

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

Thierry Guenoun, M.D.,\* Olivier Montagne, M.D.,† Monique Laplace,‡ Bertrand Crozatier, M.D., Ph.D.§

**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  $\text{Ca}^{2+}$  accumulation and cardiomyocytes.

**Methods:** The effects of propofol on intracellular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  uptake from homogenates of rat ventricles.

**Results:** The  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  transient, which resulted in an increase of diastolic  $\text{Ca}^{2+}$  when the diastolic interval was decreased. The increased diastolic  $\text{Ca}^{2+}$  also resulted in a decrease in  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  uptake capacity impairment of the sarcoplasmic reticulum. This is probably responsible for a slowing of the decrease of the  $\text{Ca}^{2+}$  transient, which in turn increases the diastolic  $\text{Ca}^{2+}$  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,<sup>1,2</sup> depending on the species (negative inotropic effect in guinea pigs<sup>3</sup> and no

effects in rats<sup>4</sup>). The depressant myocardial effects of propofol also are greater during heart failure than during the normal state,<sup>5</sup> although the negative inotropic effect was not greater in hamsters with hypertrophic cardiomyopathy than in normal hamsters.<sup>6</sup>

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

The goal of our study was to evaluate whether cellular  $\text{Ca}^{2+}$  homeostasis was impaired by propofol. The  $\text{Ca}^{2+}$  transient of isolated cardiomyocytes was studied in rats because the SR plays a major role in the excitation-contraction coupling.<sup>13</sup>

## 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

\* Assistant Professor, Department of Anesthesiology.

† Assistant Professor, Department of Internal Medicine.

‡ Research Technician INSERM U400.

§ Research Director INSERM U400.

Received from Unité Institut National de la Santé et de la Recherche Médicale (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 Santé et de la Recherche Médicale (INSERM), Paris, France. Dr. Guenoun was the recipient of a fellowship grant from Zeneca, Cergy, France.

Address reprint requests to Dr. Guenoun: Département d'Anesthésie-Réanimation Chirurgicale, Hôpital Laennec, 42, rue de Sèvres, Paris, France. Address electronic mail to: tguenoun@invivo.edu

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,<sup>14</sup> with slight modifications.<sup>15</sup> 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  $\text{Ca}^{2+}$ -free solution, followed by the infusion of the same buffer, to which 1.2 mg/ml collagenase A (Boehringer-Mannheim, 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  $\text{Ca}^{2+}$ -free buffer. Cells were filtered and washed, and  $\text{Ca}^{2+}$  (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  $\mu\text{g}/\text{ml}$ ). 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  $\mu\text{l}$  culture medium containing 10  $\mu\text{g}$  Indo-1 AM, 180  $\mu\text{l}$  bovine serum albumin, and 5  $\mu\text{l}$  dimethylsulfoxide with 5  $\mu\text{g}$  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  $\text{KH}_2\text{PO}_4$ , 1.7 mM  $\text{MgCl}_2$ , 4.4 mM  $\text{NaHCO}_3$ , 1.25 mM  $\text{CaCl}_2$ , 10 mM glucose, 10 mM HEPES, 10 mM creatine, and 20 mM taurine and was buffered to pH 7.4.

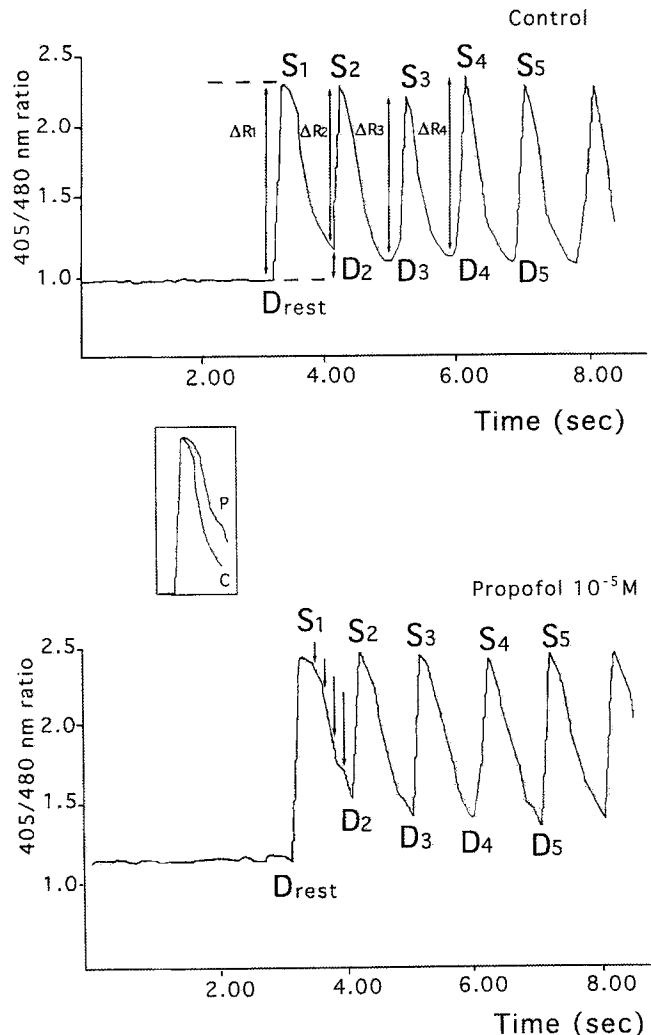
**Fluorescence Measurements, Compound Application, and Electrical Stimulation.** Cytosolic  $\text{Ca}^{2+}$  measurements were performed by dual-emission microfluorometry (Hamamatsu, Massy, France) with the Indo-1 AM probe, as previously described.<sup>15</sup> Cells loaded with the fluorescent probe 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 stim-

ulator (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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  concentrations takes into account dissociation constant ( $K_d$ ) values and concentrations of  $\text{Ca}^{2+}$  but also those of other ions, particularly  $\text{H}^+$ . Because ion concentrations are different in different parts of the cells and in different



**Fig. 1.** Typical recordings of intracellular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  transient ( $\Delta$  ratio), which is the difference between the systolic and the diastolic value of the 405:480 nm ratio. (*Top*)  $D_{\text{rest}}$  = diastolic ratio after a long pause;  $\Delta R_1 = \Delta$  ratio of the first beat after a long pause,  $D_{2-5}$  and  $\Delta R_{2-5}$  = diastolic ratio and  $\Delta$  ratio of beats 2 through 5. (*Bottom*) Analysis of the slope of the decrease of intracellular  $\text{Ca}^{2+}$ . This analysis was performed by measurement of the 405:480 ratio 120, 240, 360, and 480 ms after the peak of systolic  $\text{Ca}^{2+}$ , 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  $\text{Ca}^{2+}$  transient after its peak during propofol infusion.

conditions, the use of a fixed formula to obtain absolute values of  $\text{Ca}^{2+}$  concentration may be misleading. Therefore, rather than trying to express cellular  $\text{Ca}^{2+}$  concentration in absolute values, we preferred to present only the 405:480 ratio values as indices of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  rising) after at least 2 min of pacing interruption ( $D_{\text{rest}}$ ) and the peak of the  $\text{Ca}^{2+}$  ratio (systolic  $\text{Ca}^{2+}$ , or  $S_1$ ). The end-diastolic and end-systolic  $\text{Ca}^{2+}$  ratios after the first stimulation were called  $D_2$  through  $D_5$  and  $S_2$  through  $S_5$ , respectively. The  $\text{Ca}^{2+}$  transient (or  $\Delta$  ratio) was defined as the difference between the systolic  $\text{Ca}^{2+}$  ratio and the end-diastolic  $\text{Ca}^{2+}$  ratio (systolic-diastolic ratio). We determined (fig. 1) the difference between  $D_2$  and  $D_{\text{rest}}$ , which gives information about the ability of the cell to bring back diastolic  $\text{Ca}^{2+}$  to normal resting values after a short pause and thus tests the SR pump or the  $\text{Na}^+-\text{Ca}^{2+}$  exchanger activity. The time course of the decrease of the  $\text{Ca}^{2+}$  ratio after the peak of  $\text{Ca}^{2+}$  transient was measured every 120 ms, corresponding to the sampling rate for image acquisitions (fig. 1). Four time points were measured during each  $\text{Ca}^{2+}$  decrease, allowing an adequate analysis. The mean value of the  $\text{Ca}^{2+}$  ratio at 480 ms, (corresponding to a  $\text{Ca}^{2+}$  value close to resting  $\text{Ca}^{2+}$ ) 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  $\text{Ca}^{2+}$  adenosine triphosphatase by measuring the rate of oxalate-stimulated  $\text{Ca}^{2+}$  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.<sup>16,17</sup>

$\text{Ca}^{2+}$  uptake was measured, according to De la Bastie *et al.*,<sup>18</sup> at 30°C in 0.5 ml of a medium containing 100 mM KCl, 5 mM adenosine triphosphate, 6 mM  $\text{MgCl}_2$ , 15 mM K-oxalate, 0.2 mM EGTA, 30 mM Tris-HCl buffer (pH = 6.85), and 0.15 mM  $^{45}\text{CaCl}_2$ , giving a free  $\text{Ca}^{2+}$  concentration of 1.25  $\mu\text{M}$  as determined using the Fabiato pro-

## PROPOFOL AND SARCOPLASMIC RETICULUM FUNCTION

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

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

$D_{\text{rest}}$  = diastolic 405:480 ratio after a long (2- to 3- min) rest;  $\Delta$  Ratio 1 = difference between the diastolic and the systolic 405:480 ratio of the first beat after a long rest;  $D_{2-4}$  and  $\Delta$  ratio 2-4 = diastolic 405:480 ratios and  $\Delta$  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  $\pm$  1 SD.

gram.<sup>19</sup> Na-azide (5 mM) also was added to inhibit mitochondrial  $\text{Ca}^{2+}$  uptake. The reaction was initiated by adding 20–50  $\mu\text{g}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $D_{\text{rest}}$ ,  $D_2$ ,  $D_3$ , 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  $\Delta$  ratios. In contrast, a  $10^{-5}$  M infusion of propofol induced a significant decrease in  $\text{Ca}^{2+}$  transient as assessed by the  $\text{Ca}^{2+}$   $\Delta$  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  $\text{Ca}^{2+}$  transient ( $\Delta$  ratio) recorded at  $S_1$  (from  $0.88 \pm 0.22$  to  $0.54 \pm 0.15$ ) and decreased, by 56%, the  $\text{Ca}^{2+}$  transient recorded at  $S_2$  (from  $0.76 \pm 0.15$  to  $0.34 \pm 0.18$ ). This effect was significantly different from that produced either by 10% Intralipid or by the

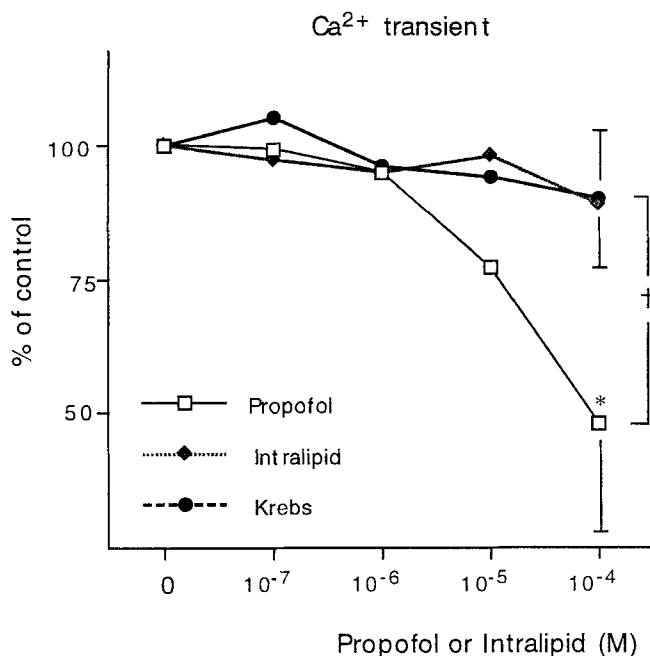


Fig. 2. Effects of propofol ( $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$  M), 10% Intralipid at corresponding doses, and Krebs solution on  $\text{Ca}^{2+}$  transients ( $\Delta$  ratios) recorded during systole 2 ( $S_2$ ), as defined in figure 1 and expressed as percentage of the control value. Results are given as the mean  $\pm$  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  $\Delta$  ratio in the  $S_2$  beat are presented in figure 2.

#### Microfluorometric Study of Intracellular $\text{Ca}^{2+}$ 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 ( $2.0 \pm 0.18$  vs.  $2.15 \pm 0.2$  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  $D_2 - D_{\text{rest}}$ . A  $10^{-5}$  M

concentration of propofol increased the difference  $D_2 - D_{\text{rest}}$  ( $+0.13$ ,  $P < 0.05$ ) compared with the control value. Krebs solution or 10% Intralipid did not change the difference  $D_2 - D_{\text{rest}}$  at any concentration.

#### Effects of Propofol and Intralipid on the $\text{Ca}^{2+}$ Uptake

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

$\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  uptake, from

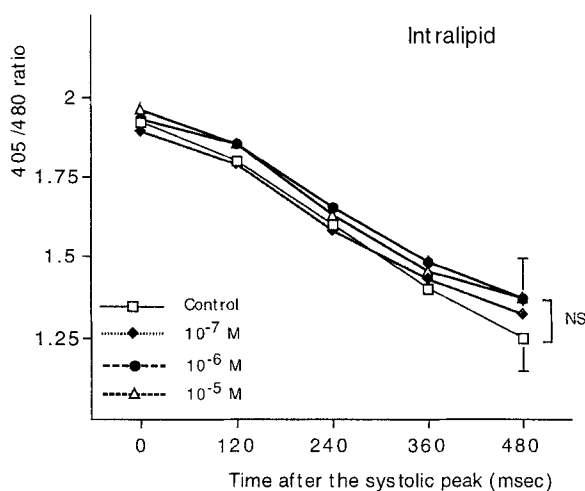
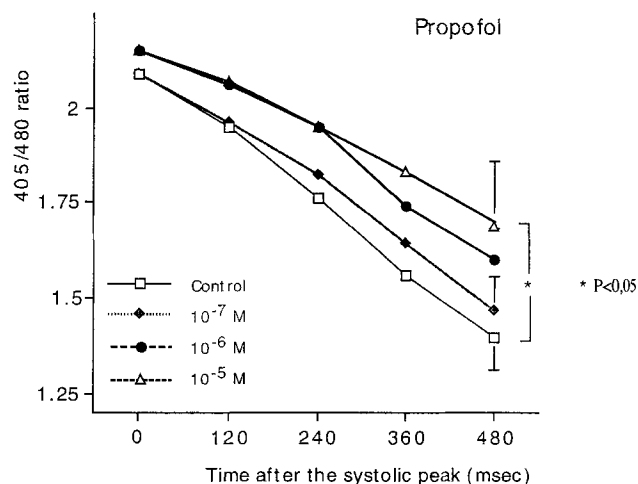


Fig. 3. Effects of propofol (top) and 10% Intralipid (bottom) on the decrease of the 405:480 ratio 480 ms after the peak of  $\text{Ca}^{2+}$  transient in the first beat after a long rest (2 to 3 min). Results are given as the mean  $\pm$  1 SD. \* $P < 0.05$  with control value.

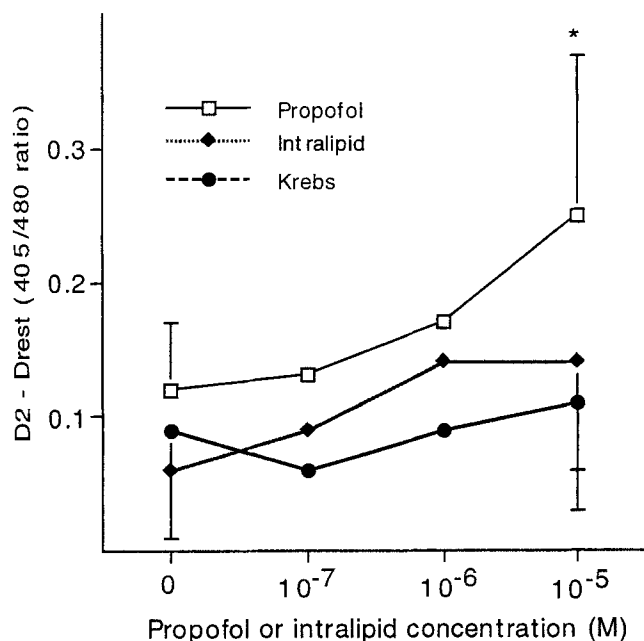


Fig. 4. Effects of propofol, 10% Intralipid, and Krebs solution on the absolute diastolic difference  $D_2 - D_{rest}$ . Results are given as the mean  $\pm$  1 SD. \* $P < 0.05$  with control value.

$11.8 \pm 1.5$  protein to  $8.3 \pm 2.6$   $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$  protein ( $70 \pm 5\%$  of the control value). In contrast, 10% Intralipid did not induce changes in  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  after its systolic peak.
2. This effect produced a diastolic  $\text{Ca}^{2+}$  elevation, after a short diastolic interval, compared with the  $\text{Ca}^{2+}$  concentration at rest.
3. Propofol ( $10^{-4}$  M) induced a decrease in  $\text{Ca}^{2+}$  uptake capacity of the SR, which was probably responsible for these changes.
4. Propofol also decreased the  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  uptake capacity of the SR was suggested previously by an alteration of mechanical diastolic performance of left ventricular pap-

illary muscles of rats.<sup>4</sup> More recently, a prolonged time to 50% recovery for intracellular  $\text{Ca}^{2+}$  and cell relengthening was observed on rat ventricular myocytes in response to supraclinical concentration of propofol, indicating a possible action of the drug on  $\text{Ca}^{2+}$  handling by the SR.<sup>8</sup>

However, to our knowledge, the direct effect of propofol on SR  $\text{Ca}^{2+}$  uptake never has been evaluated. Our results clearly show a rate-dependent alteration of the  $\text{Ca}^{2+}$  transient and, in rat ventricular homogenates, a decrease of the rate of  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  uptake (fig. 5). Therefore, the current study shows that, as was suggested indirectly,<sup>4,8</sup> SR function is indeed impaired by propofol.

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

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

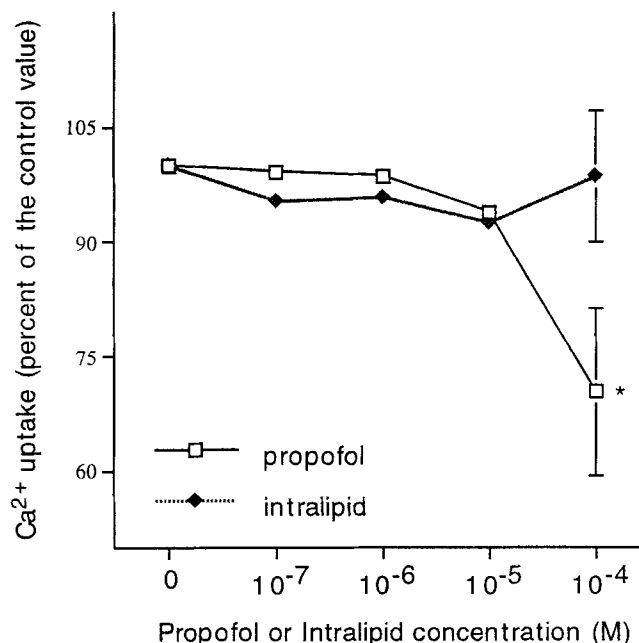


Fig. 5. Effect on  $\text{Ca}^{2+}$  uptake of propofol ( $10^{-7}$ ,  $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$  M) and 10% Intralipid at corresponding doses. Results are given as the mean  $\pm$  1 SD. \* $P < 0.05$  with control value.

mechanisms:  $\text{Ca}^{2+}$  extrusion out of the cell through the  $\text{Na}^+ - \text{Ca}^{2+}$  exchanger, and  $\text{Ca}^{2+}$  reuptake by the SR. These two mechanisms are balanced and may have variable relative importance according to the experimental conditions.<sup>20</sup> Although a propofol-induced alteration of the  $\text{Na}^+ - \text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  decrease during systole.

The slower SR  $\text{Ca}^{2+}$  uptake is probably responsible for the increase in diastolic  $\text{Ca}^{2+}$  values observed after a short diastolic pause (the difference  $D_2 - D_{\text{rest}}$  in fig. 4). A slower  $\text{Ca}^{2+}$  uptake does not have any influence on the level of diastolic  $\text{Ca}^{2+}$  measured after a long pause ( $D_{\text{rest}}$ ) but produces a larger  $D_2$  value if the pause is short (1 s), not long enough for  $\text{Ca}^{2+}$  to reach the basal value. The long duration of the diastolic pause (4 s) in the study of Cook and Housmans<sup>7</sup> may explain why no SR abnormality was found. In that study,<sup>7</sup> 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  $\text{Ca}^{2+}$ , even in case of impaired  $\text{Ca}^{2+}$  uptake.

The reduced  $\text{Ca}^{2+}$  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<sup>6,21</sup> or decreased contractility<sup>3,7</sup> were found. In the current study, if the beat after a long pause was considered, a decrease in the  $\text{Ca}^{2+}$  transient was observed only with the  $10^{-4}$ -M dose of propofol. After a long pause, SR loading is not altered if  $\text{Ca}^{2+}$  uptake is slowed. It is thus likely that, in this case, the decrease in  $\text{Ca}^{2+}$  transient can be attributed to a decreased trans-sarcolemmic  $\text{Ca}^{2+}$  entry, which impairs myocyte contractility. A propofol-induced decreased contractility already has been attributed to a reduced trans-sarcolemmic  $\text{Ca}^{2+}$  entry,<sup>3,7</sup> especially by  $\text{Ca}^{2+}$  L-type current inhibition.<sup>10,11</sup> If the beat after a short pause ( $S_2$ , table 1) was considered, the decrease in  $\text{Ca}^{2+}$  transient appeared for smaller doses of propofol ( $10^{-5}$  M). It is likely that the increased diastolic  $\text{Ca}^{2+}$  in this beat, associated with an incomplete filling of the  $\text{Ca}^{2+}$  release stores, was responsible for the decreased  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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.  $\text{Ca}^{2+}$  uptake experiments have been performed at 30°C because this method was validated previously<sup>18</sup> showing that  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  (high load) and relaxation (low load).<sup>22</sup> 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  $\text{Ca}^{2+}$  uptake, inducing a slower decrease in systolic  $\text{Ca}^{2+}$  after the peak, and, for relatively short diastolic pauses, this effect produces a diastolic increase in  $\text{Ca}^{2+}$  at rest and a decrease in  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ <sup>23</sup> 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 Hebbbar *et al.*,<sup>5</sup> 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  $5 \cdot 10^{-5}$  M,<sup>24</sup> close to the dose for which we show a depressant effect on  $\text{Ca}^{2+}$  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.<sup>25</sup> Indeed,

# PROPOFOL AND SARCOPLASMIC RETICULUM FUNCTION

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

## References

1. Ismail EF, Kim SJ, Salem R, George JC: Direct effects of propofol on myocardial contractility in *in situ* canine hearts. *ANESTHESIOLOGY* 1992; 77:964-72
2. Pagel PS, Warltier CC: Negative inotropic effects of propofol as evaluated by the regional preload recruitable stroke force relationship in chronically instrumented dogs. *ANESTHESIOLOGY* 1993; 78:100-8
3. Park WK, Lynch CI: Propofol and thiopental depression of myocardial contractility: A comparative study of mechanical and electrophysiologic effects in isolated guinea-pig ventricular muscle. *Anesth Analg* 1992; 74:395-405
4. Riou B, Besse S, Lecarpentier Y, Viars P: In vitro effects of propofol on rat myocardium. *ANESTHESIOLOGY* 1992; 76:609-16
5. Hebbar L, Dorman DH, Clair MJ, Roy RC, Spinale FG: Negative and selective effects of propofol on isolated swine myocyte contractile function in pacing-induced congestive heart failure. *ANESTHESIOLOGY* 1997; 86:649-59
6. Riou B, Lejay M, Lecarpentier Y, Viars P: Myocardial effects of propofol in hamsters with hypertrophic cardiomyopathy. *ANESTHESIOLOGY* 1995; 82:566-73
7. Cook DJ, Housmans PR: Mechanism of the negative inotropic effect of propofol in isolated ferret ventricular myocardium. *ANESTHESIOLOGY* 1994; 80:859-71
8. Kanaya N, Murray PA, Damron DS: Propofol and ketamine only inhibit intracellular  $\text{Ca}^{2+}$  transients and contraction in rat ventricular myocytes at supraclinical concentrations. *ANESTHESIOLOGY* 1998; 88:781-91
9. Takahashi H, Puttick RM, Terrar DA: The effects of propofol and enflurane on single calcium channel currents of guinea-pig isolated ventricular myocytes. *Br J Pharmacol* 1994; 111:1147-53
10. Yang CY, Wong CS, Yu CC, Luk HN, Lin CI: Propofol inhibits cardiac L-type calcium current in guinea-pig ventricular myocytes. *ANESTHESIOLOGY* 1996; 84:626-35
11. Zhou W, Fontenot HJ, Liu S, Kennedy RH: Modulation of cardiac calcium channels by propofol. *ANESTHESIOLOGY* 1997; 86:670-5
12. Fabiato A: Calcium release in skinned cardiac cells: variations with species, tissues and development. *Fed Proc* 1982; 41:2238-42
13. Bers DM: Calcium influx and sarcoplasmic reticulum release in cardiac muscle activation during postrest recovery. *Am J Physiol* 1985; 248:H366-81
14. Powell T, Twist VW: A rapid technique for the isolation and purification of adult cardiac muscle cells having respiratory control and a tolerance to calcium. *Biochem Biophys Res Com* 1976; 72:327-33
15. Laplace M, Guenoun T, Montagne O, Roser F, Crozatier B: Cytosolic myocardial calcium modulation by ATP-dependent potassium channel openers and NO donors. *J Cardiovasc Pharmacol* 1999; 33:394-400
16. Feher JJ, Briggs FN, Hess ML: Characterization of cardiac sarcoplasmic reticulum from ischemic myocardium: Comparison of isolated sarcoplasmic reticulum with unfractionated homogenates. *J Mol Cell Cardiol* 1980; 12:427-32
17. Champeil P, Buschlen S, Guillain F: Pressure-induced inactivation of sarcoplasmic reticulum adenosine triphosphatase during high speed centrifugation. *Biochemistry* 1981; 20:1520-4
18. De la Bastie D, Levitsky D, Rappaport L, Mercadier JJ, Marotte F, Wisniewsky C, Brovkovich V, Schwartz K, Lompré AM: Function of the sarcoplasmic reticulum and expression of its  $\text{Ca}^{2+}$ -ATPase gene in pressure overload-induced cardiac hypertrophy in the rat. *Circ Res* 1990; 66:554-64
19. Fabiato A, Fabiato D: Calculator programs for computing the composition of the solution containing multiples metals and ligands used for the experiments in skinned muscle cells. *J Physiol* 1979; 75:463-505
20. Schlant RC, Sonnenblick EH, Katz AM: Normal physiology of the cardiovascular system, Hurst's The Heart. Edited by Alexander RW, Fuster V, New York, Mc Graw-Hill, 1998, pp 81-124
21. Gelissen HP, Epema AH, Henning RH, Krijnen HJ, Hennis PJ, Den Hertog A: Inotropic effects of propofol, thiopental, midazolam, and ketamine on isolated human atrial muscle. *ANESTHESIOLOGY* 1996; 84:397-403
22. Lecarpentier Y, Chuck LH, Housmans PR, De Clerk NM, Brusaert DL: Nature of load dependence of relaxation in cardiac muscle. *Am J Physiol* 1979; 237:45-52
23. Fabiato A, Fabiato F: Calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cells from adult human, dog, cat, rabbit, rat, and frog hearts and newborn rat ventricles. *Ann NY Acad Sci* 1978; 307:491-522
24. Morgan DJ, Campbell GA, Crankshaw DP: Pharmacokinetics of propofol when given by intravenous infusion. *Br J Clin pharmacol* 1990; 30:144-8
25. Lompré AM, Anger M, Levitsky D: Sarcoplasmic reticulum calcium pumps in the cardiovascular system: Function and gene expression. *J Mol Cell Cardiol* 1994; 26:1109-21