

Vasodilation and Mechanism of Action of Propofol in Porcine Coronary Artery

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Background: A decrease in myocardial perfusion pressure may reduce myocardial blood flow. However, it may not significantly affect myocardial perfusion when in presence of a concurrent coronary artery vasodilation. However, the effects of propofol in coronary arteries are not well determined. In this study, the effects of propofol on porcine coronary artery responses to vasoactive agents that operate through voltage- and receptor-mediated calcium mechanisms were investigated.

Methods: Hearts of adult pigs ($n = 103$) were obtained from a slaughter house, and the left anterior descending coronary arteries were dissected. The arteries were cut into vessel rings and prepared with and without the endothelium organ chambers filled with buffered salt solution. The effect of propofol (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M) on vascular smooth muscle contraction caused by intracellular Ca^{2+} -influx through voltage- and receptor-mediated mechanism also was studied at a cellular level.

Results: Propofol relaxed coronary rings that were contracted by KCl, norepinephrine (NE), serotonin (5-HT), or carbachol (CCh). The minimal concentrations of propofol that produced significant vasorelaxation ranged from 3.16×10^{-7} M to 3.16×10^{-6} M. Vasodilation was more pronounced in rings contracted by NE, 5-HT, and CCh than by KCl. Propofol (10^{-5} M) attenuated coronary vasoconstriction in response to cumulative concentrations of KCl, NE, 5-HT, and acetylcholine. Maximal contractions produced by NE and 5-HT were inhibited to a greater degree than contractions produced by KCl. Propofol at concentrations of 10^{-5} M and higher attenuated a contraction in response to $CaCl_2$ in vascular rings depolarized by KCl, but concentrations of 10^{-6} M did not attenuate contractions. Vasoconstriction in response to calcium entry in the presence of NE (and nifedipine 10^{-6} M) was attenuated by propofol at concentrations of 10^{-6} M and higher. Caffeine-induced con-

traction, caused by intracellular calcium release, was attenuated only at 10^{-4} M of propofol.

Conclusions: Propofol possesses vasodilator effect and attenuates the effects of vasoconstrictor agents in porcine coronary artery. Further, an antagonism of calcium channels may be responsible for these effects of propofol. (Key words: Anesthetics, intravenous: propofol. Artery: coronary. Ions: calcium.)

PROPOFOL, 2,6-di-isopropylphenol, is a short-acting, potent intravenous anesthetic agent that possesses many desirable characteristics. Because its effects fade quickly after emergence from anesthesia,¹ it has been widely used in outpatient surgery. It also is reported to be safe and effective in coronary artery bypass surgery.^{2,3}

The effects of propofol on the coronary circulation have not been established. The decrease of arterial pressure and cardiac index observed with propofol may reduce myocardial blood flow.⁴ However, Vermeyer reported that coronary sinus flow and indicators of global myocardial perfusion did not change in patients anesthetized with propofol.⁵

Propofol was shown to have a biphasic vasoactive effect on canine coronary arteries. Although it caused relaxation at higher concentrations, propofol contracted the canine coronary artery at clinical anesthetic levels.^{6,7} In swine, Coetzee *et al.* suggested that propofol has an inotropic negative effect that is associated to peripheral vasoconstriction. Others have suggested that propofol may interfere with serotonin-mediated coronary vasoconstriction in pigs.⁸ Propofol also attenuates calcium-induced contraction of rat portal veins and relaxes rat aortic rings contracted by KCl.^{9,10} Park *et al.* showed that propofol is a vasodilator that may interfere with endothelium-dependent mechanisms in rat aorta.¹¹ In contrast, Chang and Davis showed that the vasodilator effect of propofol was not endothelium-dependent in rat aorta, and that this effect was caused mainly by a blockade of voltage-gated influx of extracellular calcium.¹⁰ However, if propofol also interferes with receptor-mediated calcium entry mechanisms,

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then the study done by Park *et al.* would not be in contradiction to the work of Chang and Davis.¹⁰ A non-specific calcium blocking effect of propofol would also interfere with the production of endothelium-dependent relaxing factor as studied by Park *et al.*¹¹ Depending of the animal model, technique and emphasis of the study one may obtain apparently discordant results. For example, the rat aorta is known to respond with greater calcium influx to depolarizing agents than to receptor-mediated mechanisms.¹²

An arterial smooth muscle contraction is triggered by an increase in cytosolic free Ca^{2+} that is derived either from the extracellular space or intracellular Ca^{2+} stores, such as the sarcoplasmic reticulum (SR).^{13,14} Ca^{2+} influx from the extracellular space have been known to occur through voltage-dependent or receptor-operated channels. Although it is well accepted that membrane depolarization opens calcium channels, the effect of the interaction receptor-agonist on calcium channels is more complex and may depend also on the amplitude of the calcium stores and voltage-mediated mechanisms.¹⁵ Nelson *et al.* have showed that vasoconstriction caused by norepinephrine can open voltage-dependent calcium channels.¹⁶

These observations suggest that the vasodilator effects of propofol may be caused by interference in a common voltage- and receptor-operated mechanism of intracellular calcium mobilization in vascular smooth muscle.

The purpose of this study was to determine whether the calcium blocking effects of propofol affected coronary artery vasoresponsiveness and to clarify how cellular Ca^{2+} mobilizations through the sarcoplasmic reticulum, voltage-dependent and receptor-mediated mechanisms are affected by this anesthetic.

Materials and Methods

This study was approved by the Cleveland Clinic Animal Care and Use Committee. Hearts of adult pigs ($n = 103$) of either sex were obtained immediately post-mortem at a nearby slaughterhouse (Mahan Packing, Bristoville, OH) and transported to the laboratory in ice-cold buffered salt solution.

Isolated Vessel Preparation

Left anterior descending coronary arteries were dissected from the hearts and stored in cold buffered salt solution (millimolar composition: NaCl, 118.3; KCl, 4.7; CaCl_2 , 2.5; MgSO_4 , 1.2; KH_2PO_4 , 1.2; NaHCO_3 ,

25.0; EDTA calcium disodium, 0.026; and glucose, 11.1). The arteries were cleaned of surrounding fat and connective tissue and cut into 3 to 4 mm-wide rings. In some rings, the endothelium was deliberately removed by gently rubbing a fine stainless steel rod against the vessel intima. Rings were suspended between two stainless steel wires in organ chambers filled with 25 ml of buffered salt solution at 37°C and bubbled with a 95% O_2 -5% CO_2 gas mixture. One of the wires was anchored in the organ chamber and the other was connected to a force transducer (Grass FT03, Quincy, MA).

Before the experiment, the preparations were progressively stretched and repeatedly exposed to 40 mM of KCl to induce contraction at each level of stretching, until a maximal contractile response was obtained. The rings were then allowed to equilibrate for 45 min. Contractile responses to norepinephrine were measured after a pre incubation with propranolol (5×10^{-6} M). The effectiveness of the endothelial denudation was assessed by exposing the rings (contracted by KCl) to 10^{-7} M of bradykinin. Concentrations of drugs were expressed in molar (M).

Experimental Protocol

1. Study of Vasodilator Effects. Cumulative concentration-response curves for propofol (10^{-8} up to 3.16×10^{-3} M) were obtained from tension measurements of rings contracted by KCl (40 mM), norepinephrine (10^{-6} M), serotonin (10^{-6} M), or carbachol (3.16×10^{-7} M). The vasodilator response of propofol was expressed as a percentage of the initial contraction induced by each vasoconstrictor. The effect of the vehicle solution (Intralipid; 10% soy bean oil, 1.2% egg phospholipids, and 2.25% glycerin) at equivalent concentrations was also examined.

2. Study of Modulator Effects. Cumulative concentration-response curves to KCl, norepinephrine, serotonin, and acetylcholine were obtained in the presence or absence of propofol (10^{-5} M), added 20 min before the measurements. The response was expressed as a percentage of KCl (40 mM)-induced contraction of each ring. Contractions caused by the same vasoconstrictor agonists were also examined in the presence or absence of the vehicle at a concentration equivalent to that in 10^{-5} M of propofol.

3. Study of the Mechanisms of Action on Ca^{2+} Mobilization.

3.1. Ca^{2+} Influx through Voltage-mediated Mechanisms. A control contractile response to KCl (40 mM)

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in buffered salt solution was obtained first. The rings were then washed in buffered salt solution for 45 min. Thereafter, the rings were incubated for 30 min in Ca^{2+} -free salt solution with EGTA (0.01 mM). The Ca^{2+} -free solution was prepared by omitting CaCl_2 from regular buffered salt solution, KCl (40 mM) was added 20 min after the beginning of the incubation in Ca^{2+} -free solution in the presence (10^{-7} , 10^{-6} , 10^{-5} , or 10^{-4} M) or absence of propofol. A contractile response to CaCl_2 (2 mM) was measured. This contraction was expressed as a percentage of the control KCl-induced contraction of each ring in regular buffered salt solution.

3.2. Ca^{2+} Influx through Receptor-mediated Mechanisms. A control contractile response to norepinephrine (10^{-5} M) in buffered salt solution was obtained first. The rings were then washed in buffered salt solution for 45 min. Thereafter, the rings were incubated for 30 min in Ca^{2+} -free solution with EGTA (0.01 mM) and nifedipine (10^{-6} M). Norepinephrine (10^{-5} M) was added in the presence (10^{-7} , 10^{-6} , 10^{-5} , or 10^{-4} M) or absence of propofol. A contractile response to CaCl_2 (2 mM) was measured. This contraction was expressed as a percentage of the control norepinephrine-induced contraction of each ring in the regular buffered salt solution.

3.3. Caffeine-induced Ca^{2+} Release from Sarcolemmic Reticulum. A control contractile response to caffeine (20 mM) in buffered salt solution was obtained first. Thereafter, the rings were incubated in Ca^{2+} -free solution with 0.01 mM EGTA and 10^{-6} M nifedipine for 20 min, and a second contractile response to caffeine was obtained in the presence (10^{-7} , 10^{-6} , 10^{-5} , or 10^{-4} M) or absence of propofol. The second response to caffeine was expressed as a percentage of the control caffeine-induced contraction of each ring in the regular buffered salt solution.

Drugs

The following drugs were used: propofol (Stuart Pharmaceuticals, Wilmington, DE), acetylcholine, carbachol, norepinephrine, serotonin, caffeine, nifedipine, ethyleneglycol-bis-(β -aminoethyl ether) N,N'-tetraacetic acid (EGTA; Sigma, St. Louis, MO), and Intralipid (KabiVitrum, Clayton, NC). The drugs were prepared daily in distilled deionized water or in 0.1% solution of ascorbic acid in water and kept on ice during the experiments. Nifedipine was initially dissolved in alcohol and further diluted in distilled water. The final concentration of alcohol in organ chambers was less than 0.01%.

Statistical Analysis

In these experiments, "n" indicates the number of animals (hearts). Each animal is represented by a pair of rings used simultaneously in parallel for each experiment. The results were expressed as mean \pm SEM. When appropriate, Student's *t*-test was used for paired observations. To compare more than two mean values among groups, one-way analysis of variance, and subsequently Duncan's multiple range tests were used. Values were considered statistically significant when $P < 0.05$.

Results

1. Vasodilator Effects

The maximal contractile response of the porcine coronary arteries to 40 mM KCl was in average 19.9 ± 2 g (1 g = 10 mN), to acetylcholine was 18.8 ± 2 g, to norepinephrine was 6.0 ± 1.2 g and to serotonin was 5.0 ± 0.7 g. Propofol relaxed porcine coronary arterial rings contracted by KCl, norepinephrine, serotonin, and carbachol (fig. 1). The vasodilator effects were not significantly different between rings with intact (E+) and denuded (E-) endothelium for these agents. The magnitude of the vasodilation produced by propofol was significantly different among rings contracted by different vasoconstrictor agents. The relaxation in rings contracted by norepinephrine (at concentrations of 3.16×10^{-6} M and higher), serotonin (3.16×10^{-6} M and higher), or carbachol (3.16×10^{-5} M and higher) was larger than the relaxation observed in rings contracted by KCl. The vehicle exerted no significant response on rings contracted by any of the vasoconstrictor agents used.

2. Modulator Effects

Propofol (10^{-5} M) attenuated porcine coronary contractions provoked by all the vasoconstrictor agents examined (fig. 2). The degree of this attenuation was not significantly different between endothelium intact (E+) and endothelium denuded (E-) rings. The attenuation of the maximal contraction was significantly larger in the response produced by norepinephrine and serotonin than in those by KCl (table 1). No significant modulation of the contraction caused by the vasoactive agents was observed in the presence of the vehicle.

3.1. The Effect of Propofol on Ca^{2+} Influx through Voltage-mediated Mechanisms

Ca^{2+} produced a tonic contraction of porcine coronary arteries in Ca^{2+} -free solution in the presence

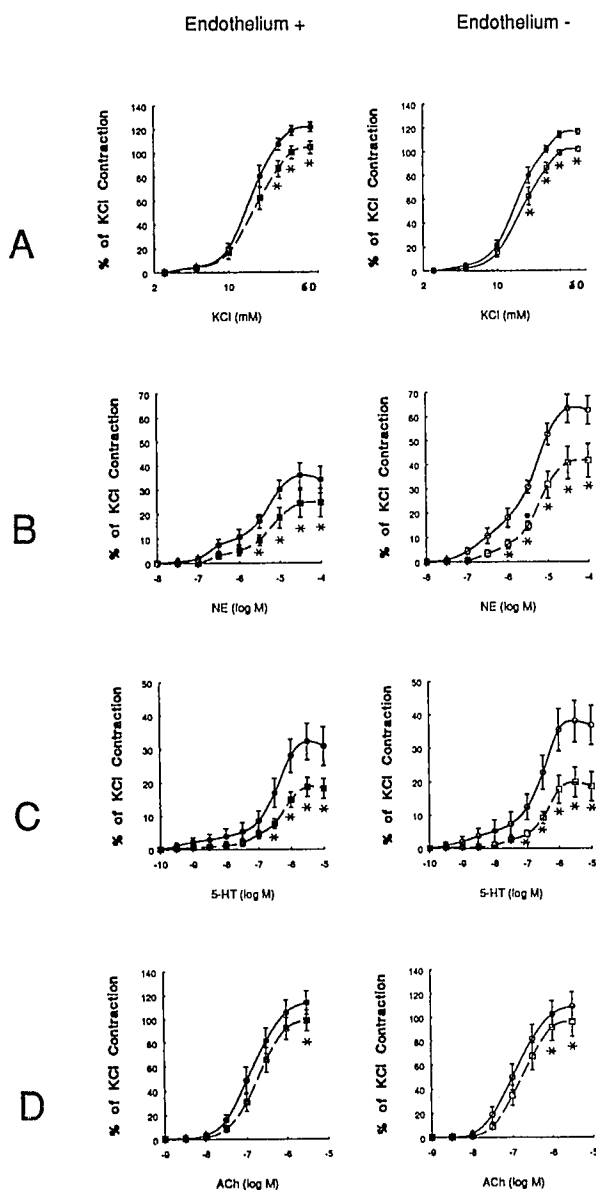


Fig. 1. Concentration-response curves to KCl (A), norepinephrine (NE, B), serotonin (5-HT, C) and acetylcholine (ACh, D) in the presence (squares) and absence (circles) of propofol (10^{-5} M) in porcine coronary arteries with intact (left panels) and denuded (right panels) endothelium ($n = 8$). Contractions are expressed as percentages to control contractions caused by 40 mM of KCl (190 ± 27 mN). Propofol attenuated coronary contractions provoked by all the vasoconstrictors examined. * $P < 0.05$ from control.

of KCl (12.4 ± 0.8 g, 68.8% of KCl-induced control contractions in regular solution, fig. 3A). Propofol significantly attenuated this contraction at 10^{-4} M and

10^{-5} M, whereas 10^{-6} M and 10^{-7} M had no effect (fig. 4A).

3.2. The Effect of Propofol on Ca^{2+} Influx through Receptor-mediated Mechanisms

Ca^{2+} produced a tonic contraction of porcine coronary arteries in Ca^{2+} -free solution in the presence of norepinephrine (3.9 ± 0.5 g; 45.2% of norepinephrine-induced control contractions in regular solution, fig. 3B). This contraction was significantly attenuated by propofol at concentrations of 10^{-6} M and higher (fig. 4B).

3.3. The Effect of Propofol on the Caffeine-induced Ca^{2+} Release from Sarcoplasmic Reticulum

Caffeine (20 mM) produced a phasic contraction of porcine coronary arteries in buffered salt solution. In Ca^{2+} -free solution, caffeine also produced a phasic contraction (1.9 ± 0.2 g, 44.6% of control contractions in regular solution, fig. 3C). This contraction was attenuated only by the highest concentration of propofol (10^{-4} M, fig. 4C).

Discussion

The current study shows that propofol directly relaxes porcine coronary arteries and attenuates the vasoconstriction caused by neurohumoral substances in these blood vessels. The vascular relaxation observed in this study in response to propofol is caused by this agent because the vehicle was devoid of vasoactive effects.

Other investigators showed that propofol contracts canine coronary arteries at anesthetic concentration and relaxes at concentrations (10^{-4} M), which are not comparable to plasma levels observed in clinical uses.^{6,7} In contrast, our present data on porcine coronary arteries reveal solely a vasodilator effect of propofol at concentrations that may be detectable in patients' plasma. Clinical plasma levels of propofol range from 1 to $10 \mu\text{g} \cdot \text{ml}^{-1}$.^{17,18} During nitrous oxide anesthesia in humans, the EC_{50} values of propofol range from 1.66 to $2.5 \mu\text{g} \cdot \text{ml}^{-1}$, and the EC_{95} values range from 3.39 to $5.92 \mu\text{g} \cdot \text{ml}^{-1}$.^{19,20} Taking into consideration that the plasma protein binding for propofol is 97% to 98%,²¹ the concentration of 10^{-6} M ($0.18 \mu\text{g} \cdot \text{ml}^{-1}$) of propofol in organ chambers is comparable to approximately 5 to $9 \mu\text{g} \cdot \text{ml}^{-1}$ *in vivo* (protein-bound plus -unbound fraction), the range usually seen

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in patients' plasma. Consequently, the vasodilator effect of propofol as shown by us in porcine coronary arteries may be observed within clinically detectable levels.

Ozahn *et al.* reported preliminary data showing that propofol attenuated porcine coronary contraction caused by serotonin.⁸ Our study confirms this observation. In addition, we showed that propofol similarly attenuates the contraction induced by other vasoconstrictor agents, such as KCl, norepinephrine, and acetylcholine.

The vasodilator and the modulator effects of propofol were independent of the endothelium, implying that no significant participation of endothelium-derived relaxing factors occurred with these agents. Neither is it likely that the vasodilator effect of propofol is mediated through an antagonism of a specific receptor, because the effects are seen in rings contracted by all different agonists examined. In this study we did not investigate the influences of propofol on endothelium-dependent vasorelaxing agents such as substance P, bradykinin or calcium ionophore. Therefore, it may still be shown by others that the calcium blocking effect of propofol also opposes endothelium-dependent vasorelaxation.¹¹ Considering all these possibilities we propose that the prevailing mechanism underlying the effects of propofol in blood vessels is an alteration in Ca^{2+} mobili-

Table 1. The Attenuation by Propofol of Porcine Coronary Artery Contractions (n = 8) Induced by Various Vasoconstrictors

Vasoconstrictor	Decrease in Tension (%) ^a	
	Endothelium Intact Rings	Endothelium Denuded Rings
KCl	14.0 ± 3.4	11.9 ± 2.0
Norepinephrine	37.3 ± 9.8†	36.7 ± 5.3†
Serotonin	38.7 ± 4.9†	50.8 ± 7.5†
Acetylcholine	12.7 ± 2.0	12.8 ± 3.7

Data are mean ± SD.

^a Percentage of tension decrease in rings in the presence of propofol (10^{-6} M) compared to the contractions in control rings provoked by each vasoconstrictor.

† Significantly different from KCl groups $P < 0.05$ by Duncan's multiple range test after ANOVA.

zation. This view is also supported by Bunting *et al.*⁹ who showed that propofol attenuates a calcium-induced contraction of murine portal veins, and by Chang *et al.*,¹⁰ who showed that propofol relaxes murine aortic rings contracted by KCl.

In isometric tension studies using isolated vessels, vasodilation in rings contracted by KCl indicates a decrease of Ca^{2+} influx through voltage-mediated mechanisms, whereas vasodilation in rings contracted by re-

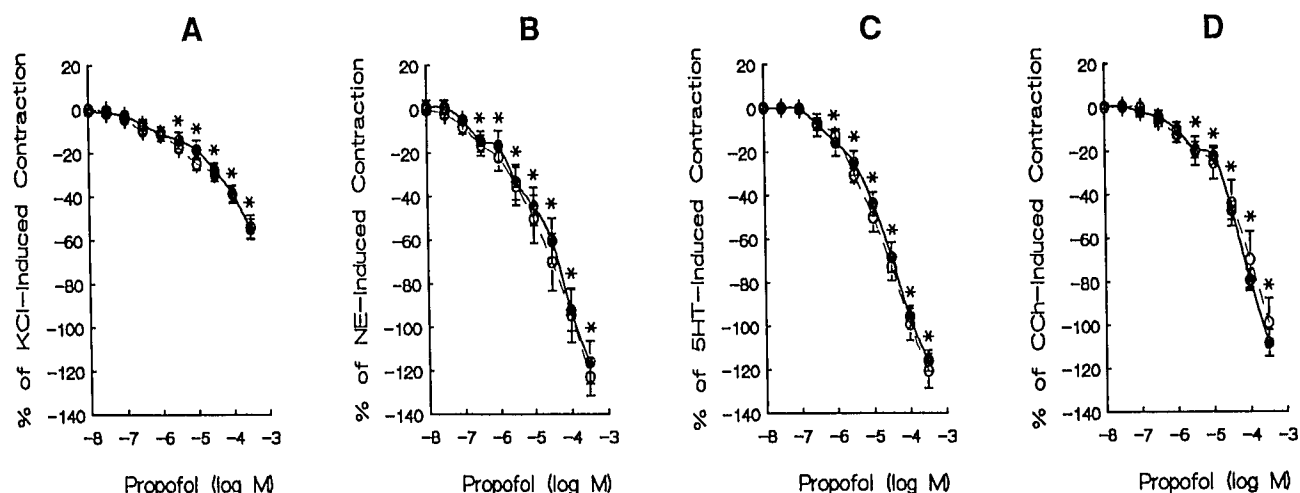


Fig. 2. Concentration-response curves to propofol in porcine coronary arteries (n = 7). Rings were contracted by KCl (40 mM, A), norepinephrine (NE; 10^{-6} M, B), serotonin (5-HT; 10^{-6} M, C) or carbachol (CCh; 3.16×10^{-7} M, D). Closed and open symbols show data in endothelium-intact (E+) and endothelium-denuded (E-) rings, respectively. Relaxations are expressed as percent responses to initial contractions produced by each vasoconstrictor agent. The average maximal tension generated by these agents at their respective concentrations are KCl = 190 ± 27 , NE = 60 ± 10 , 5HT = 50 ± 7 and CCh = 180 ± 20 (in mN). Propofol had vasodilator effects on rings contracted by the vasoconstrictors. The vasodilator effects are not significantly different between E+ and E- rings. The vasodilator effects were larger in rings contracted by norepinephrine, serotonin, or carbachol than in those contracted by KCl. * $P < 0.05$ from the baseline contractions.

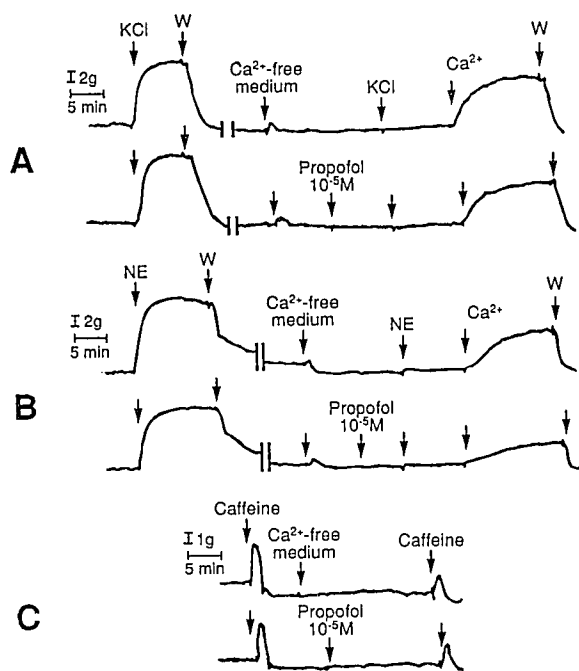


Fig. 3. Representative traces of paired experiments for examining the contractile effect of propofol on calcium mobilization. (A) Tonic contractile responses of porcine coronary artery to CaCl_2 (2 mM) in Ca^{2+} -free, EGTA (0.01 mM)-containing solution in the presence of KCl (40 mM). This contraction represents a Ca^{2+} -influx through voltage-dependent calcium channels. (B) Tonic contractile responses to CaCl_2 (2 mM) in Ca^{2+} -free solution with nifedipine (NE: 10^{-6} M) and EGTA (0.01 mM) in the presence of norepinephrine (10^{-5} M). This contraction represents a Ca^{2+} influx through receptor-operated mechanisms. (C) Phasic contractile responses to caffeine (20 mM) in Ca^{2+} -free solution with nifedipine (10^{-6} M) and EGTA (0.01 mM) were obtained, and the effect of propofol was examined. This contraction represents the release of Ca^{2+} from the SR pool that is responsive to Ca^{2+} , that is the Ca^{2+} -induced Ca^{2+} releasable pool that is particularly sensitive to caffeine. W; wash.

ceptor-agonists, such as norepinephrine, suggests a decrease of Ca^{2+} influx through receptor-mediated mechanisms.^{22,23} In the first protocol of our study, the vasodilator effect of propofol was significantly larger in rings contracted by the receptor-agonists than in rings contracted by KCl-induced depolarization. This, together with the observation that preincubation with propofol attenuated the maximum contractions caused by norepinephrine and serotonin more than the maximum contraction caused by KCl, suggests an important effect of this anesthetic on receptor-mediated mechanisms. We therefore propose that, in porcine coronary arteries, interference with receptor-mediated calcium

mobilization plays an important role in the mechanisms of vasodilation caused by propofol.

We further assessed the importance of receptor-mediated mechanisms in the propofol-induced coronary vasodilation. The sustained norepinephrine-induced contraction triggered by adding Ca^{2+} in Ca^{2+} -free solution with a blockade of voltage-dependent calcium entry by nifedipine represents mainly a Ca^{2+} influx through receptor-mediated calcium influx.^{24,25} Our result shows that this contraction is inhibited by propofol at 10^{-6} M, further corroborating the concept that an interference with receptor-mediated mechanisms plays an important role in the effect of propofol. Although propofol also inhibited the KCl-induced contraction triggered by adding Ca^{2+} in Ca^{2+} -free solution, this inhibitory effect occurred only at concentrations of 10^{-5} M and higher. Because this contraction represents a Ca^{2+} influx through voltage-operated calcium mechanisms,^{26,27} we suggest that, in the coronary arteries, propofol interferes with both, voltage- and receptor-mediated calcium mobilization. Chang and Davis also demonstrated a significant blockade of KCl-induced vasoconstriction and voltage-mediated calcium influx in isolated rat aorta with propofol at concentrations 10^{-5} M or higher. In contrast to our findings in the porcine coronary artery, the authors showed that even higher concentration of propofol (10^{-4} M) was necessary to relax rat aortic rings contracted by receptor-mediated mechanism with phenylephrine.¹⁰ Because of that, the authors suggested that propofol acts mainly by blocking voltage-dependent calcium channels.¹⁰ We confirmed in our laboratories the results from Chang and Davis regarding rat aorta (unpublished observations). However, we believe that species and vascular heterogeneity account for the differences observed between our work and theirs. For example, a possible predominant presence of voltage triggered calcium mechanisms in the rat aorta may have enhanced the calcium blocking effects of propofol toward voltage mechanism.¹²

The concentration of propofol in the plasma may be approximately 5×10^{-5} M 2 min after a bolus injection of $2 \text{ mg} \cdot \text{kg}^{-1}$.²⁸ Since as much as 98% of propofol may be bound to protein this is not equivalent to the same concentration of propofol used in protein free salt solutions during experiments in vitro. Only a concentration of 10^{-6} M or less would represent the free "unbound" plasma level of propofol observed in patients. Therefore, the effect of propofol on voltage-dependent calcium channels, as observed for the coronary arteries

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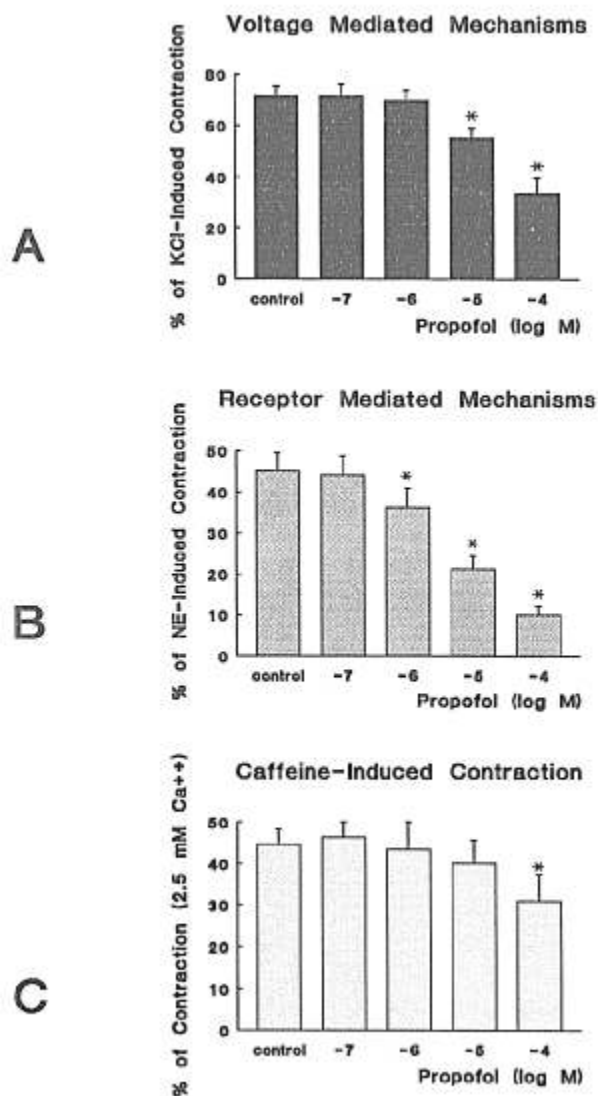


Fig. 4. Effects of propofol concentrations on Ca^{2+} -mobilization in porcine coronary arteries. Residual contractions of each response in the presence and absence of propofol are shown as follows; (A) Tonic contractile responses of porcine coronary artery to CaCl_2 (2 mM) in Ca^{2+} -free, EGTA (0.01 mM)-containing solution in the presence of KCl at 40 mM ($n = 7$ or 8). This contraction represents a Ca^{2+} influx through voltage-dependent mechanisms, and was inhibited by propofol at 10^{-4} and 10^{-5} M, (B) Tonic contractile response to CaCl_2 (2 mM) in Ca^{2+} -free solution with nifedipine (10^{-6} M) and EGTA (0.01 mM) in the presence of norepinephrine (NE; 10^{-5} M) ($n = 8$ or 9). This contraction represents a Ca^{2+} influx through receptor-operated mechanisms, and was inhibited by propofol at 10^{-6} M and higher concentrations. (C) Phasic contractile responses to caffeine (20 mM) in Ca^{2+} -free solution with nifedipine (10^{-6} M) and EGTA (0.01 mM) ($n = 9$ or 10). This contraction represents an intracellular Ca^{2+} release from the sarcoplasmic reticulum and was inhibited by propofol only at 10^{-4} M. Each contraction was expressed as a percentage to the control contraction, in regular buffered salt solution, produced by KCl (124 ± 8 mN), norepinephrine (39 ± 5 mN), and caffeine (19 ± 2 mN), respectively. * $P < 0.05$ from control.

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the receptor-operated mechanisms of calcium mobilization.¹⁵

The nature of the second messenger that links the receptors to calcium channels is still unsolved. Receptor activation may open calcium channels directly or indirectly *via* the inositol phosphate turnover or guanylate-tri-phosphate (GTP)-binding proteins (G-protein).²⁷ In our study, the vasodilator and the modulator effects of propofol were observed in coronary rings contracted by any of the agonists used. Some calcium antagonists, as well as propofol, possess also slight anti-adrenergic, antiserotonergic, or antimuscarinic effects.²⁷⁻³⁰

To examine another possible site of action, the effect of propofol on intracellular Ca^{2+} release was also tested. Two methods are usually used to assess this response. One is the evaluation of inositol phosphate gated calcium release from the SR. This could be evaluated by assessing the drug effect on the residual contraction induced by norepinephrine in Ca^{2+} -free solution. In some blood vessels, this method produces a phasic contraction, implying the release of intracellular Ca^{2+} from SR in response to norepinephrine.^{24,31} However, this method was not feasible in the current study because of the extremely small residual contractions to norepinephrine at zero calcium in porcine coronary arteries. The peak contraction was 0.4 ± 0.2 g, which was only 4.0% of the norepinephrine-induced contraction in a normal medium ($n = 6$). The other method is to test the

in our study, may not be detected at clinically relevant concentrations.

The effects of receptor-ligand interaction on transmembrane calcium mobilization are complex. For example, some investigators have proposed that activation of voltage-dependent calcium channels may participate in the vasoactive effects of serotonin and angiotensin II.¹⁵ However, these agents are not known to activate calcium channels by causing depolarization. Nevertheless, in whole cell preparations the activation of currents through voltage-dependent Ca-channels by norepinephrine was observed without change in membrane potential. Therefore, it is difficult to separate as independent phenomenon the voltage-mediated from

response to caffeine that represents the Ca^{2+} -induced Ca^{2+} releasable pool of the SR. A caffeine-induced contraction of the vessel at zero Ca^{2+} concentration may represent the Ca^{2+} -induced Ca^{2+} releasable pool.^{32,33} The effect of propofol in caffeine-induced contraction indicates a possible effect of this anesthetic on the intracellular Ca^{2+} release although to a lesser degree than that observed on the Ca^{2+} entry. Caffeine challenge, however, does not represent the totality of releasable calcium from the SR but only the amount sensible to ryanodine or calcium-induced-calcium release. The intracellular pool of calcium that is responsive to inositol phosphate was not totally explored in our study. Nevertheless it is our suggestion that only concentrations much higher than clinical plasma levels of propofol may have an effect on intracellular Ca^{2+} release.

Another possible mechanism for the effect of propofol is an interference with the action of Ca^{2+} at intracellular sites. Certain vasodilators may interfere with smooth muscle contraction by altering myosin light chain phosphorylation,³⁴ altering calcium binding to calmodulin or calcium uptake by mitochondria.³⁵ It is unknown whether propofol also affects directly the action of Ca^{2+} at these intracellular sites.

Ljung *et al.*³⁶ observed that removal of extracellular calcium depressed norepinephrine-induced contraction of rat portal veins, whereas the same intervention did not depress the contraction of rabbit aorta. This mobilization supports the notion that the degree of receptor-operated Ca^{2+} mobilization may differ among species and different types of blood vessels. Vasodilator effects of calcium antagonists in vessel rings contracted by norepinephrine vary to a great extent among blood vessels, whereas these effects in rings contracted by KCl are not different among those vessels.^{37,38} Assuming propofol acts as a non-specific calcium channel blocking agent, it is reasonable to assume that it may predominantly affect receptor-mediated calcium mechanisms in some vessels and voltage-activated calcium channels in others, depending on the prevalence of the type of calcium channels in the blood vessel. In the rat aorta the receptor-operated Ca^{2+} -channels share the same structural characteristic as the voltage-operated Ca^{2+} channels but are gated separately.¹² Therefore, the receptor- and voltage-operated channels may be identical and may be only different aspects of the same phenomenon.¹⁵

References

1. Grant IS, Mackenzie N: Recovery following propofol ("Diprivan") anaesthesia—a review of three different anaesthetic techniques. *Postgrad Med J* 61(suppl 3):133–137, 1985
2. Vermeyer KM, Erpels FA, Janssen LA, Buckman CP, Hamegraef GH: Propofol-fentanyl anesthesia for coronary bypass surgery in patients with good left ventricular function. *Br J Anaesth* 59:1115–1120, 1987
3. Kaplan JA, Gaffin AV, Mikula S, Dolman J, Profeta J: Comparative hemodynamic effect of propofol and thiamylal sodium during anesthetic induction for myocardial revascularization. *J Cardiothorac Anesth* 2:297–302, 1988
4. Stephan H, Sonntag H, Schenk HD, Kettler D, Khambatta HJ: Effect of propofol on cardiovascular dynamics, myocardial blood flow and myocardial metabolism in patients with coronary artery disease. *Br J Anaesth* 58:969–975, 1986
5. Vermeyer KM, DeHert SG, Erpels FA, Adriaensen HF: Myocardial metabolism during anesthesia with propofol-low dose fentanyl for coronary artery bypass surgery. *Br J Anaesth* 66:504–508, 1991
6. Nakamura K, Hatano Y, Hirakawa H, Nishiwada M, Toda H, Mori K: Direct vasoconstrictor and vasodilator effects of propofol in isolated dog arteries. *Br J Anaesth* 68:193–197, 1992
7. Coughlan MG, Flynn NM, Bosnjak ZJ, Kampine JP, Waltier DC: Differential relaxant effect of high concentration of intravenous anesthetics on endothelin-constricted proximal and distal canine coronary arteries. *Anesth Analg* 74:378–383, 1992
8. Ozhan M, Sill JC, Katusic Z, Roric D, Uhl C: Propofol, thiopental, and contractile responses in isolated pig coronary arteries and cultured vascular smooth muscle cells (abstract). *ANESTHESIOLOGY* 73:A583, 1990
9. Bunting P, Ramsay TM, Pleuvry BJ: An in-vitro study of the interactions between intravenous induction agents and the calcium antagonists verapamil and nifedipine. *J Pharm Pharmacol* 41:840–843, 1989
10. Chang KSK, Lacy MO, Davis RF: Propofol produces endothelium-independent vasodilation and may act as a calcium channel blocker (abstract). *ANESTHESIOLOGY* 75:A535, 1991
11. Park WK, Lynch III C, Johns RA: Effects of propofol and thiopental in isolated rat aorta and pulmonary artery. *ANESTHESIOLOGY* 77:956–963, 1992
12. Chiu AT, McCall DE, Timmermans PB: Pharmacological characteristics of receptor-operated and potential-operated Ca^{2+} channels in rat aorta. *Eur J Pharmacol* 7(127):1–8, 1986
13. Bolton TB: Mechanisms of action of transmitter and other substances on smooth muscle. *Physiol Rev* 59:606–718, 1979
14. van Breemen C, Aaronson P, Loutzenhiser R: Na^+ , Ca^{2+} interactions in mammalian smooth muscle. *Pharmacol Rev* 30:167–208, 1979
15. Nelson MT, Patlak JB, Worley JF, Standen NB: Calcium Channels, potassium channels and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 259(Cell Physiol 28):C3–C18, 1990
16. Nelson MT, Standen NB, Brayden JE, Worley JF: Noradrenaline contracts arteries by activating voltage dependent calcium channels. *Nature (Lond)* 336:382–385, 1988
17. Cockscott ID: Propofol ("Diprivan") pharmacokinetics and metabolism—an overview. *Postgrad Med J* 61(suppl 3):45–50, 1985
18. Herregods L, Golly G, Versichelen L, Rossel MT: Propofol combined with nitrous oxide-oxygen for induction and maintenance of anaesthesia. *Anaesthesia* 42:360–365, 1987

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19. Spelina KR, Coates DP, Monk CR, Prys-Roberts C, Norley I, Turtle MJ: Dose requirements of propofol by infusion during nitrous oxide anaesthesia in man: I. Patients premedicated with morphine sulfate. *Br J Anaesth* 58:1080–1084, 1986
20. Turtle MJ, Cullen P, Pres-Robert C, Coates D, Monk CR, Farogui MH: Dose requirement of propofol by infusion during nitrous oxide anaesthesia in man. *Br J Anaesth* 59:283–287, 1987
21. Servin F, Desmots JM, Haberer JP, Cockshott ID, Plummer GF, Farinotti R: Pharmacokinetics and protein binding of propofol in patients with cirrhosis. *ANESTHESIOLOGY* 69:887–891, 1988
22. Bohr DH: Vascular smooth muscle: Dual effect of calcium. *Science* 139:597–599, 1963
23. Hudgins PM, Weiss GB: Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J Pharmacol Exp Ther* 159:91–99, 1968
24. Hester RK: The effects of 2-nicotinamidoethyl nitrate on agonist-sensitive Ca^{2+} release and Ca^{2+} entry in rabbit aorta. *J Pharmacol Exp Ther* 233:100–111, 1985
25. Shibata S, Wakabayashi S, Sakata N, Hester PK, Ueda S, Tomiyama A: Mode of vasorelaxing action of 5-[3-[[2-(3,4-dimethoxyphenyl)-ethylamino]-1-oxopropyl]-2,3,4,5-tetrahydro-1,5-benzothiazepine fumarate (KT-362), a new intracellular calcium antagonist. *J Pharmacol Exp Ther* 240:16–22, 1987
26. Godfraind T, Kaba A: Blockade or reversal of the contraction induced by calcium and adrenaline in depolarized arterial smooth muscle. *Br J Pharmacol* 36:549–560, 1969
27. Barrett RJ, Appell KC, Kilpatrick BF, Proakis AG, Nolan JC, Walsh DA: AHR-16303B, a novel antagonist of 5-HT₂ receptors and voltage-sensitive calcium channels. *J Cardiovasc Pharmacol* 17:41–53, 1991
28. Boer F, Ros P, Bovill JG, van Brummelen P, van der Krogt J: Effect of propofol on peripheral vascular resistance during cardiopulmonary bypass. *Br J Anaesth* 65:184–189, 1990
29. van Breemen C, Saida K: Cellular mechanisms regulating $[Ca^{2+}]_i$ smooth muscle. *Annu Rev Physiol* 51:315–329, 1989
30. Fairhurst AS, Whittaker ML, Ehlert FJ: Interactions of D600 (methoxyverapamil) and local anesthetics with rat brain α -adrenergic and muscarinic receptors. *Biochem Pharmacol* 29:155–162, 1980
31. Karaki H, Kubota H, Urakawa N: Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. *Eur J Pharmacol* 56:237–245, 1979
32. Itoh T, Kuriyama H, Suzuki H: Difference and similarities in the noradrenaline- and caffeine-induced mechanical responses in rabbit mesenteric artery. *J Physiol (Lond)* 337:609–629, 1983
33. Jeijten PAA, van Breemen C: The effect of caffeine on the noradrenaline-sensitive calcium store in rabbit aorta. *J Physiol (Lond)* 357:327–339, 1984
34. Silver PJ, Dachiw J, Ambrose JM: Effects of calcium antagonists and vasodilators on arterial myosin phosphorylation and actin-myosin interactions. *J Pharmacol Exp Ther* 230:141–148, 1984
35. Weishaar RE, Quade M, Schenden JA, Kaplan HR: The methylenedioxyindenes, a novel class of "intracellular calcium antagonists": Effects on contractility and on processes involved in regulating intracellular calcium homeostasis. *J Pharmacol Exp Ther* 227:767–778, 1983
36. Ljung B, Kjellstedt A: Functional antagonism of noradrenaline responses by felodipine and other calcium antagonists in vascular smooth muscles. *J Cardiovasc Pharmacol* 10(suppl 1):S82–S88, 1987
37. van Breemen C, Siegel B: The mechanisms of α -adrenergic activation of the dog coronary artery. *Circ Res* 46:426–429, 1980
38. Cauvin C, Saida K, van Breemen C: Extracellular Ca^{2+} dependence and diltiazem inhibition of contraction in rabbit conduit arteries and mesenteric resistance vessels. *Blood Vessels* 21:23–31, 1984