

Mechanism of the Negative Inotropic Effect of Propofol in Isolated Ferret Ventricular Myocardium

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Background: The aim of this study was to investigate propofol's effect on myocardial contractility and relaxation and examine its underlying mechanism of action in isolated ferret ventricular myocardium.

Methods: The effects of propofol on variables of contractility and relaxation and on the free intracellular Ca^{++} transient detected with the Ca^{++} -regulated photoprotein aequorin were analyzed. Propofol's effects were evaluated in a preparation in which the sarcoplasmic reticulum function was impaired by ryanodine. The effects of propofol's solvent, intralipid, on myocardial contractility, relaxation, and the intracellular Ca^{++} transient also were examined.

Results: Propofol, at concentrations of 10 μM or greater, decreased contractility and, at concentrations of 30 μM or greater, decreased the amplitude of the intracellular Ca^{++} transient. At equal peak force, control peak aequorin luminescence in $[\text{Ca}^{++}]_o = 2.25 \text{ mM}$ and peak aequorin luminescence in 300 μM propofol in $[\text{Ca}^{++}]_o > 2.25 \text{ mM}$ did not differ, which suggests that propofol does not alter myofibrillar Ca^{++} sensitivity. After inactivation of sarcoplasmic reticulum Ca^{++} release with 1 μM ryanodine, a condition in which myofibrillar activation depends almost exclusively on transsarcolemmal Ca^{++} influx, propofol caused a decrease in contractility and in the amplitude of the intracellular Ca^{++} transient. Under these conditions, propofol's relative negative inotropic effect did not differ from that in control muscles not exposed to ryanodine. Propofol's solvent, 10% intralipid, exerted a modest positive inotropic effect in this preparation. The intracellular Ca^{++} transient was unchanged by intralipid. Neither propofol nor intralipid altered the load sensitivity of relaxation.

Conclusions: These findings suggest that the negative inotropic effect of propofol results from a decrease in intracellular Ca^{++} availability with no changes in myofibrillar Ca^{++} sensitivity. At least part of propofol's action is attributable to inhibition of transsarcolemmal Ca^{++} influx. (Key words: Aequorin. Anesthetics, intravenous: propofol. Heart: contractility; intracellular Ca^{++} transient.)

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THE intravenous anesthetic propofol (2,6-diisopropyl phenyl) has achieved wide clinical acceptance because of rapid emergence that follows even prolonged administration.^{1,2} This latter characteristic is in part a function of the drug's extremely high octanol-blood partition coefficient.³

Unfortunately, the induction of anesthesia with propofol can result in marked decreases in systemic arterial blood pressure.⁴⁻⁶ Many of the early studies on propofol's hemodynamic effects were carried out in whole animals, and it became clear that propofol decreased systemic blood pressure through a reduction in systemic vascular resistance with consequent decreases in cardiac output secondary to decreasing preload.⁷⁻⁹ The effects of propofol on intrinsic myocardial contractility have been studied only more recently. The results of these studies, though showing variability between species, demonstrate an intrinsic negative inotropic effect of propofol independent of changes in preload, afterload, and systemic vascular resistance.¹⁰⁻¹³ In isolated guinea pig myocardium, propofol decreased peak twitch force and selectively depressed the distinct late peaking tension observed with "slow" action potentials in partially depolarized myocardium more than early peak tension.¹² This was taken as evidence that propofol depresses Ca^{++} entry across the sarcolemma and that some effect of propofol on sarcoplasmic reticulum (SR) Ca^{++} release may contribute to contractile depression. In rabbit ventricular muscle, propofol exerted a significant negative inotropic effect *via* depression of both sarcolemmal and SR function, the former being most affected.¹¹ In rat ventricular myocardium, propofol exerted minimal inotropic effects and impaired isotonic relaxation, suggesting that it decreased SR Ca^{++} uptake.¹³ The aim of this study is to evaluate the effects of propofol on contractility and relaxation in ferret papillary muscle and to investigate the mechanism by which propofol exerts its negative inotropic effect by studying the intracellular Ca^{++} transient with the Ca^{++} -regulated photoprotein aequorin.¹⁴ Potential differences of re-

sults in myocardium of different species will shed more light on the mechanism(s) of the inotropic effects of propofol.

Materials and Methods

After approval by the Animal Care and Use Committee of the Mayo Foundation, we used papillary muscles from the right ventricle of adult male ferrets (weight 1,100–1,500 g, age 16–19 weeks). The animals were anesthetized with sodium pentobarbital (100 mg/kg, intraperitoneally), and the heart was quickly removed through a left thoracotomy. The right ventricle was opened, and suitable papillary muscles were excised and mounted in a temperature-controlled (30°C) muscle chamber that contained a physiologic salt solution of the following composition (mM): Na⁺ 135; K⁺ 5; Ca⁺⁺ 2.25; Mg²⁺ 1; Cl⁻ 103.5; HCO₃⁻ 24; HPO₄²⁻ 1; SO₄²⁻ 1; acetate 20; glucose 10. This solution was equilibrated with 95% O₂ and 5% CO₂ (pH 7.4). Suitable preparations were selected on the basis of previously used criteria.¹⁵ The muscles were held between the lever of a force-length transducer (Innovi, Zaventem, Belgium) and a miniature Lucite clip with a built-in punctate stimulation electrode. Muscles were stimulated at a stimulus frequency of 0.25 Hz, with rectangular pulses of 5-ms duration and an intensity 10% above threshold. Muscles were made to contract in alternating series of four isometric and four isotonic twitches at preload only during a 2-h stabilization period before the onset of the experiment. All experiments were carried out with the initial muscle length set at L_{max}, *i.e.*, the muscle length at which active force development is maximal.

Experimental Design

Four protocols were used to examine the mechanism of propofol's direct inotropic effect. Two protocols assessed intralipid's contribution to the net drug effect. Each muscle served as its own control.

In group 1 muscles (n = 8), propofol's effect on contractility and relaxation were defined. Papillary muscles were exposed to cumulative concentrations of propofol (in intralipid) 3, 10, 30, 100, and 300 μM. The peak plasma concentration of propofol after an intravenous induction dose may be as high as 44 μM,¹⁶ whereas stable plasma levels of approximately 10–20 μM are observed during a maintenance infusion.¹⁷ Though the doses studied span those of other investigators, the concen-

tration range tested in this study must be considered high, because more than 90% of propofol is protein-bound in blood.³ After 15 min of equilibration at a particular concentration, variables of contraction and relaxation were determined from three types of twitches. The first contraction was an isotonic twitch at the preload of L_{max} from which peak shortening (DL), peak velocity of shortening (+V), peak velocity of lengthening (-V), and time to peak shortening (TDL) were measured. The second contraction was a "zero-load-clamp" twitch, that is, an isotonic twitch at the preload of L_{max} when load was rapidly (<3 ms) reduced to zero just before the stimulus was applied. Maximal unloaded velocity of shortening (MUVS) and time to maximal unloaded velocity of shortening (TMUVS) were measured from zero-load-clamped twitches. The third contraction was an isometric twitch, from which peak developed force (DF), maximal rate of rise (+dF/dt), and fall of force (-dF/dt), time to peak force (TPF), and time from DF to half-isometric relaxation (RTH) were measured. Each of these three contractions was separated by seven isotonic twitches at the preload of L_{max} to eliminate effects of loading history of preceding contractions.^{18,19} Load sensitivity of relaxation was determined as in earlier studies,²⁰ from an isometric twitch and six afterloaded isotonic twitches. In brief, the ratio of time to initiation of isometric relaxation in afterloaded isotonic twitches relative to time for the isometric twitch to decline to corresponding force levels was plotted against the ratio of force of the afterloaded isotonic twitches relative to DF of the isometric twitch. The slope of this time ratio *versus* force relationship is a quantitative measure of load sensitivity of relaxation.

In group 2 muscles (n = 8), the effects of propofol's solvent, 10% intralipid, were examined in concentrations equivalent to those obtained to papillary muscles exposed to the propofol-intralipid combination. Effects of intralipid on contractility under the loading conditions described for group 1 and load sensitivity of relaxation were determined as in group 1.

Detection of the Intracellular Ca⁺⁺ Transient

After the initial 2-h stabilization period, electrical stimulation was stopped, and multiple superficial cells were microinjected with the Ca⁺⁺-regulated photoprotein aequorin,¹⁴ to allow for subsequent detection of the intracellular Ca⁺⁺ transient. It usually was necessary to microinject 30–100 cells. After microinjection, muscles were not stimulated for 2 h to allow for re-sealing of the plasma membranes of the injected cells.

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The muscles were carefully transferred to and mounted in a vertical muscle chamber that allows for simultaneous detection of variables of contractility and of aequorin luminescence (AL).²¹⁻²³ The aequorin-injected muscle was positioned in a narrow glass extension at the base of the organ chamber at one focal point of a bifocal ellipsoidal reflector. The photocathode of a bialkali photomultiplier (EMI 9235QA, Fairfield, NJ) was located at the other focal point. Muscles were made to contract isometrically at L_{\max} throughout experiments in which AL was measured. It usually was necessary to average luminescence and force signals of 16–256 twitches to obtain a satisfactory signal-to-noise ratio in AL signals. This was accomplished on a digital oscilloscope (Nicolet 4094C, Madison, WI).

In group 3 muscles ($n = 8$), we determined possible changes in the intracellular Ca^{++} transient during exposure to propofol. Isometrically contracting papillary muscles previously microinjected with aequorin were exposed to five cumulative concentrations of propofol (3, 10, 30, 100, 300 μM), and an AL dose-response curve was obtained in each of eight muscles. The muscles were exposed to each concentration of propofol until a steady state of force and systolic AL was obtained. Steady-state force and luminescence signals were averaged until a satisfactory signal-to-noise ratio was obtained. In group 4 muscles ($n = 5$), the effects of 10% intralipid on the intracellular Ca^{++} transient were examined as in group 3.

In group 5 muscles ($n = 8$), we determined whether propofol alters myofibrillar Ca^{++} sensitivity. Each of eight muscles was subjected to "Ca⁺⁺ back-titration" experiments: after measurement of control variables of the isometric twitch, muscles were exposed to 300 μM propofol. Extracellular Ca^{++} was rapidly increased by adding small aliquots of a concentrated $CaCl_2$ solution (112.5 mM) to the bathing solution, until DF was equal to that in the control twitch. This protocol allows a comparison of AL, relaxation, and time variables in control and in the presence of 300 μM propofol at equal DF.

In group 6 muscles ($n = 5$), we attempted to determine whether transsarcolemmal Ca^{++} exchange is affected by propofol. Pretreatment with the plant alkaloid ryanodine was used to exclude the contribution of the SR to Ca^{++} release. Each of five muscles was exposed to 1 μM ryanodine, after which the effects of 3, 10, 30, 100, and 300 μM propofol, respectively, on contractile variables and AL were assessed. Propofol's effects on contractility and AL in the presence of functional SR

(group 3) were compared to its effects in muscles lacking a functional SR (group 6).

A separate set of experiments (group 7) was performed to assess the stability of the ryanodine-treated preparation with time ($n = 5$). At time 0, papillary muscles pre-injected with aequorin were exposed to 1 μM ryanodine, and DF and AL were repeatedly recorded over the next 6 h. Plots of force and AL *versus* time were generated in ryanodine-exposed muscles and compared to dose-response curves of group 6 muscles (propofol and ryanodine).

All waveforms of AL, force, length, and velocity were displayed as a function of time on a four-channel digital oscilloscope (Nicolet 4094C, Madison, WI), stored permanently on 5¼-inch floppy disks, and recorded at slow speed on a four-channel pen recorder (Honeywell 1400, Minneapolis, MN). All waveforms of interest recorded on the digital oscilloscope were transferred to a computer (Reason Technology 486/33 MHz, Minneapolis, MN), so that variables of contraction and relaxation, AL, and corresponding time values were determined automatically.

To determine whether propofol influences the Ca^{++} sensitivity of aequorin, we used an aequorin *in vitro* assay apparatus¹⁴ to measure AL in the presence and absence of 600 μM propofol in a solution containing 150 mM KCl, 5 mM PIPES (piperazine-N,N bis-2-ethanesulfonic acid), 2 mM EGTA [ethylene glycol bis (β -aminoethylether)-N,N,N',N'-tetraacetic acid], and 2 mM CaEGTA, pH 7.0. This solution approximates the ionic composition of the intracellular milieu and produces, at 22°C, a pCa of 6.4, which is in the range of myoplasmic-free Ca^{++} concentrations encountered during a twitch. The effects of 600 μM propofol on the Ca^{++} sensitivity of AL also was assessed in a Ca^{++} -free solution containing only KCl and PIPES in the same concentration as noted above to determine whether propofol alters Ca^{++} -independent AL.

Statistical Analysis

For experiments in groups 1, 2, 3, 4, and 6, the dose-related effect of propofol and intralipid on contractility, relaxation, and AL was assessed using a repeated-measures analysis of variance (ANOVA). When appropriate, Dunnett's test was used to compare effects of individual drug concentration with control. All values are expressed as mean \pm SEM. For group 5 muscles, AL and contractile variables of control twitches and after Ca^{++} back-titration were compared by Student's paired *t*-tests. $P < 0.05$ was considered significant. To compare

the dose-response curves to propofol (in intralipid) with those to intralipid alone and ryanodine time controls (group 7) to ryanodine plus propofol-exposed muscles (group 6), the following procedure was used. First, the inotropic responses in each muscle were expressed as percent of control. Second, the sums of percent effect over the entire drug concentration range were compared between corresponding groups (groups 1 and 2; groups 3 and 4; groups 6 and 7) with a one-way ANOVA followed by Student-Neuman-Keuls test when appropriate.

Results

Among the muscle groups, there were no statistically significant differences in muscle length at L_{max} , mean cross-sectional area, DF, and ratio of resting to total peak isometric force at L_{max} (one-way ANOVA). When all muscle characteristics are pooled, L_{max} was 5.3 ± 0.1 mm, cross-sectional area was 0.87 ± 0.07 mm²; DF was 31.6 ± 1.9 mN · mm⁻², and ratio of resting to total peak isometric force at L_{max} was 0.20 ± 0.01 ($n = 42$). Propofol (600 μ M in intralipid), a twofold higher concentration than the highest concentration used in these experiments, did not alter the Ca⁺⁺-independent AL, nor the Ca⁺⁺-sensitivity of aequorin at pCa 6.4 (22°C, pH 7.0) in *in vitro* assays.

Contractility

Table 1 shows the absolute values of variables of contractility in control conditions at the onset of the experiment for groups 1 and 2. Figures 1 and 2 show

measurements of contractility in the dose-response experiments to propofol (in intralipid; group 1; $n = 8$) and to 10% intralipid alone (group 2; $n = 8$). Figure 1 illustrates that 30 μ M propofol or more decreased DF and peak shortening and decreased maximal unloaded velocity of shortening at concentrations of 100 μ M or greater. By contrast, intralipid alone had a small but significant positive inotropic effect as it increased DF, peak shortening (≥ 10 μ M equivalent), and maximal unloaded velocity of shortening (≥ 30 μ M equivalent). Propofol caused a dose-dependent decrease in the maximal rate of force development (+dF/dt) and decline (-dF/dt; fig. 2) of isometric twitches. In isotonic twitches, propofol decreased peak velocity of shortening and peak velocity of lengthening in a dose-dependent fashion. Intralipid caused small increases in +dF/dt, -dF/dt, peak velocity of shortening, and peak velocity of lengthening; fig. 2). Figure 3 summarizes the effects of propofol and intralipid on kinetics of contraction and relaxation. Propofol shortened TPF and RTH of isometric twitches. Time to maximal unloaded velocity of shortening of zero-load clamped twitches was increased by propofol. Propofol had no effect on time to peak shortening. Intralipid caused no significant changes in time variables.

Relaxation of ferret papillary muscle is sensitive to load during control conditions. Neither propofol nor intralipid alone had any significant effect on load sensitivity of relaxation (fig. 4).

Intracellular Ca⁺⁺ Transient

Effects of propofol and intralipid on contractility and AL are demonstrated in figures 5 and 6 and summarized

Table 1. Variables of Contractility and Relaxation of Ferret Right Ventricular Papillary Muscles of Groups 1 and 2 at the Onset of the Experiment

	Group 1 (propofol, n = 8)	Group 2 (Intralipid, n = 8)
Peak shortening ($L \cdot L_{max}^{-1}$)	0.11 \pm 0.01	0.09 \pm 0.01
Peak velocity of shortening ($L_{max} \cdot s^{-1}$)	0.90 \pm 0.07	0.68 \pm 0.07
Peak velocity of lengthening ($L_{max} \cdot s^{-1}$)	2.66 \pm 0.40	1.66 \pm 0.20
Maximal unloaded velocity of shortening ($L_{max} \cdot s^{-1}$)	1.50 \pm 0.08	1.36 \pm 0.12
Peak developed force (mN · mm ⁻²)	29.5 \pm 3.4	40.0 \pm 8.0
Maximal rate of rise of force (mN · mm ⁻² · s ⁻¹)	205 \pm 25	268 \pm 56
Maximal rate of force decline (mN · mm ⁻² · s ⁻¹)	156 \pm 27	175 \pm 37
Time to peak shortening (ms)	245 \pm 9	298 \pm 18
Time to maximal unloaded velocity of shortening (ms)	60 \pm 3	95 \pm 11
Time to peak force (ms)	241 \pm 9	291 \pm 20
Time to half relaxation (ms)	168 \pm 15	192 \pm 20

Data are mean \pm SEM.

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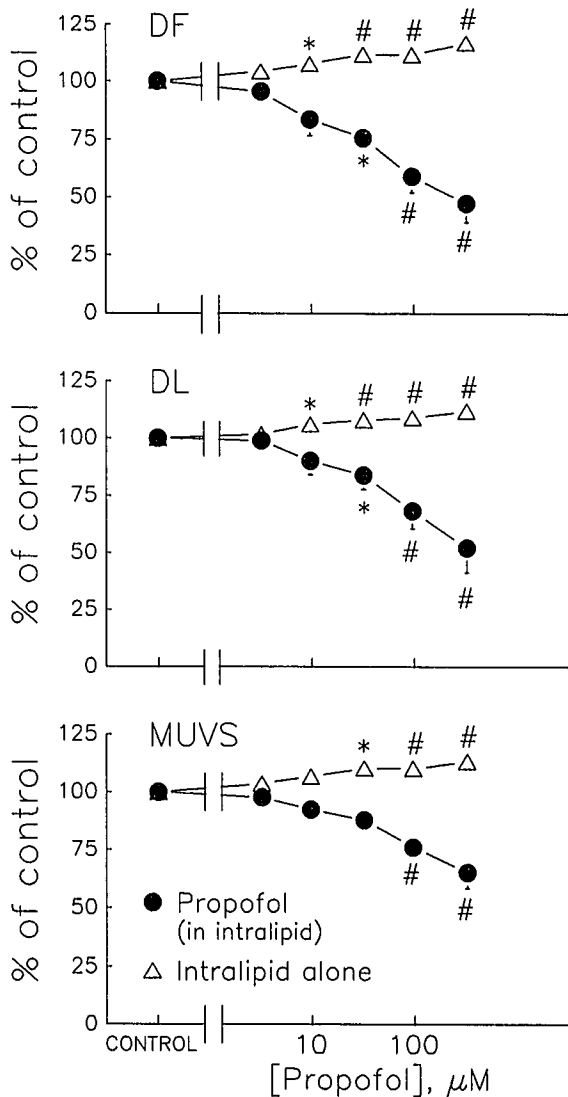


Fig. 1. Effect of propofol in intralipid ($n = 8$, ●) and intralipid alone ($n = 8$, △) on peak force (DF; *top*), peak shortening (DL; *middle*), and maximum unloaded velocity of shortening (MUVS; *bottom*). Data are mean \pm SEM. * $P < 0.05$ and # $P < 0.01$ for comparison with control within each group.

in table 2. These illustrate that propofol, at concentrations of 30 μM or greater, decreased both DF and AL. By contrast, intralipid alone increased DF at concentration equivalents of 10 μM or greater, yet intralipid did not change AL. Neither propofol nor intralipid changed the diastolic AL at any concentration ($P = 0.59$ and $P = 0.35$ by repeated-measures ANOVA, respectively). Time to AL was increased by 100 and 300 μM propofol ($131.3 \pm 10.7\%$ and $157.9 \pm 13.7\%$ of con-

trol) but was unchanged by intralipid alone ($99.1 \pm 4.3\%$ and $99.3 \pm 3.0\%$ of control).

To determine whether propofol alters myofibrillar Ca^{++} responsiveness, AL was measured in eight muscles (group 5) and compared at equal DF in control conditions (fig. 7, left) and after exposure to 300 μM propofol in elevated $[\text{Ca}^{++}]_o$ (fig. 7, right). Table 3 lists the values of AL and of contractile variables in control, in 300 μM propofol, and in 300 μM propofol in higher $[\text{Ca}^{++}]_o$. The $[\text{Ca}^{++}]_o$ achieved during Ca^{++} back-titration to equal control peak force was $3.25 \pm 0.10 \text{ mM}$ ($n = 8$). As observed for group 1 and 3 muscles, propofol decreased DF, TPF, RTH, and AL. When propofol's negative inotropic effect on DF was corrected by raising $[\text{Ca}^{++}]_o$, AL was not significantly different from control ($P = 0.72$, Student's paired t -test). Diastolic AL, TPF, and RTH were unchanged from control (table 3).

Ryanodine Effects

To assess the effects of propofol on contractility independent of the SR Ca^{++} release, AL and contractility

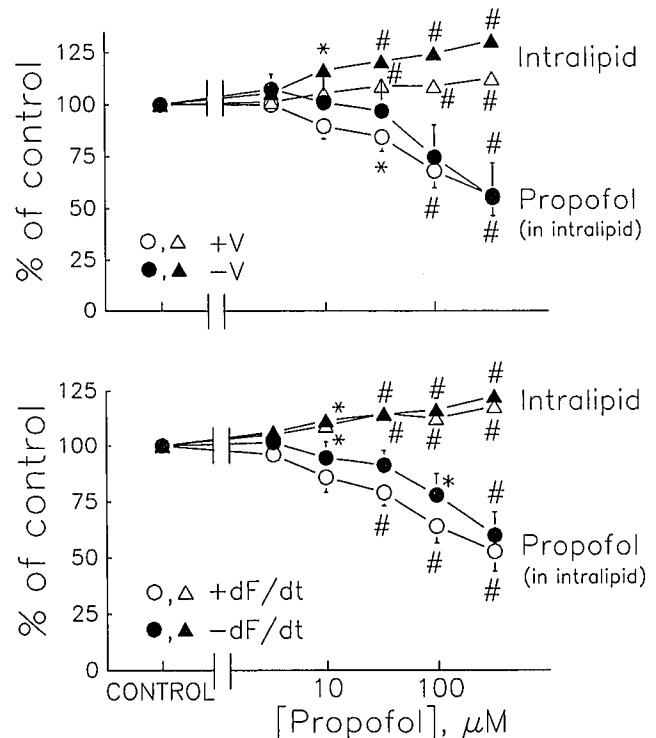


Fig. 2. Effects of propofol in intralipid (circles) and of intralipid alone (triangles) on peak isotonic velocity of shortening and of lengthening (upper panel) and on maximal rates of force development and force decline (lower panel). Data are mean \pm SEM ($n = 8$). * $P < 0.05$ and # $P < 0.01$ for comparison with control values within each group.

Table 2. Aequorin Luminescence and Variables of Contractility during Cumulative Dose-Response Experiments to Propofol (in Intralipid) (Group 3; n = 8) and to Intralipid Alone (Group 4; n = 5)

	Control	Propofol					Propofol + Intralipid Versus Intralipid (<i>t</i> test on Sums)
		3 μ M	10 μ M	30 μ M	100 μ M	300 μ M	
Diastolic aequorin luminescence (nA)							
Propofol + intralipid	0.72 \pm 0.30	0.63 \pm 0.08	0.51 \pm 0.12	0.43 \pm 0.11	0.45 \pm 0.11	0.43 \pm 0.13	NS
Intralipid only	0.56 \pm 0.02	0.79 \pm 0.35	1.17 \pm 0.69	0.74 \pm 0.25	2.30 \pm 1.83	0.39 \pm 0.17	
Peak systolic aequorin luminescence (nA)							
Propofol + intralipid	14.7 \pm 5.3	11.9 \pm 4.2	11.2 \pm 4.5	9.6 \pm 3.6	6.9 \pm 2.3*	5.2 \pm 1.7*	<i>P</i> < 0.05
Intralipid only	7.1 \pm 1.3	7.2 \pm 1.4	8.3 \pm 2.1	15.2 \pm 4.4	9.7 \pm 2.0	10.4 \pm 3.0	
Time to peak aequorin luminescence (ms)							
Propofol + intralipid	49 \pm 5	52 \pm 3	51 \pm 4	50 \pm 4	61 \pm 3	76 \pm 8†	NS
Intralipid only	45 \pm 2	43 \pm 3	43 \pm 4	44 \pm 3	42 \pm 1	41 \pm 1	
Peak developed force (mN \cdot mm ⁻²)							
Propofol + intralipid	35.3 \pm 6.1	32.7 \pm 6.7	33.3 \pm 5.9	30.7 \pm 5.2	24.7 \pm 4.3†	19.6 \pm 3.7*	<i>P</i> < 0.001
Intralipid only	15.5 \pm 2.9	18.4 \pm 4.0	18.2 \pm 3.9	23.2 \pm 7.2	23.5 \pm 6.7	23.9 \pm 6.8	
Time to peak force (ms)							
Propofol + intralipid	224 \pm 19	211 \pm 9†	203 \pm 8*	197 \pm 9*	194 \pm 9*	199 \pm 10*	NS
Intralipid only	209 \pm 9	201 \pm 8	200 \pm 8	208 \pm 10	207 \pm 9	210 \pm 9	
Time to half isometric relaxation (ms)							
Propofol + intralipid	132 \pm 21	121 \pm 13	115 \pm 11†	110 \pm 11*	101 \pm 9*	101 \pm 8*	NS
Intralipid only	138 \pm 18	125 \pm 10	127 \pm 11	138 \pm 13	141 \pm 13	139 \pm 13	

Data are mean \pm SEM.

* *P* < 0.01; †*P* < 0.05; for comparison with control by repeated measures analysis of variance and Dunnett's test.

variables were measured under isometric conditions in ferret papillary muscle after exposure to 1 μ M ryanodine (group 6). Consistent with its effects on the SR, ryanodine decreased DF and AL from control conditions (table 4 and fig. 8, left). In muscles pretreated with 1 μ M ryanodine, force and AL were decreased further by propofol (fig. 8, right; table 4).

The role of the SR in propofol's negative inotropic effect was evaluated further by comparing the relative effects of propofol on DF and AL in group 1 muscles with intact SR (fig. 9, upper curves) with those in group 6 muscles with ryanodine-inactivated SR (fig. 9, lower curves). The relative effects of propofol on DF and AL with or without functional SR did not differ (DF, *P* = 0.49; AL; *P* = 0.8; Student's *t*-test on sums of effects over entire concentration range).

Ryanodine-treated muscles (group 7) were stable over time. At 6 h, DF remained 107.4 \pm 12.3% of control measurements and AL at 97.5 \pm 12.4% of control measurements (mean \pm SEM). Ryanodine time control curves were significantly different from group 6 curves for both DF (*P* \leq 0.05) and AL (*P* \leq 0.01).

Discussion

Propofol is a short-acting intravenous anesthetic induction agent with significant cardiovascular effects.⁴⁻⁶ Marked decreases in systemic arterial blood pressure often are observed with its use. Although the clinical effects on the cardiovascular system appeared to be largely the result of reductions in systemic vascular resistance and decreasing preload,⁷⁻⁹ this *in vitro*

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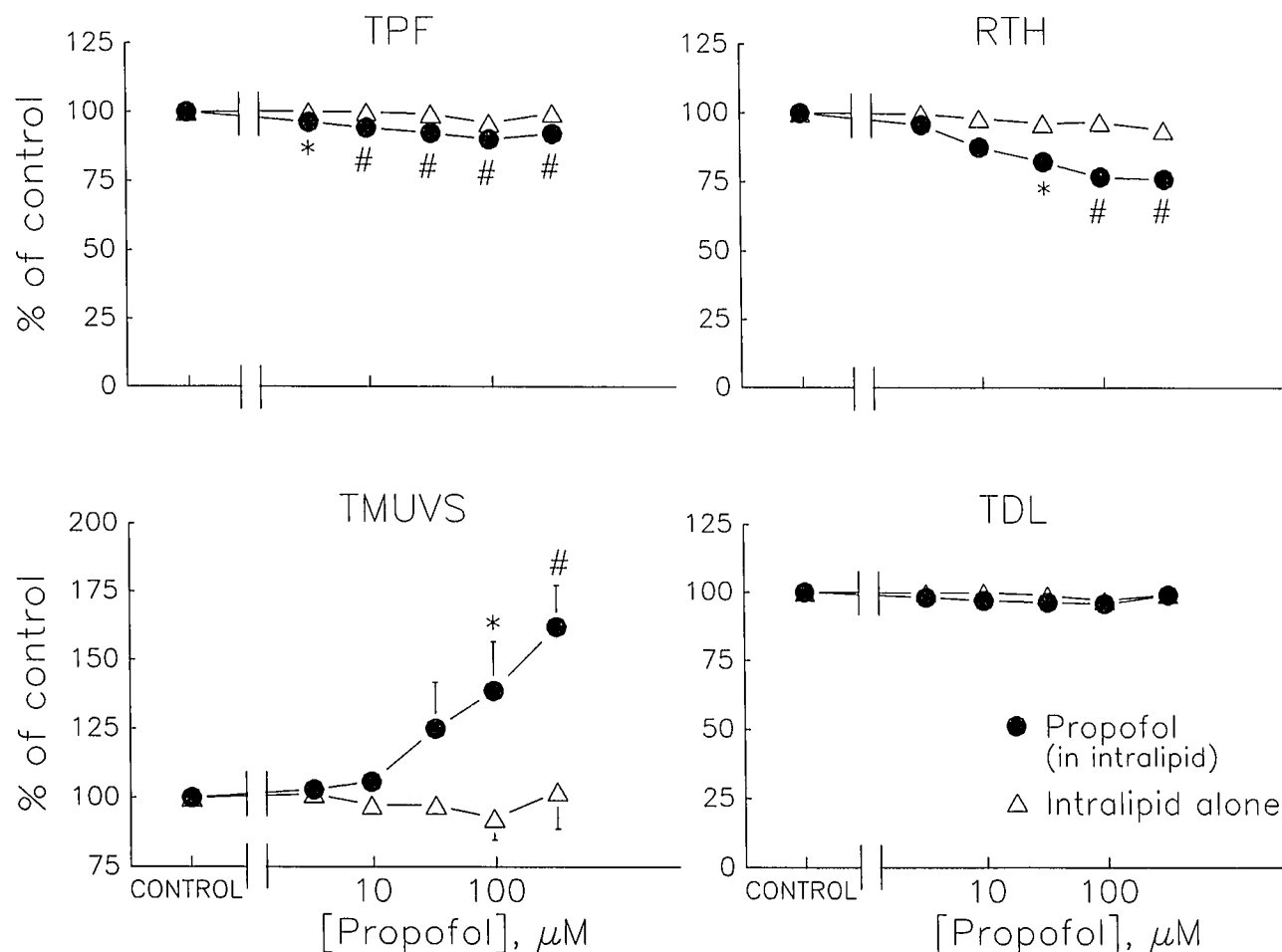


Fig. 3. Effects of propofol in intralipid (filled circles) and of intralipid alone (open triangles) on time variables: time to peak force (TPF) and time to half-isometric relaxation (RTH) of isometric twitches, time to maximal unloaded velocity of shortening (TMUVS) of zero-load clamp twitches, and time to peak shortening (TDL) of preload isotonic twitches. Data are mean \pm SEM ($n = 8$). * $P < 0.05$ and # $P < 0.01$ for comparison with corresponding control.

study demonstrates the negative inotropic effect of propofol in isolated ferret ventricular myocardium at concentrations of $30 \mu\text{M}$ and greater. Though the propofol concentration found after an intravenous bolus injection of propofol may reach $44 \mu\text{M}$ because of greater than 90% protein binding,^{3,16,17} the free plasma concentration of propofol should not exceed $2 \mu\text{M}$. Therefore, the concentration range studied here must be considered as higher than likely to be encountered during clinical practice. Propofol exerted a negative inotropic effect in guinea pig¹² and rabbit ventricular myocardium,^{10,11} yet had limited effects in rat papillary muscle.¹³ Of different species, ferret ventricular myocardium is a good model to study drug actions because it shares certain physiologic characteristics with human

ventricular myocardium. The density of sympathetic innervation of the ferret right ventricle parallels that of human right ventricle.²⁴ The regulation of activator Ca^{++} by sarcolemma and SR is similar in the two species. This is expressed in the mechanical characteristics of the two tissues exhibiting similar force-frequency relationships and mechanical restitution curves.^{25,26} Furthermore, information from experiments carried out in different species can help identify the predominant site of drug action because of different contributions to excitation-contraction coupling by SR Ca^{++} release and transsarcolemmal Ca^{++} entry in rat, ferret, rabbit, and guinea pig ventricular myocardium.

The results of this study must be interpreted in the context of the experimental conditions in which they

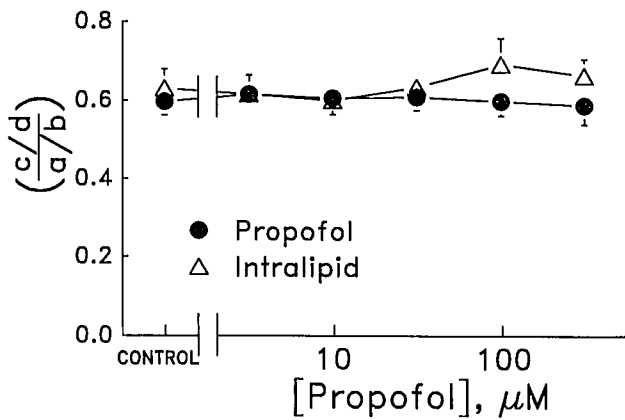


Fig. 4. Effects of propofol in intralipid (filled circles) and intralipid alone (open triangles) on the load sensitivity of relaxation.

were obtained. Results obtained here at 30°C and a stimulus interval of 4 s may differ from those that could be obtained at the more physiologic conditions of the animal, 37–38°C and 200 beats/min particularly because, at low stimulation rates, the sarcolemma may play a more prominent role in providing the Ca⁺⁺ of activation.

Drugs that exert negative inotropic effects either decrease the amount of Ca⁺⁺ available for contraction,

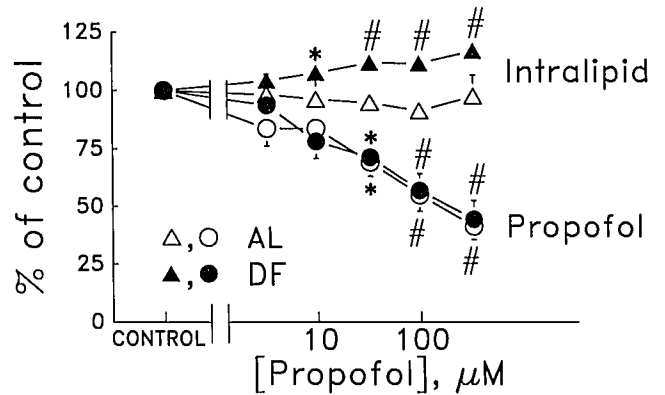


Fig. 6. Summary of dose-response experiments to propofol in intralipid (circles) and to intralipid (triangles) on peak aequorin luminescence (AL) and peak force development (DF). Data are mean ± SEM (n = 8). **P* < 0.05 and #*P* < 0.01 for comparison with corresponding control.

decrease the sensitivity of the myofibrillar apparatus to Ca⁺⁺, or both. The loss of contractility we describe is accompanied by a decrease in the amplitude of the aequorin signal. Thus, propofol's negative inotropic effect is at least in part the result of decreased intracellular Ca⁺⁺ availability. A decrease in Ca⁺⁺ availability can result from either an impairment of sarcolemmal or SR function. To differentiate these potential sites of

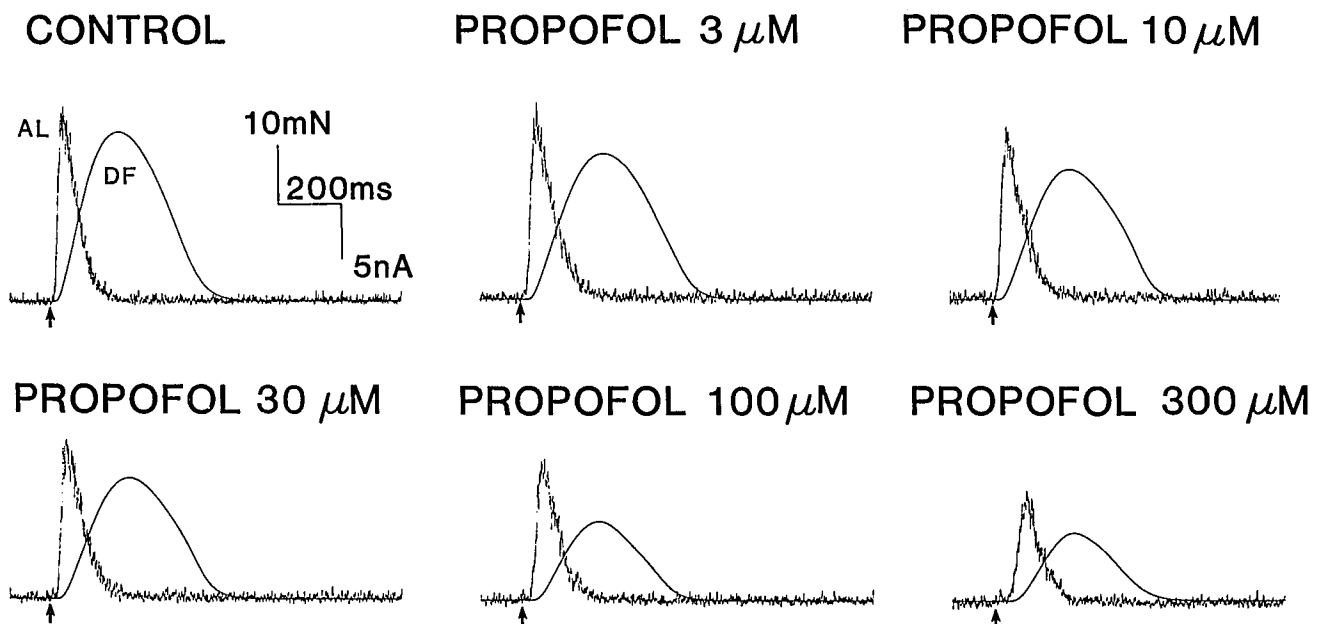


Fig. 5. Aequorin luminescence and force traces during isometric twitch contractions of a right ventricular ferret papillary muscle in control and during exposure to each of five propofol concentrations. Sixty-four twitches were averaged in each panel.

PROPOFOL AND MYOCARDIAL CONTRACTILITY

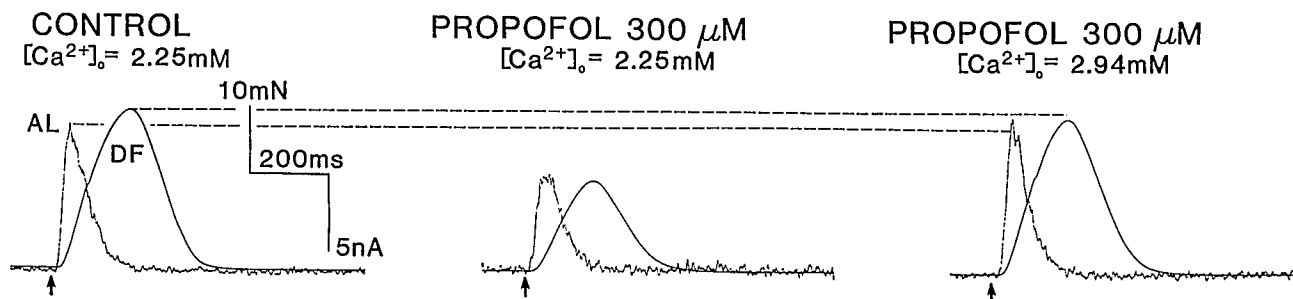


Fig. 7. Aequorin luminescence and force traces of isometric twitches during a typical Ca^{++} back-titration experiment. After an initial control (left), muscles were exposed to $300 \mu\text{M}$ propofol (middle), and $[\text{Ca}^{++}]_o$ was subsequently rapidly raised (right) so that peak force equaled that in the control. At equal peak force with (right) and without (left) propofol, there was no difference in peak aequorin luminescence. Sixty-four twitches were averaged in each panel.

action, we examined propofol's effect in myocardial tissue pretreated with the plant alkaloid ryanodine. Ryanodine abolishes SR Ca^{++} release^{27,28} but has no effect on the SR Ca^{++} uptake pump or Na^+ - Ca^{++} exchanger²⁹⁻³¹ and therefore can be used as a tool to make contractility depend on transsarcolemmal Ca^{++} movement.³² The significant reduction in all contractile variables from control measurements after $1 \mu\text{M}$ ryanodine is similar in magnitude to that reported in similar³² and identical³³ experimental conditions and reflects the substantial contribution of the SR to the activator Ca^{++} pool in ferret ventricular myocardium. Changes in contractility and in the intracellular Ca^{++} transient caused by a drug that occur over and above those produced by ryanodine reflect the action of that drug on sarcolemmal function.^{32,33} In the presence of $1 \mu\text{M}$ ryanodine, propofol caused a further reduction in contractility and AL. The relative loss of contractility produced by propofol after ryanodine pretreatment (no SR Ca^{++} release) did not differ from that in muscles not pretreated with ryanodine (SR Ca^{++} release unim-

paired). This strongly suggests that propofol decreases intracellular Ca^{++} availability by interfering with transsarcolemmal Ca^{++} influx.

While propofol profoundly affects intracellular Ca^{++} availability, we also examined its effects on myofibrillar Ca^{++} sensitivity. We measured AL in Ca^{++} back-titration experiments in which AL was measured in the absence and presence of $300 \mu\text{M}$ propofol, at equal force obtained by adjusting the extracellular Ca^{++} concentration upward in the presence of propofol. The assumption implicit to this type of analysis is that the Ca^{++} occupancy of troponin C at peak force is the same in either condition, so that myofibrillar Ca^{++} sensitivity can be assessed from the relationship between $[\text{Ca}^{++}]_i$ and Ca^{++} occupancy of troponin C. If propofol alters reaction mechanisms "downstream" from the binding of Ca^{++} to troponin C and modifies the relationship between Ca^{++} occupancy of troponin C and force, our approach would be invalid. Yet, so far there is no evidence that this occurs. Since there was no change in the relation between AL and peak force under propofol, it is un-

Table 3. Aequorin Luminescence and Isometric Force Variables in Ferret Papillary Muscle During Ca^{++} Back-Titration Experiments (Group 3; n = 8) in the Presence of $300 \mu\text{M}$ Propofol in Intralipid

	Control, $[\text{Ca}^{++}]_o = 2.25 \text{ mM}$	$300 \mu\text{M}$ Propofol, $[\text{Ca}^{++}]_o = 2.25 \text{ mM}$	$300 \mu\text{M}$ Propofol, $[\text{Ca}^{++}]_o > 2.25 \text{ mM}$
Diastolic aequorin luminescence (nA)	0.5 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
Peak systolic aequorin luminescence (nA)	28.9 ± 10.9	$9.6 \pm 3.3^*$	24.2 ± 7.6
Time to peak aequorin luminescence (ms)	48 ± 5	$69 \pm 5^*$	$57 \pm 4^\dagger$
Peak developed force ($\text{mN} \cdot \text{mm}^{-2}$)	40.0 ± 5.2	$21.3 \pm 3.4^*$	41.1 ± 5.3
Time to peak force (ms)	226 ± 19	202 ± 10	214 ± 10
Time to half isometric relaxation (ms)	138 ± 20	109 ± 7	124 ± 10

Data are mean \pm SEM.

* $P < 0.01$; $\dagger P < 0.05$; for comparison with control by Student's paired t test.

Table 4. Aequorin Luminescence and Contractile Variables in Ferret Papillary Muscles (Group 4; n = 5) in Control Conditions and during Dose-Response Experiments to Propofol in the Presence of Ryanodine

	1 μM Ryanodine						
	Control	Control	Propofol				
			3 μM	10 μM	30 μM	100 μM	300 μM
Aequorin luminescence (nA)	7.23 \pm 1.49	0.62 \pm 0.05	0.52 \pm 0.04	0.43 \pm 0.05*	0.33 \pm 0.05*	0.20 \pm 0.04†	0.10 \pm 0.04†
Time to peak aequorin luminescence (ms)	66 \pm 11	163 \pm 26	168 \pm 16	168 \pm 16	176 \pm 23	146 \pm 13	130 \pm 18
Peak developed force (mN \cdot mm ⁻²)	22.8 \pm 7.9	4.5 \pm 0.7	4.2 \pm 0.6	3.8 \pm 0.7*	2.9 \pm 0.8*	1.9 \pm 0.8†	1.1 \pm 0.5†
Time to peak force (ms)	257 \pm 26	353 \pm 44	334 \pm 22	334 \pm 27	330 \pm 29	275 \pm 26	271 \pm 27
Time to half isometric relaxation (ms)	169 \pm 28	164 \pm 26	145 \pm 19	144 \pm 20	136 \pm 19	124 \pm 19	123 \pm 14

Data are mean \pm SEM.

* $P < 0.05$; † $P < 0.01$; for comparison with control in ryanodine by repeated measures analysis of variance and Dunnett's test.

likely that the myofibrillar Ca^{++} sensitivity is changed by propofol. The negative inotropic effect of propofol therefore can be attributed to an interference with cellular mechanisms that regulate intracellular Ca^{++} availability.

Two recent reports describing propofol's inotropic effect are difficult to compare to our own. Mouren *et al.*³⁴ monitored intraventricular pressure changes in an isolated perfused rabbit heart and did not demonstrate changes in maximum or minimum dP/dt following propofol exposure. Ismail *et al.*³⁵ assessed propofol's inotropic effect in an open chest dog model. At clinically relevant concentrations, propofol did not change segmental shortening as measured by ultrasonic crys-

tals, although supratherapeutic concentrations caused cardiac depressions. Work in isolated myocardium, in which loading conditions are easier to control, may provide more insight as to the mechanisms of propofol's effects.

Our findings confirm Lin *et al.*'s¹⁰ hypothesis that propofol's negative inotropic effect is a result of a decrease in intracellular Ca^{++} availability. Our results also agree with those of Rusy *et al.*,¹¹ who studied propofol's negative inotropic effect in rested, steady-state, and potentiated-state contractions in rabbit papillary muscle. Those authors suggested that both sarcolemmal function and SR function were altered by propofol but that propofol's sarcolemmal activity was probably the

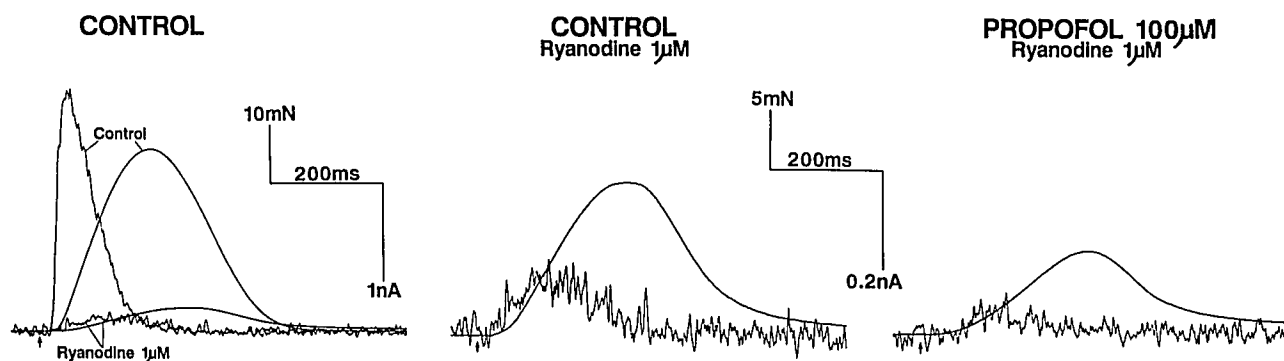


Fig. 8. Effect of propofol on force and aequorin luminescence before and after inactivation of sarcoplasmic reticulum Ca^{++} release by 1 μM ryanodine. The left panel shows superimposed traces of force and aequorin luminescence during isometric twitches before and after 1 μM ryanodine. Note alteration in vertical scale in the post-ryanodine-treated panels (center and right). One hundred twenty-eight twitches were averaged in control, and 256 twitches were averaged in ryanodine.

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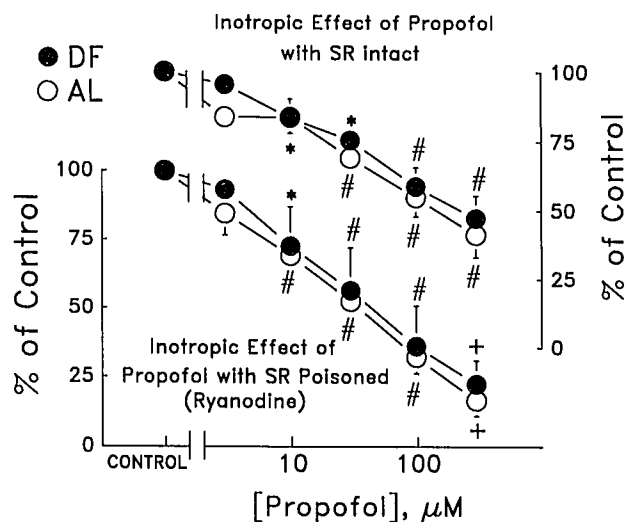


Fig. 9. Relative dose-related effects of propofol on force development (DF) and on aequorin luminescence (AL) in ferret right ventricular papillary muscles (mean \pm SEM; $n = 8$) with an intact sarcoplasmic reticulum (upper curves; data from group 1) and after inactivation of sarcoplasmic reticulum Ca^{++} release (lower curves) in muscles (mean \pm SEM; $n = 5$) exposed to 1 μM ryanodine. * $P < 0.05$, # $P < 0.01$, and † $P < 0.001$ versus corresponding control before the propofol dose-response experiments.

more important site of action. Park and Lynch¹² also observed a negative inotropic effect of propofol in guinea pig papillary muscle. Propofol depressed selectively the late-peaking tension during "slow" action potentials in partially depolarized muscles. This was taken to suggest that propofol depresses transsarcolemmal Ca^{++} entry, yet an effect on SR Ca^{++} release was not discounted.¹² Our results primarily differ from those of Riou *et al.*,¹³ who described only an impairment of isotonic relaxation in rat heart and attributed this effect to an impairment in SR function.

The results of Riou *et al.*¹³ may differ from Rusy *et al.*¹¹ and our own because the SR is highly developed in the rat, and the sarcolemma plays a less prominent role in provision of activator Ca^{++} . Rabbit and ferret myocardium are more similar in that transsarcolemmal Ca^{++} movement provides a relatively greater contribution to the Ca^{++} of activation than in the rat.³⁶ A drug with sarcolemmal effects might be expected to have negligible effect in a species like the rat and significant effects in species more dependent on sarcolemmal function to provide activator Ca^{++} . Though these experiments clearly identify a sarcolemmal site of action, a specific Ca^{++} current is not identified, nor are pro-

propofol potential effects on the relationship between sarcolemmal Ca^{++} entry and SR Ca^{++} release.

The effect of propofol's solvent, 10% intralipid, on contractility and the intracellular Ca^{++} transient also was examined. In contrast to propofol, intralipid has small but significant positive inotropic properties. Force development, muscle shortening, and contraction kinetics were increased by intralipid. A ceiling effect was demonstrated in that contractility was increased approximately 10% at an intralipid concentration equivalent to 30 μM propofol and was not increased further by higher intralipid concentrations. This increase in contractility was not associated with a change in AL; therefore, intralipid's positive inotropic effect is not achieved by increasing intracellular Ca^{++} availability. Based on these findings, we speculate that the intralipid may serve as a metabolic substrate for the myocardial tissue in our preparation. In this way, a small non-dose-dependent inotropic effect might be seen in the absence of changing intracellular Ca^{++} concentrations.

It would be oversimplistic to suggest that the negative inotropic effect of pure propofol, independent of its commercial solvent, is 10–15% greater than we report—pharmacodynamics are not necessarily additive—although in our experimental preparation, intralipid probably compensated to some extent for pure propofol's negative inotropic effect.

Neither propofol nor intralipid significantly altered the load sensitivity of relaxation, although contractility was changed. This again illustrates that relaxation is a physiologically separate process from contraction and is consistent with the postulated mechanism of propofol's negative inotropic effect. In human³⁷ and other mammalian species,^{38–40} relaxation rate and afterload are directly related; relaxation occurring more quickly under low-load conditions. This property is a function of Ca^{++} reuptake by the SR, the release of Ca^{++} from troponin C, the myofibrillar responsiveness to Ca^{++} , and the rate of actomyosin cross-bridge detachment. We were unable to document any change in myofibrillar Ca^{++} sensitivity in our back-titration experiments, and propofol's negative inotropic effect is preserved largely in a preparation devoid of an SR able to release Ca^{++} . These data point to the sarcolemma as the primary site of propofol's action. Because propofol's primary site of action at the sarcolemma is more removed from the primary processes that determine relaxation, one need not anticipate an alteration in relaxation with this drug, although contractility is impaired.

In conclusion, the evidence from this study suggests that propofol exerts a mild to moderate direct negative inotropic effect by decreasing transsarcolemmal Ca^{++} influx without altering myocardial relaxation. The depressant effect may be due to an inhibition of the sarcolemmal slow inward L-type Ca^{++} current. Possible effects of propofol on other membranous Ca^{++} exchange mechanisms (e.g., $\text{Na}^+/\text{Ca}^{++}$ exchange, Ca^{++} ATPase export pump) cannot be excluded and may necessitate further study. The concentrations of propofol at which significant inotropic effects are observed (30 μM) are at least tenfold those observed in humans after an intravenous bolus of propofol.^{3,16} Propofol's solvent, intralipid, also was demonstrated to possess a small positive inotropic effect. This may be relevant to pharmacologic studies but probably has little clinical significance.

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