Anesthesiology
2000; 92:542-9

Propofol-induced Modifications of Cardiomyocyte Calcium Transient and Sarcoplasmic Reticulum Function in Rats

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Background: **Propofol is considered to be an anesthetic agent with few or no negative inotropic effects. This study evaluated a possible direct depressant effect of propofol on sarcoplasmic reticulum Ca^{2+} accumulation and cardiomyocytes.**

Methods: **The effects of propofol on intracellular Ca^{2+} transients were evaluated in isolated rat cardiomyocytes using a microfluorometric technique with Indo-1. Sarcoplasmic reticulum function was also assessed by measuring the oxalate-stimulated Ca^{2+} uptake from homogenates of rat ventricles.**

Results: **The Ca^{2+} uptake capacity of the sarcoplasmic reticulum was decreased by propofol (10^{-4} M). Large concentrations of propofol decreased the rate of decrease of the intracellular Ca^{2+} transient, which resulted in an increase of diastolic Ca^{2+} when the diastolic interval was decreased. The increased diastolic Ca^{2+} also resulted in a decrease in Ca^{2+} transient. This effect appeared for lower doses (10^{-5} M) after a short diastolic pause rather than after a long (2- to 3-min) rest (appearing at 10^{-4} M).**

Conclusions: **For doses more than 10^{-5} M, propofol induces a Ca^{2+} uptake capacity impairment of the sarcoplasmic reticulum. This is probably responsible for a slowing of the decrease of the Ca^{2+} transient, which in turn increases the diastolic Ca^{2+} for high heart rate. These diastolic modifications may participate in the slight negative inotropic effect of the drug. (Key words: Contraction; heart; ventricle.)**

THE short-acting anesthetic agent propofol induces few or no negative inotropic effects,[1,2] depending on the species (negative inotropic effect in guinea pigs[3] and no effects in rats[4]). The depressant myocardial effects of propofol also are greater during heart failure than during the normal state,[5] although the negative inotropic effect was not greater in hamsters with hypertrophic cardiomyopathy than in normal hamsters.[6]

An absence of change in myofibrillar affinity for Ca^{2+} has been shown,[7] but a more recent study[8] strongly suggested that myofibrillar Ca^{2+} sensitivity may be increased by propofol. Most studies attributed the negative inotropic effect of propofol to a decrease in transarcolemmal Ca^{2+} entry inside the cell.[9-11] Riou *et al.*[4] suggested that Ca^{2+} uptake by the sarcoplasmic reticulum (SR) also was impaired by propofol, as shown by a slowing of isotonic relaxation. Similar results were published recently.[8] However, a propofol-induced impairment of SR function was not observed in another study.[7] A possible explanation for the differing conclusions of these studies is that, in the work of Cook and Housmans,[7] the stimulation rate of the papillary muscles was very slow (0.25 Hz). Possible abnormalities of SR Ca^{2+} uptake could have been masked, because each contraction took place after a 4-s diastolic pause, allowing for a complete return to baseline diastolic Ca^{2+} levels. Another possible explanation is that the study of Cook and Housmans[7] was performed with ferrets, a species with a less-developed SR than rats.[12]

The goal of our study was to evaluated whether cellular Ca^{2+} homeostasis was impaired by propofol. The Ca^{2+} transient of isolated cardiomyocytes was studied in rats because the SR plays a major role in the excitation-contraction coupling.[13]

Methods

Microfluorometric Cytosolic Calcium Measurements

Cell Isolation. Cardiac myocytes were obtained from the hearts of male Wistar rats (270-300 g). All procedures were performed according to the Guiding Principles in the Care and Use of Animals of the American

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Received from Unité Institut National de la Sante et de la Recherche Medicale (INSERM) U400, Faculté de Médecine, Créteil, France. Submitted for publication December 28, 1998. Accepted for publication September 30, 1999. Support was provided by the Institut National de la Sante et de las Recherche Medicale (INSERM), Paris, France. Dr. Guenoun was the recipient of a fellowship grant from Zeneca, Cergy, France.

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Physiologic Society, Bethesda, Maryland, with the agreement of the French Ministry of Agriculture, Paris, France. Cardiomyocytes were isolated using the procedure of Powell and Twist,[14] with slight modifications.[15] After anesthetization of the rat (thiopental 100 mg/kg intraperitoneally), a median thoracotomy was performed. The heart was excised, the aorta was cannulated, and the heart was perfused under a Langendorff column, allowing a retrograde perfusion of the heart for 5 min by a Ca^{2+}-free solution, followed by the infusion of the same buffer, to which 1.2 mg/ml collagenase A (Bohringer-Manheim, Meylan, France) was added.

After a 45-min perfusion period, the heart was taken from the column and cut into small (1-mm) pieces in the same Ca^{2+}-free buffer. Cells were filtered and washed, and Ca^{2+} (1.2 mM) was reintroduced. At the end of this procedure, approximately 80% of myocytes showed normal architecture, were quiescent, and could be electrically stimulated. Cardiomyocytes were then resuspended in a culture medium (BM86 Wisler; IMV Lab, L'Aigle, France) and placed on Petri dishes previously coated with laminin (30 μg/ml). Cells were incubated for 3 h at 37°C in the presence of 5% carbon dioxide on these dishes. At the end of the period, cells adhered to the bottom of the dish, and the culture medium was changed. Cells were used 12 h later for the study.

Loading with Indo-1 AM. Before each experiment, the cells were preloaded for 15 min at room temperature with Indo-1 AM by incubation in 200 μl culture medium containing 10 μg Indo-1 AM, 180 μl bovine serum albumin, and 5 μl dimethylsulfoxide with 5 μg pluronic acid. The cells were then washed and maintained at room temperature for 40 min in the culture medium before the fluorescence measurements. The nutrient medium was replaced with a Krebs solution containing 117 mM NaCl, 5.7 mM KCl, 1.2 mM KH_2PO_4, 1.7 mM $MgCl_2$, 4.4 mM $NaHCO_3$, 1.25 mM $CaCl_2$, 10 mM glucose, 10 mM HEPES, 10 mM creatine, and 20 mM taurine and was buffered to pH 7.4.

Fluorescence Measurements, Compound Application, and Electrical Stimulation. Cytosolic Ca^{2+} measurements were performed by dual-emission microfluorometry (Hamamatsu, Massy, France) with the Indo-1 AM probe, as previously described.[15] Cells loaded with the fluorescent probe were excited at 360 nm. Emission spectra were divided in two halves using interference filters at 405 and 480 nm. The fluorescence ratio 405:480 nm, which is independent of the probe concentration, was calculated directly from the two signals.

Electrical pacing was performed using a Harvard stimulator (Harvard, Les Ulis, France) delivering 40-V stimuli of a duration of 10 ms at a frequency of 1 Hz. Cells were perfused continuously at a rate of 2 ml/min by the Krebs solution at room temperature. The solution was bubbled previously by a gas mixture (95% oxygen, 5% carbon dioxide). We verified previously that if this procedure is used, pH is not modified for at least 2 h.

Experimental Protocol for Fluorescence Measurements. Each cell isolation allowed the seeding of approximately 30 Petri dishes. One to three cells could be observed in each field of the microscope. Data were recorded for 8.52 s (72 image acquisitions separated by 120-ms intervals). We verified previously that this procedure did not produce a significant attenuation of the measured peak of the Ca^{2+} transient by comparing it with that obtained at the maximal rate of acquisition of the system (50 Hz; that is, 72 points separated by 20-ms intervals). In the time interval separating two recording periods, the electrical stimulation was interrupted and the pacing was initiated after 2 s of recording (fig. 1). After recording of the control state, the bath inside the Petri dish was aspirated and replaced by the solution of the drug to be tested, which was continuously infused for 5 min. Each Petri dish was used for either propofol or its solvent, 10% Intralipid (Kabi Pharmacia, Noisy le Grand, France) at cumulative drug concentrations of 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M. Intralipid contained 100 g purified soybean oil, 12 g purified egg phospholipids, 22.5 g glycerol USP, and 1,000 ml water. We called 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M doses of Intralipid the concentrations of Intralipid corresponding to those used for 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M doses of propofol. Drugs (propofol, 10% Intralipid) were infused in random order. A similar protocol using Krebs solution was also performed to verify the effect of repeated measurements on cytosolic Ca^{2+} concentration. Lastly, it was verified that propofol and its solvent did not interfere with Indo-1 fluorescence. Doses of 10^{-4} M of propofol, or Intralipid at corresponding doses, did not induce significant change in autofluorescence levels compared with those measured in the Krebs solution.

Data Analysis. The mean ratio image of each cell was calculated by the average of all points included in the area of the cell. In each cell, three to five areas that included approximately 2,500 pixels were selected. The transformation of 405:480 ratio values into Ca^{2+} concentrations takes into account dissociation constant (Kd) values and concentrations of Ca^{2+} but also those of other ions, particularly H^+. Because ion concentrations are different in different parts of the cells and in different

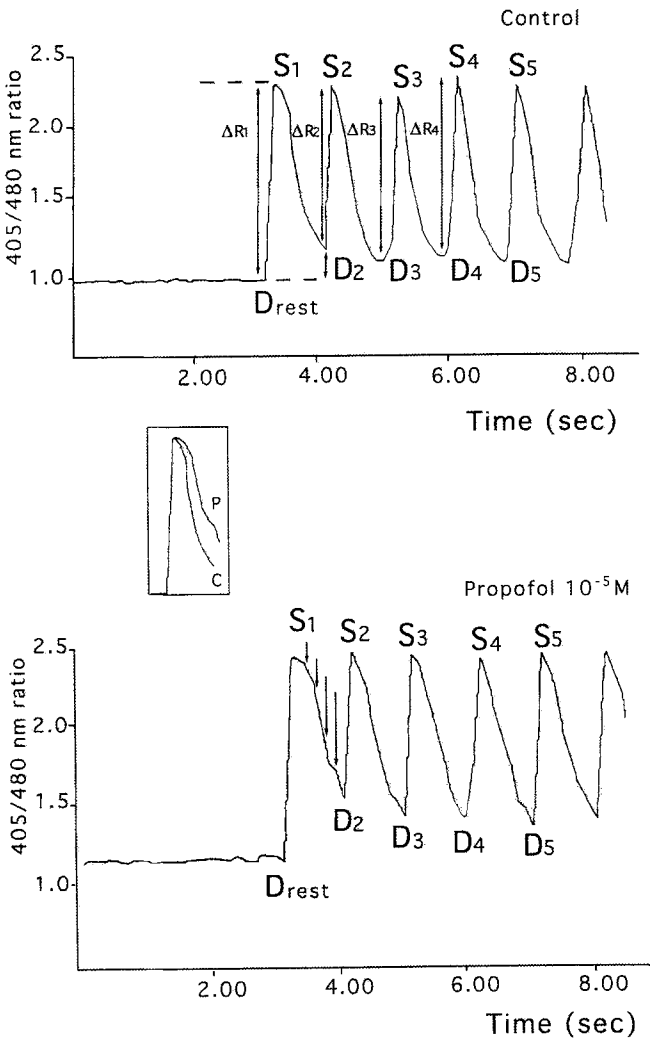

Fig. 1. Typical recordings of intracellular Ca^{2+} measurements in one cell obtained by the 405:480-nm wavelength ratio during control conditions (*top*) and after an infusion of 10^{-5} M propofol (*bottom*). Different 405:480-nm wavelength ratios are defined: the diastolic ratio, the systolic ratio, and the Ca^{2+} transient (Δ ratio), which is the difference between the systolic and the diastolic value of the 405:480 nm ratio. (*Top*) D_{rest} = diastolic ratio after a long pause; ΔR1 = Δ ratio of the first beat after a long pause, D_{2-5} and ΔR_{2-5} = diastolic ratio and Δ ratio of beats 2 through 5. (*Bottom*) Analysis of the slope of the decrease of intracellular Ca^{2+}. This analysis was performed by measurement of the 405:480 ratio 120, 240, 360, and 480 ms after the peak of systolic Ca^{2+}, as indicated by the arrows showing the corresponding time points in the S_1 beat. (*Insert*) The superimposition of S_1 transients obtained during control condition (C) and during propofol infusion (P), showing a slower decrease in Ca^{2+} transient after its peak during propofol infusion.

conditions, the use of a fixed formula to obtain absolute values of Ca^{2+} concentration may be misleading. Therefore, rather than trying to express cellular Ca^{2+} concentration in absolute values, we preferred to present only the 405:480 ratio values as indices of Ca^{2+} concentration.

If different cells, obtained from the same cell isolation, had the same drug infusion, the mean value for each measurement was calculated so that each cell isolation gave only one value for each intervention. Several time points were measured (fig. 1), including the end-diastolic ratio (at the bottom of the Ca^{2+} rising) after at least 2 min of pacing interruption (D_{rest}) and the peak of the Ca^{2+} ratio (systolic Ca^{2+}, or S_1). The end-diastolic and end-systolic Ca^{2+} ratios after the first stimulation were called D_2 through D_5 and S_2 through S_5, respectively. The Ca^{2+} transient (or Δ ratio) was defined as the difference between the systolic Ca^{2+} ratio and the end-diastolic Ca^{2+} ratio (systolic-diastolic ratio). We determined (fig. 1) the difference between D_2 and D_{rest}, which gives information about the ability of the cell to bring back diastolic Ca^{2+} to normal resting values after a short pause and thus tests the SR pump or the Na^+-Ca^{2+} exchanger activity. The time course of the decrease of the Ca^{2+} ratio after the peak of Ca^{2+} transient was measured every 120 ms, corresponding to the sampling rate for image acquisitions (fig. 1). Four time points were measured during each Ca^{2+} decrease, allowing an adequate analysis. The mean value of the Ca^{2+} ratio at 480 ms, (corresponding to a Ca^{2+} value close to resting Ca^{2+}) was compared in a control situation and during propofol infusion (10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M). The same analysis was performed for 10% Intralipid.

Biochemical Study of the Calcium Uptake

For biochemical experiments, rats were killed by heart excision after administration of the same anesthesia as used for cell isolation.

We studied the function of the SR Ca^{2+} adenosine triphosphatase by measuring the rate of oxalate-stimulated Ca^{2+} uptake. Crude homogenates were used because only a minor fraction of SR could be isolated by standard procedures and, moreover, marked loss of activity has been reported on isolated vesicles of SR.[16,17]

Ca^{2+} uptake was measured, according to De la Bastie *et al.*,[18] at 30°C in 0.5 ml of a medium containing 100 mM KCl, 5 mM adenosine triphosphate, 6 mM $MgCl_2$, 15 mM K-oxalate, 0.2 mM EGTA, 30 mM Tris-HCl buffer (pH = 6.85), and 0.15 mM $^{45}CaCl_2$, giving a free Ca^{2+} concentration of 1.25 μM as determined using the Fabiato pro-

Table 1. Effects of Propofol, 10% Intralipid, and Krebs Solution on Diastolic 405:480 Ratio, Systolic 405:480 Ratio and Ca^{2+} Transient (Δ Ratio)

	D_{rest}	Δ Ratio 1	D_2	Δ Ratio 2	D_3	Δ Ratio 3	D_4	Δ Ratio 4
Propofol								
control	1.1 ± 0.11	0.96 ± 0.22	1.22 ± 0.08	0.76 ± 0.19	1.15 ± 0.11	0.83 ± 0.19	1.14 ± 0.11	0.83 ± 0.25
10^{-7} M	1.12 ± 0.14	0.95 ± 0.25	1.25 ± 0.14	0.78 ± 0.19	1.2 ± 0.14	0.78 ± 0.28	1.19 ± 0.14	0.76 ± 0.28
10^{-6} M	1.16 ± 0.11	0.99 ± 0.25	1.33 ± 0.11	0.74 ± 0.09	1.26 ± 0.08	0.81 ± 0.22	1.25 ± 0.08	0.74 ± 0.31
10^{-5} M	1.24 ± 0.11*	0.92 ± 0.17	1.49 ± 0.19*	0.57 ± 0.25*	1.38 ± 0.17*	0.68 ± 0.25	1.38 ± 0.14	0.68 ± 0.19
Intralipid								
control	1.08 ± 0.11	0.86 ± 0.31	1.14 ± 0.14	0.73 ± 0.31	1.1 ± 0.11	0.75 ± 0.11	1.11 ± 0.11	0.75 ± 0.25
10^{-7} M	1.09 ± 0.11	0.78 ± 0.22	1.18 ± 0.14	0.64 ± 0.19	1.13 ± 0.14	0.69 ± 0.25	1.15 ± 0.11	0.7 ± 0.19
10^{-6} M	1.11 ± 0.11	0.83 ± 0.22	1.25 ± 0.17	0.66 ± 0.28	1.2 ± 0.17	0.66 ± 0.28	1.18 ± 0.14	0.68 ± 0.25
10^{-5} M	1.18 ± 0.11*	0.81 ± 0.22	1.32 ± 0.17	0.62 ± 0.25	1.23 ± 0.11*	0.7 ± 0.22	1.25 ± 0.14	0.72 ± 0.19
Krebs								
T1	1.1 ± 0.08	0.78 ± 0.15	1.19 ± 0.13	0.61 ± 0.15	1.15 ± 0.1	0.66 ± 0.15	1.15 ± 0.1	0.69 ± 0.18
T2	1.12 ± 0.1	0.83 ± 0.26	1.18 ± 0.13	0.7 ± 0.21	1.17 ± 0.13	0.71 ± 0.23	1.16 ± 0.1	0.68 ± 0.4
T3	1.13 ± 0.08	0.82 ± 0.26	1.22 ± 0.1	0.68 ± 0.21	1.19 ± 0.1	0.66 ± 0.23	1.18 ± 0.08	0.67 ± 0.29
T4	1.19 ± 0.1	0.75 ± 0.26	1.3 ± 0.1	0.58 ± 0.26	1.26 ± 0.15	0.62 ± 0.23	1.25 ± 0.13	0.6 ± 0.21

D_{rest} = diastolic 405:480 ratio after a long (2- to 3- min) rest; Δ Ratio 1 = difference between the diastolic and the systolic 405:480 ratio of the first beat after a long rest; D_{2-4} and Δ ratio 2–4 = diastolic 405:480 ratios and Δ ratios of beats 2–4, as defined in Figure 1.

* $P < 0.05$, as compared with control value.

T1, T2, T3, T4 represent the values of four successive recordings during Krebs solution infusion. Values are the mean ± 1 SD.

gram.[19] Na-azide (5 mM) also was added to inhibit mitochondrial Ca^{2+} uptake. The reaction was initiated by adding 20–50 μg total protein. After 4 min, the reaction was stopped by rapid dilution of the incubation mixture with a cold solution containing 100 mM KCl, 1 mM EGTA, and 10 mM histidine (pH = 6.4), followed by rapid filtration under vacuum through glass-fiber filters and radioactivity was counted in an LS 6000 SE Beckman scintillation counter (Beckman, Gagny, France).

For each animal, the value of Ca^{2+} transport, expressed in nanomoles per minute per milligram of protein, was the mean value of two determinations.

Statistical Analysis

Results are presented as the mean (SD). The significant dose-dependent effect of a drug (or time for Krebs infusion) in the same cell, used as its own control, was determined using one-way analysis of variance. If different groups of cells were compared (comparison of the effect of two drugs at different concentrations), two-way analysis of variance was performed. If the results of the analysis of variance were significant, multiple comparisons were performed using a *post hoc* Scheffé test. For the 10^{-4} M doses of propofol, the 405:480 ratios could not be obtained in all cells and were analyzed separately. A P value < 0.05 was necessary to reject the null hypothesis.

Results

Inotropic Effects of Propofol and 10% Intralipid

The Ca^{2+} transient measurements in the dose-response experiments with propofol (n = 8), 10% Intralipid (n = 8), and Krebs solution (n = 7) are summarized in table 1. There was no change in the diastolic 405:480 ratio during Krebs infusion, for the 10^{-7} and 10^{-6} M doses of propofol and for corresponding doses of 10% Intralipid. The 10^{-5} M infusion of propofol increased the diastolic 405:480 ratios recorded at D_{rest}, D_2, D_3, and D_4. The 10^{-5} M corresponding dose of 10% Intralipid also increased these diastolic ratios, but to a lower extent than propofol.

Neither Krebs solution nor 10% Intralipid at any concentration, nor propofol in doses less than 10^{-5} M significantly changed the Δ ratios. In contrast, a 10^{-5} M infusion of propofol induced a significant decrease in Ca^{2+} transient as assessed by the Ca^{2+} Δ ratios recorded at S_2, S_3, and S_4, but not at S_1 (table 1, fig. 2).

In a group of seven cells analyzed separately, the 10^{-4}-M dose of propofol significantly ($P < 0.05$) decreased, by 38%, the Ca^{2+} transient (Δ ratio) recorded at S_1 (from 0.88 ± 0.22 to 0.54 ± 0.15) and decreased, by 56%, the Ca^{2+} transient recorded at S_2 (from 0.76 ± 0.15 to 0.34 ± 0.18). This effect was significantly different from that produced either by 10% Intralipid or by the

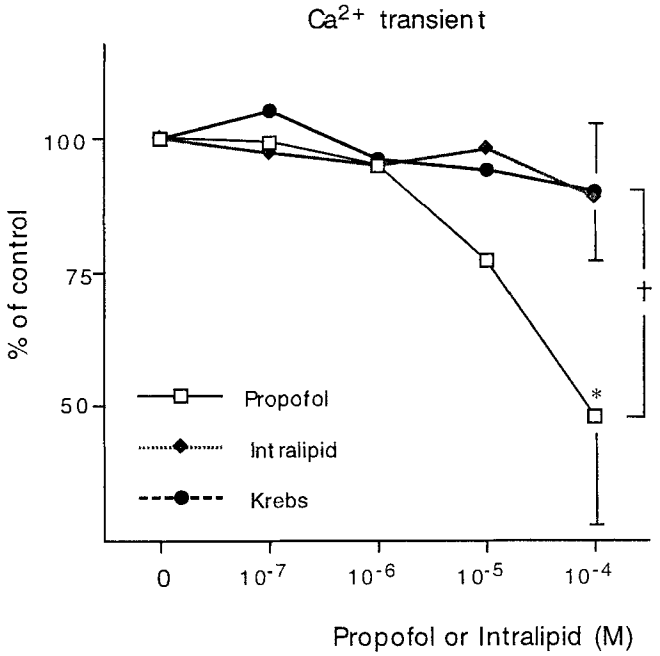

Fig. 2. Effects of propofol (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M), 10% Intralipid at corresponding doses, and Krebs solution on Ca^{2+} transients (Δ ratios) recorded during systole 2 (S_2), as defined in figure 1 and expressed as percentage of the control value. Results are given as the mean ± 1 SD. Comparison with the control value: *P < 0.05. Comparison of propofol with Krebs and the corresponding 10% Intralipid: †P < 0.05.

Krebs solution. The changes in the Δ ratio in the S_2 beat are presented in figure 2.

Microfluorometric Study of Intracellular Ca^{2+} Behavior in Diastole

The effects of propofol compared with those of its solvent, 10% Intralipid, are shown in figures 3 and 4.

Figure 3 shows that propofol (upper) but not 10% Intralipid infusion (lower) induced a significant (P < 0.05) dose-dependent slowing of the slope of the decrease of the 405:480 ratio, measured after the systolic peak after a long rest.

The 10^{-4} M dose of propofol and the corresponding dose of 10% Intralipid are not shown in figure 3 because the peak value for this dose of propofol was lower than the control value (2.0 ± 0.18 *vs.* 2.15 ± 0.2 for the Krebs-solution value). The slope of the decrease was even slower than the 10^{-5} M dose: The mean decrease in the 405:480 ratio 480 ms after the peak was 0.18, compared with 0.69 during control before propofol infusion.

As shown in figure 1 in a typical example and in figure 4, cumulative concentrations (10^{-7}, 10^{-6}, 10^{-5} M) of propofol increased the difference $D_2 - D_{rest}$. A 10^{-5} M concentration of propofol increased the difference $D_2 - D_{rest}$ (+0.13, $P < 0.05$) compared with the control value. Krebs solution or 10% Intralipid did not change the difference $D_2 - D_{rest}$ at any concentration.

Effects of Propofol and Intralipid on the Ca^{2+} Uptake

The effects of propofol and 10% Intralipid on Ca^{2+} transport are presented in figure 5.

Ca^{2+} uptake was not modified by propofol between 10^{-7} and 10^{-5} M. A 10^{-4} M concentration of propofol significantly decreased the rate of Ca^{2+} uptake, from

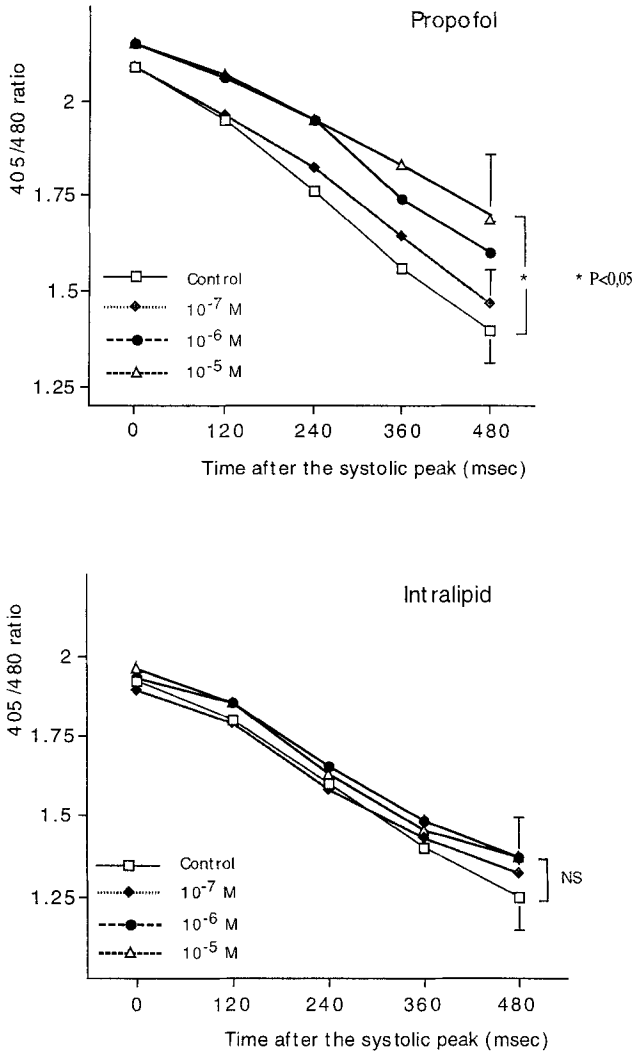

Fig. 3. Effects of propofol (*top*) and 10% Intralipid (*bottom*) on the decrease of the 405:480 ratio 480 ms after the peak of Ca^{2+} transient in the first beat after a long rest (2 to 3 min). Results are given as the mean ± 1 SD. *P < 0.05 with control value.

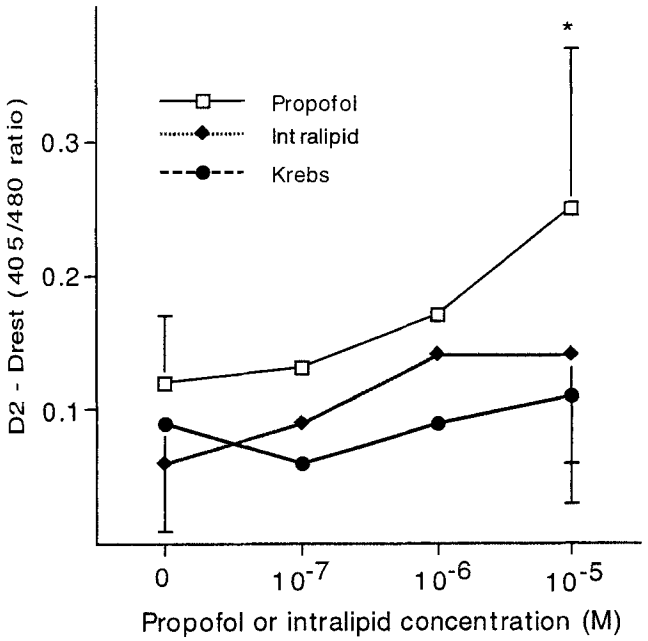

Fig. 4. Effects of propofol, 10% Intralipid, and Krebs solution on the absolute diastolic difference $D_2 - D_{rest}$. Results are given as the mean ± 1 SD. *$P < 0.05$ with control value.

11.8 ± 1.5 protein to 8.3 ± 2.6 nmol · min^{-1} · mg^{-1} protein (70 ± 5% of the control value). In contrast, 10% Intralipid did not induce changes in Ca^{2+} transport at any concentration.

Discussion

The principal results of this study may be summarized as follows:

1. Large concentrations of propofol decreased the slope of the decrease of the intracellular Ca^{2+} after its systolic peak.
2. This effect produced a diastolic Ca^{2+} elevation, after a short diastolic interval, compared with the Ca^{2+} concentration at rest.
3. Propofol (10^{-4} M) induced a decrease in Ca^{2+} uptake capacity of the SR, which was probably responsible for these changes.
4. Propofol also decreased the Ca^{2+} transient. This effect appeared for lower doses (10^{-5} M) after short diastolic pauses rather than after a long (2- to 3-min) rest (appearing at 10^{-4} M).

A propofol-induced impairment in Ca^{2+} uptake capacity of the SR was suggested previously by an alteration of mechanical diastolic performance of left ventricular papillary muscles of rats.[4] More recently, a prolonged time to 50% recovery for intracellular Ca^{2+} and cell relengthening was observed on rat ventricular myocytes in response to supraclinical concentration of propofol, indicating a possible action of the drug on Ca^{2+} handling by the SR.[8]

However, to our knowledge, the direct effect of propofol on SR Ca^{2+} uptake never has been evaluated. Our results clearly show a rate-dependent alteration of the Ca^{2+} transient and, in rat ventricular homogenates, a decrease of the rate of Ca^{2+} uptake induced by propofol. There was a slight and nonsignificant tendency toward a decrease with a 10^{-5} M dose, but the decrease induced by a 10^{-4} M dose was significant (30%). This effect was not caused by the solvent because Intralipid did not induce any change in SR Ca^{2+} uptake (fig. 5). Therefore, the current study shows that, as was suggested indirectly,[4,8] SR function is indeed impaired by propofol.

The reduction in the slope of the systolic Ca^{2+} decrease (fig. 3) may be caused by several different factors.

1. An increase in myofibrillar affinity for Ca^{2+} could produce a slower myofibrillar Ca^{2+} release during relaxation, as suggested by Kanaya *et al.*[8]
2. There may be a direct effect of propofol on the Na^{+}-Ca^{2+} exchanger because cytosolic Ca^{2+} decreased during relaxation results from two different

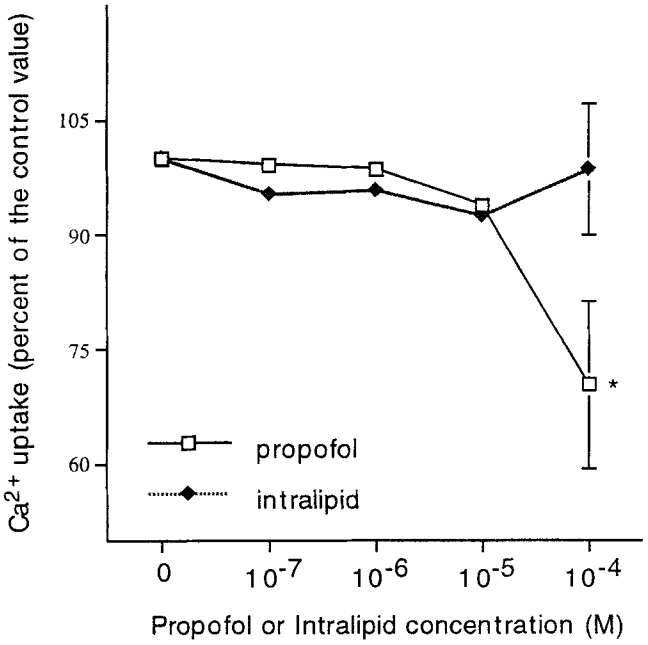

Fig. 5. Effect on Ca^{2+} uptake of propofol (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M) and 10% Intralipid at corresponding doses. Results are given as the mean ± 1 SD. *$P < 0.05$ with control value.

mechanisms: Ca^{2+} extrusion out of the cell through the Na^{+}-Ca^{2+} exchanger, and Ca^{2+} reuptake by the SR. These two mechanisms are balanced and may have variable relative importance according to the experimental conditions.[20] Although a propofol-induced alteration of the Na^{+}-Ca^{2+} exchanger cannot be excluded in our study, such an effect has never been shown.

3. There may be a reduction in SR uptake capacity. This was found in our study and appears to be the major mechanism inducing the reduction in the slope of systolic Ca^{2+} decrease during systole.

The slower SR Ca^{2+} uptake is probably responsible for the increase in diastolic Ca^{2+} values observed after a short diastolic pause (the difference $D_2 - D_{rest}$ in fig. 4). A slower Ca^{2+} uptake does not have any influence on the level of diastolic Ca^{2+} measured after a long pause (D_{rest}) but produces a larger D_2 value if the pause is short (1 s), not long enough for Ca^{2+} to reach the basal value. The long duration of the diastolic pause (4 s) in the study of Cook and Housmans[7] may explain why no SR abnormality was found. In that study,[7] ryanodine injection was used to evaluate SR contribution to muscle contraction with and without propofol. The long rest (2 to 3 min) probably allowed the SR stores to be refilled with an unchanged releasable Ca^{2+}, even in case of impaired Ca^{2+} uptake.

The reduced Ca^{2+} uptake induced by propofol probably also plays a role in the decrease in myocyte contractile function. The existence of a negative inotropic effect of the drug depends on species and pathophysiologic status. In isolated muscles, no negative inotropic effect[6,21] or decreased contractility[3,7] were found. In the current study, if the beat after a long pause was considered, a decrease in the Ca^{2+} transient was observed only with the 10^{-4}-M dose of propofol. After a long pause, SR loading is not altered if Ca^{2+} uptake is slowed. It is thus likely that, in this case, the decrease in Ca^{2+} transient can be attributed to a decreased trans-sarcolemmic Ca^{2+} entry, which impairs myocyte contractility. A propofol-induced decreased contractility already has been attributed to a reduced trans-sarcolemmic Ca^{2+} entry,[3,7] especially by Ca^{2+} L-type current inhibition.[10,11] If the beat after a short pause (S_2, table 1) was considered, the decrease in Ca^{2+} transient appeared for smaller doses of propofol (10^{-5} M). It is likely that the increased diastolic Ca^{2+} in this beat, associated with an incomplete filling of the Ca^{2+} release stores, was responsible for the decreased Ca^{2+} transient.

A possible limitation of the current study was that microfluorometric and biochemical studies were performed at different temperatures (22° and 30°C respectively). Obviously, the temperature at which experiments are performed may influence cellular Ca^{2+} handling, and this may modify the results. We chose these procedures because pacing isolated cells at physiologic temperature may lead to an imbalance between energy supply and demand, and physiologic experiments in isolated cells usually are performed at room temperature. Ca^{2+} uptake experiments have been performed at 30°C because this method was validated previously[18] showing that Ca^{2+} transport in the presence of oxalate is linear for several minutes at this temperature. Another potential limitation of the study is that cardiomyocytes contracted without load (preload and afterload). Changes in loading conditions affect myofilament affinity for Ca^{2+} (high load) and relaxation (low load).[22] Experiments with no load performed in this study allowed us to make more apparent relaxation abnormalities caused by SR.

In conclusion, this study showed that propofol decreases SR Ca^{2+} uptake, inducing a slower decrease in systolic Ca^{2+} after the peak, and, for relatively short diastolic pauses, this effect produces a diastolic increase in Ca^{2+} at rest and a decrease in Ca^{2+} transient. Clinical implications of this study must be drawn with caution because it is always difficult to extrapolate results obtained *in vitro* in another species to humans. However, this study shows that propofol impairs SR function, with potential functional consequences. Although SR functional importance is lesser in humans than in rats, it represents at least 50% of the origin of systolic Ca^{2+}[23] and is therefore not negligible for cardiac contraction. The doses of propofol that produced a negative inotropic effect in the study are larger than those used in clinical practice. Furthermore, propofol is highly bound (approximately 98%) to proteins *in vivo*, so that only a minor fraction of the drug is pharmacologically active if injected intravenously. As discussed by Hebbar *et al.*,[5] concentrations in experimental solution may not reflect effective plasma concentration because propofol binding to proteins, lipid microsomes, and tissue is not taken into account *in vitro*. However, the free-drug concentration obtained during a bolus injection may locally reach 5.10^{-5} M,[24] close to the dose for which we show a depressant effect on Ca^{2+} transient. In addition, if SR function is impaired, propofol clearly may be detrimental. A decreased SR function probably exists during heart failure, although this is still being debated.[25] Indeed,

propofol and Ca^{2+} uptake could contribute to a more potent negative inotropic effect during heart failure compared with the normal state, particularly if blockade of Ca^{2+} entry also contributes.

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