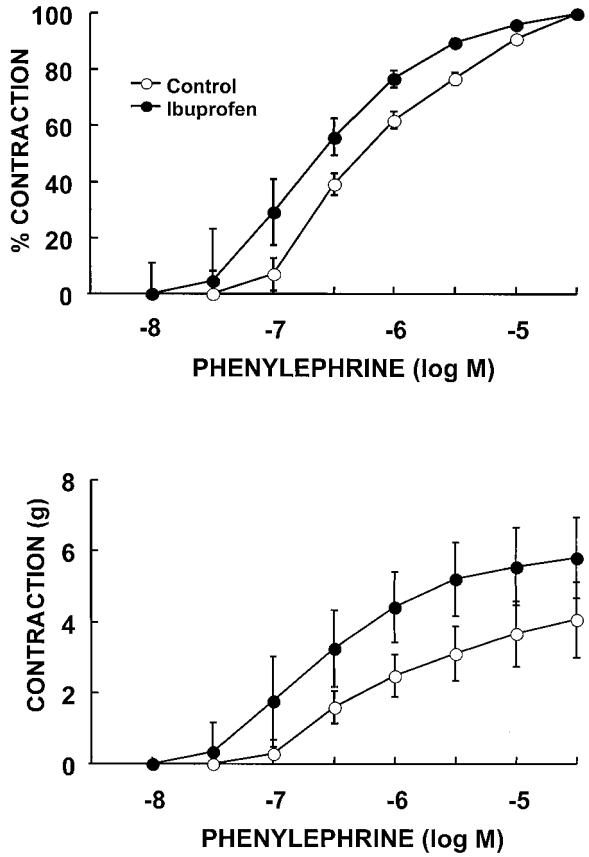

Fig. 3. Effects of ibuprofen on concentration–effect curve for phenylephrine in pulmonary arterial rings without endothelium (n = 7). Results are expressed as percentage of maximum contraction to phenylephrine (*top*) and as absolute changes in tension in response to phenylephrine (*bottom*). Ibuprofen (10^{-5} M) caused a leftward shift (*$P < 0.05$) in the phenylephrine concentration–effect curve and increased (*$P < 0.05$) absolute increases in tension in response to phenylephrine.

cyclooxygenase to synthesize prostacyclin in pulmonary artery smooth muscle.

Our first goal was to determine whether the pulmonary vasoconstrictor response to propofol was an endothelial or vascular smooth muscle effect. Propofol does inhibit endothelium-dependent pulmonary vasorelaxation,[8] which could unmask a contractile response to propofol. However, the dose-dependent increases in tension in response to propofol were similar in endothelium-intact and -denuded pulmonary arterial rings, which indicates that the contractile response to propofol is endothelium-independent. Moreover, in endothelium-denuded pulmonary arterial strips, we observed that the propofol-induced increases in tension were associated with concomitant increases in $[Ca^{2+}]_i$. These results indicate that propofol is acting directly on pulmonary arterial smooth muscle.

We next tested the hypothesis that propofol potentiates phenylephrine-induced contraction by inhibiting the synthesis and/or activity of a vasodilator metabolite of the cyclooxygenase pathway. The rationale for this hypothesis is as follows. Activation of α adrenoreceptors

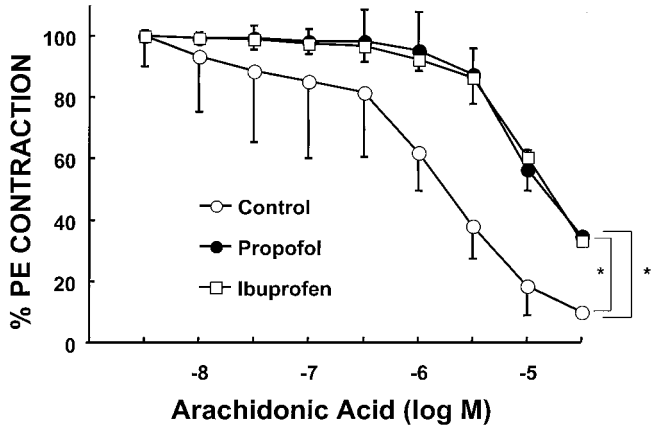

Fig. 4. Effects of propofol and ibuprofen on the pulmonary vasorelaxant response to arachidonic acid (AA) in endothelium-denuded rings (n = 6). Vasorelaxation expressed as percent reversal of phenylephrine (PE) contraction. Propofol (10^{-4} M) and ibuprofen (10^{-5} M) attenuated (*$P < 0.05$) AA-induced vasorelaxation to the same extent.

stimulates both phospholipase C and phospholipase A_2.[9,10] Stimulation of phospholipase C induces the formation of diacylglycerol and inositol 1,4,5-triphosphate, which act as second messengers for the activation of protein kinase C[11] and the release of Ca^{2+} from intracellular stores,[12] respectively. Diacylglycerol is then metabolized by diglyceride lipase to release arachidonic acid.[13] Stimulation of phospholipase A_2 also results in the release of arachidonic acid.[14,15] Arachidonic acid, in turn, is a substrate for cyclooxygenase and is metabolized to produce prostacyclin, thromboxane A_2, and other prostaglandins. Prostacyclin is the major cyclooxygenase metabolite in vascular tissue.[16] Finally, α agonists, including phenylephrine and norepinephrine, have been shown to stimulate prostacyclin synthesis in both systemic[14,15,17] and pulmonary artery[18] vascular smooth muscle.

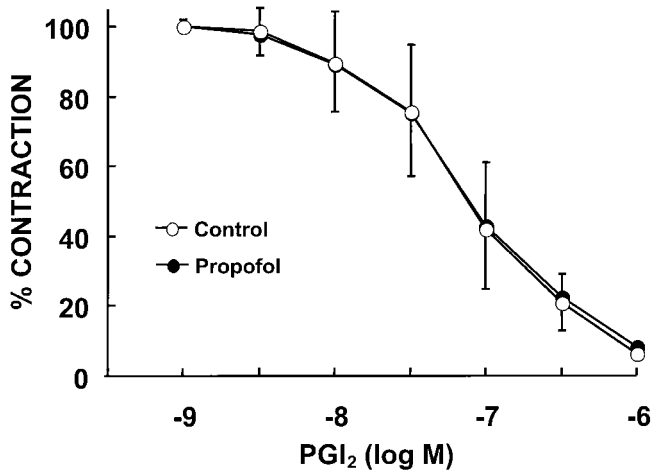

Fig. 5. Effects of propofol on the pulmonary vasorelaxant response to prostacyclin (PGI_2) in endothelium-denuded rings (n = 6) pretreated with ibuprofen (10^{-5} M). Vasorelaxation expressed as percent reversal of phenylephrine contraction. Propofol (10^{-4} M) had no effect on the vasorelaxation response to prostacyclin.

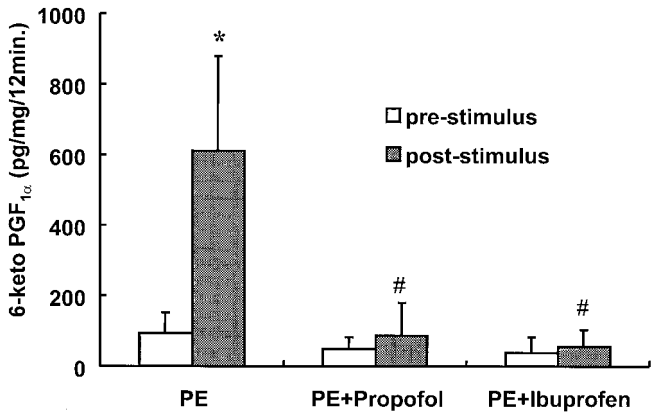

Fig. 6. Concentration of 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$), a stable metabolite of prostacyclin, released by endothelium-denuded pulmonary arterial rings. Production of 6-keto $PGF_{1\alpha}$ is expressed as picogram per milligram wet weight per 12 min (n = 7). Phenylephrine (PE; 10^{-5} M) increased (*$P < 0.01$) the production of 6-keto $PGF_{1\alpha}$ by sixfold in control rings. Pretreatment with propofol (10^{-4} M) or ibuprofen (10^{-5} M) abolished (#$P < 0.01$) the phenylephrine-induced increase in 6-keto $PGF_{1\alpha}$.

We observed that the cyclooxygenase inhibitor ibuprofen further increased tension and $[Ca^{2+}]_i$ in pulmonary arterial strips precontracted with phenylephrine. That result suggests that vasodilator metabolites of the cyclooxygenase pathway normally act to modulate the pulmonary vasoconstrictor response to α-adrenoreceptor activation. We also observed that ibuprofen abolished the propofol-induced increases in tension and $[Ca^{2+}]_i$ in phenylephrine-precontracted strips. That result supports the hypothesis that propofol potentiates phenylephrine-induced contraction by inhibiting the synthesis and/or activity of a vasodilator metabolite of the cyclooxygenase pathway. Moreover, if this hypothesis is correct, then propofol should inhibit the pulmonary vasorelaxant response to exogenous arachidonic acid. Consistent with this, we observed that propofol and ibuprofen attenuated the pulmonary vasorelaxant response to arachidonic acid to the same extent.

We next attempted to distinguish between these two possible effects of propofol on the cyclooxygenase pathway. Does propofol inhibit the vasorelaxant activity of a cyclooxygenase metabolite, or does propofol inhibit the synthesis of a vasodilator metabolite of the cyclooxygenase pathway? To address the first possibility, we assessed the effects of propofol on the pulmonary vasorelaxant response to prostacyclin. We observed that propofol had no effect on the prostacyclin concentration–effect curve, which would suggest that the contractile response to propofol is not caused by an inhibitory effect on the vasorelaxant activity of prostacyclin. To address the second putative mechanism, we assessed the effects of propofol on phenylephrine-induced prostacyclin production. Phenylephrine caused a marked increase in prostacyclin production in control pulmonary arterial rings. Moreover, both propofol and ibuprofen essentially abolished the phenylephrine-stimulated increase in prostacyclin production. These results support the concept that the propofol-induced contractile response is caused by inhibition of prostacyclin synthesis in α-adrenoreceptor–activated pulmonary arterial vascular smooth muscle.

Prostacyclin is well known as an important endothelium-derived mediator that causes vascular smooth muscle relaxation.[16] The sixfold increase in prostacyclin production induced by phenylephrine in endothelium-denuded rings clearly confirms that pulmonary vascular smooth muscle is also capable of prostacyclin production. All phenylephrine-stimulated rings and strips were pretreated with propranolol, which indicates that β-adrenoreceptor activation is not involved in any of the phenylephrine-induced effects. This is consistent with previous studies.[15,18] Prostacyclin released from vascular tissue activates adenylyl cyclase, which increases cyclic adenosine monophosphate concentration.[19] An increase in cyclic adenosine monophosphate concentration activates protein kinase A, which inhibits Ca^{2+} influx by acting on inositol 1,4,5-triphosphate or Ca^{2+}-activated K^+ channels to decrease $[Ca^{2+}]_i$ and cause vasorelaxation.[12] Thus, as predicted and as we observed, inhibition of prostacyclin synthesis by propofol or ibuprofen should result in an increase in $[Ca^{2+}]_i$ and an increase in tension.

The precise mechanism underlying the inhibition of cyclooxygenase by propofol remains to be elucidated. One possible mechanism could involve an antioxidant effect of propofol. Propofol has been shown to have an antioxidant effect and act as a free radical scavenger.[20,21] Propofol also prevents lipid peroxidation in vascular smooth muscle.[22] An antioxidant effect of propofol could be explained by its structural similarity to the active nucleus of α-tocopherol and butylhydroxytoluene, both of which are known to have antioxidant properties.[23,24] Cyclooxygenase is an enzyme that adds oxygen to arachidonic acid, producing the endoperoxide precursor of prostaglandins, including prostacyclin.[16] Because cyclooxygenase activity depends on the presence of lipid peroxides, resulting in free radical chain reactions, the presence of a free radical scavenger or an antioxidant could inhibit cyclooxygenase activity.[25–27] We will investigate this possible mechanism of action of propofol in future studies.

The plasma concentration of propofol in patients during the maintenance of general anesthesia has been reported to be in the range of 10^{-5} to 10^{-4} M.[28] Because 97–98% of propofol is bound to plasma proteins,[29] the free concentration of propofol is estimated to be 10^{-6} to 10^{-5} M. However, because protein binding of propofol *in vivo* is unlikely to be instantaneous, the free concentration associated with the bolus administration of propofol would be higher than the steady state value. In the current study, 10^{-6} and 10^{-5} M propofol potentiated the contractile response to phenylephrine in endotheli-

um-denuded pulmonary arterial rings by 12 ± 5% and 34 ± 6%, respectively. Thus, clinically relevant concentrations of propofol altered pulmonary vascular smooth muscle function, although it is acknowledged that a higher concentration (10^{-4} M) was used in some protocols.

In summary, our results suggest that propofol increases tension and $[Ca^{2+}]_i$ during α-adrenoreceptor activation by inhibiting the synthesis of prostacyclin in pulmonary vascular smooth muscle.

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