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Experimental Conditions Are Important Determinants of Cardiac Inotropic Effects of Propofol

Noriaki Kanaya, M.D., Brad Gable, B.S.,† Peter J. Wickley, B.S.,† Paul A. Murray, Ph.D.,‡ Derek S. Damron, Ph.D.§*

Background: **The rationale for this study is that the depressant effect of propofol on cardiac function *in vitro* is highly variable but may be explained by differences in the temperature and stimulation frequency used for the study. Both temperature and stimulation frequency are known to modulate cellular mechanisms that regulate intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and myofilament Ca^{2+} sensitivity in cardiac muscle. The authors hypothesized that temperature and stimulation frequency play a major role in determining propofol-induced alterations in $[Ca^{2+}]_i$ and contraction in individual, electrically stimulated cardiomyocytes and the function of isolated perfused hearts.**

Methods: **Freshly isolated myocytes were obtained from adult rat hearts, loaded with fura-2, and placed on the stage of an inverted fluorescence microscope in a temperature-regulated bath. $[Ca^{2+}]_i$ and myocyte shortening were simultaneously measured in individual cells at 28° or 37°C at various stimulation frequencies (0.3, 0.5, 1, 2, and 3 Hz) with and without propofol. Langendorff perfused hearts paced at 180 or 330 beats/min were used to assess the effects of propofol on overall cardiac function.**

Results: **At 28°C (hypothermic) and, to a lesser extent, at 37°C (normothermic), increasing stimulation frequency increased peak shortening and $[Ca^{2+}]_i$. Times to peak shortening and rate of relengthening were more prolonged at 28°C compared with 37°C at low stimulation frequencies (0.3 Hz), whereas the same conditions for $[Ca^{2+}]_i$ were not altered by temperature. At 0.3 Hz and 28°C, propofol caused a dose-dependent decrease in peak shortening and peak $[Ca^{2+}]_i$. These changes were greater at 28°C compared with 37°C and involved activation of protein kinase C. At a frequency of 2 Hz, there was a rightward shift in the dose-response relation for propofol on $[Ca^{2+}]_i$ and shortening at both 37° and 28°C compared with that observed at 0.3 Hz. In Langendorff perfused hearts paced at 330 beats/min, clinically relevant concentrations of propofol decreased left ventricular developed pressure, with the effect being less at 28°C compared with 37°C. In contrast, only a supraclinical concentration of propofol decreased left ventricular developed pressure at 28°C at either stimulation frequency.**

Conclusion: **These results demonstrate that temperature and stimulation frequency alter the inhibitory effect of propofol on cardiomyocyte $[Ca^{2+}]_i$ and contraction. In isolated cardiomyocytes, the inhibitory effects of propofol are more pronounced during hypothermia and at higher stimulation frequencies and involve activation of protein kinase C. In Langendorff perfused hearts at constant heart rate, the inhibitory effects of propofol at clinically relevant concentrations are more pronounced during normothermic conditions.**

* Research Fellow, † Graduate Student, ‡ Carl E. Wasmuth, Endowed Chair and Director, § Assistant Staff.

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Address reprint requests to Dr. Damron: Center for Anesthesiology Research, FF-40, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195. Address electronic mail to: damrond@ccf.org.

IT is widely accepted that stimulation frequency has a major impact on myocardial contractility in *in vitro* models of cardiac function.[1] In general, increasing stimulation frequency causes a positive force–frequency relation in most mammalian cardiac muscle[2,3] and cardiomyocytes.[4] In contrast, rat cardiac muscle typically exhibits a negative force–frequency relation,[2,5,6] although both positive and negative force–frequency relations have been demonstrated in isolated rat cardiomyocytes.[7,8] It seems that the final force developed by the preparation when stimulation frequency is altered depends on a number of factors, including recovery of the processes that lead to contraction (mechanical restitution). Restitution of cross-bridge cycling and the sarcoplasmic reticulum (SR) Ca^{2+} release and reuptake mechanisms, as well as the extent of "Ca^{2+} loading" of the preparation, which will have an effect on both the SR and the Na^+–Ca^{2+} exchanger, are key factors.[8–10] In addition, increasing stimulation frequency also causes an abbreviation in the time course of the intracellular Ca^{2+} transient, likely due to an acceleration in SR Ca^{2+} handling.[11]

The temperature at which *in vitro* studies are conducted also dramatically influences the contractile properties of the heart. In isolated cardiac muscle, twitch amplitude, and duration of contraction are decreased as temperature is increased.[12–16] Increasing temperature has also been reported to increase myofilament Ca^{2+} sensitivity and myofibrillar actomyosin adenosine triphosphatase activity,[16,17] suggesting an enhancement in cardiac contractility. These opposing effects of temperature and stimulation frequency on cardiac excitation–contraction coupling are likely due to effects on multiple intracellular processes, including SR Ca^{2+} handling, Ca^{2+} extrusion, and mechanical processes such as cross-bridge cycling and alterations in myofilament Ca^{2+} sensitivity.[4,8,17,18] The extent to which temperature alters frequency-dependent regulation of cardiomyocyte intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) and shortening has not been examined.

Our rationale is that many previous studies using a variety of *in vitro* cardiac preparations have assessed the extent to which the intravenous anesthetic, propofol, alters the inotropic state of the heart, but the results have been inconsistent and highly variable.[19,20] Differences in temperature and stimulation frequency of the *in vitro* cardiac preparation, as well as the cardiac muscle preparation itself (cardiomyocyte, papillary muscle, or Langendorff heart), species differences, or the perfusate likely contribute to the controversy. In light of the

above-mentioned issues regarding temperature- and frequency-dependent modulation of myocardial function, we hypothesized that temperature or stimulation frequency or both may play a role in influencing the highly variable cardiac depressant effects of propofol over a wide range of concentrations. In the current study, we used freshly isolated, individual rat ventricular myocytes to examine the extent to which temperature alters frequency-dependent modulation of cardiomyocyte $[Ca^{2+}]_i$ and shortening, and the extent to which propofol-induced alterations in myocyte $[Ca^{2+}]_i$ and shortening are dependent on stimulation frequency, temperature, or both. We also assessed the functional effects of propofol on cardiac performance in isolated perfused Langendorff hearts at different pacing frequencies and temperatures.

Materials and Methods

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

Ventricular Myocyte Preparation

Isolated adult ventricular myocytes from rat hearts were obtained as previously described.[21–23] Immediately after euthanasia, the hearts were rapidly removed and perfused in a retrograde manner at a constant flow rate (8 ml/min) with oxygenated (95% oxygen–5% carbon dioxide) Krebs-Henseleit buffer (37°C) containing the following: 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 37.5 mM $NaHCO_3$, and 16.5 mM dextrose, pH 7.35. After a 5-min equilibration period, the perfusion buffer was changed to a Ca^{2+}-free Krebs-Henseleit buffer containing collagenase type II (309 U/ml; Worthington Biochemical Corp., Freehold, NJ). After digestion with collagenase (20 min), the ventricles were minced and shaken in Krebs-Henseleit buffer, and the resulting cellular digest was washed, filtered, and resuspended in HEPES-buffered saline (23°C) containing the following: 118 mM NaCl, 4.8 mM KCl, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 11.0 mM dextrose, 25.0 mM HEPES, and 5.0 mM pyruvate, pH 7.35.

Simultaneous Measurement of $[Ca^{2+}]_i$ and Myocyte Shortening

Simultaneous measurement of $[Ca^{2+}]_i$ and myocyte shortening was performed as previously described by our laboratory.[21–23] Ventricular myocytes exhibiting a rod-shaped appearance with clear striations and less than one spontaneous contraction per minute were chosen for study. Myocytes (0.5×10^6 cells/ml) were incubated in HEPES-buffered saline containing 2 μM fura-2/AM at 37°C for 15 min. Fura-2–loaded ventricular myocytes were placed in a temperature-regulated chamber (Bioptechs, Inc., Butler, PA) mounted on the stage of an Olympus IX-70 inverted fluorescence microscope (Olympus America, Lake Success, NY). The cells were superfused continuously with HEPES-buffered saline at a flow rate of 2 ml/min and field-stimulated *via* bipolar platinum electrodes with a 5-ms pulse using a Grass SD9 stimulator (Grass Instruments, West Warwick, RI).

Fluorescence measurements were performed on individual myocytes using a dual-wavelength spectrofluorometer (Deltascan RFK6002; Photon Technology International, Lawrenceville, NJ) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. Because calibration procedures rely on a number of assumptions, the ratio of the light intensities at the two wavelengths was used to measure qualitative changes in $[Ca^{2+}]_i$. Just before data acquisition, background fluorescence was measured and automatically subtracted from the subsequent experimental measurement. The fluorescence sampling frequency was 100 Hz, and data were collected using software from Photon Technology International.

To simultaneously monitor cell shortening, the cells were also illuminated with red light. A dichroic mirror (600-nm cutoff) in the emission path deflected the cell image through a charge-coupled device video camera (Phillips VC 62505T; Marshall Electronics, Culver City, CA) into a video-edge detector (Crescent Electronics, Sandy, UT) with 16-ms resolution. The video-edge detector was calibrated using a stage micrometer so that cell lengths during shortening and relengthening could be measured.

Analysis of $[Ca^{2+}]_i$ and Shortening Data

The following conditions were calculated for each individual contraction: resting $[Ca^{2+}]_i$ and cell length; peak $[Ca^{2+}]_i$ and cell length; change in $[Ca^{2+}]_i$ (peak $[Ca^{2+}]_i$ minus resting $[Ca^{2+}]_i$) and twitch amplitude; time to peak (Tp) for $[Ca^{2+}]_i$ and shortening and time to 50% (Tr) resting $[Ca^{2+}]_i$ and relengthening. Measurements from 10 contractions were averaged to obtain mean values at baseline and in response to the various interventions. Averaging minimizes beat-to-beat variation.

Isolated Perfused Langendorff Heart Preparation

Male Sprague-Dawley rats weighing 250–300 g were given an intraperitoneal injection of heparin (200 U). After euthanasia, hearts were excised rapidly and placed in ice-cold Krebs-Henseleit buffer before being mounted on a Langendorff apparatus for perfusion with Krebs-Henseleit buffer at 330 beats/min and a constant pressure of 70 mmHg. The buffer was equilibrated with 95% oxygen and 5% carbon dioxide and had the following composition: 118 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 11 mM dextrose. A balloon-tipped catheter was inserted through the left atrium into the left ventricle, and the left

ventricular end-diastolic pressure in all hearts was adjusted to between 8 and 12 mmHg. Left ventricular developed pressure (LVDP) was monitored continuously throughout the experiment. Coronary flow was measured by timed collection of the effluent into a graduated cylinder.

Experimental Protocols

Protocol 1: Effect of Temperature and Stimulation Frequency on Cardiomyocyte $[Ca^{2+}]_i$ and Shortening. To examine the extent to which $[Ca^{2+}]_i$ and myocyte shortening are altered by increasing temperature and stimulation frequency, myocytes (28° or 37°C) were stimulated at 0.3, 0.5, 1, 2, and 3 Hz. At each stimulation frequency, changes in myocyte length and $[Ca^{2+}]_i$ were collected from individual, contracting myocytes for 1.5 min. A 20-s rest period was provided between each increase in stimulation frequency to eliminate any influence of the previous stimulation period. Summarized results are expressed as a percentage of the control value.

Protocol 2: Effect of Temperature and Stimulation Frequency on Propofol-induced Decreases in Cardiomyocyte $[Ca^{2+}]_i$ and Shortening. To determine whether temperature or stimulation frequency alters propofol-induced myocardial depression, we performed dose–response curves to propofol at 28° and 37°C using stimulation frequencies of 0.3 and 2 Hz. A stimulation frequency of 0.3 Hz was chosen to allow for comparison between previous studies from our laboratory,[21–23] and 2 Hz was chosen because it represents the top of the positive force–frequency relation. Myocytes were assigned to one of two groups (28° or 37°C) and allowed to stabilize for 10 min. Baseline measurements were collected from individual myocytes for 1.5 min at 0.3 or 2 Hz in the absence of any intervention. Myocytes were exposed to four concentrations of propofol (30, 100, 300, and 1,000 μM) by exchanging the buffer in the dish with new buffer containing propofol at the desired concentration. Data were acquired for 1.5 min after a 5-min equilibration period in the presence of each concentration of propofol. The myocytes were field stimulated throughout the entire protocol. Summarized results are expressed as a percentage of the control value.

Protocol 3: Effect of PKC Inhibition on Propofol-induced Decreases in Cardiomyocyte $[Ca^{2+}]_i$ and Shortening. To determine whether protein kinase C (PKC) plays a role in mediating the cardiodepressant effect of propofol on cardiomyocyte $[Ca^{2+}]_i$ and shortening, we pretreated the myocytes with the PKC inhibitor bisindolylmaleimide (1 μM) before exposure to propofol. Experiments were performed at both 28° and 37°C and at stimulation frequencies of 0.3 and 2 Hz. Summarized results are expressed as a percentage of control.

Protocol 4: Effect of Stimulation Frequency and Temperature on Propofol-induced Myocardial Depression in Isolated Perfused Langendorff Hearts. To determine the extent to which temperature alters propofol-induced myocardial depression, we perfused Langendorff hearts paced at 330 beats/min (normal heart rate in rats) or 180 beats/min (lowest rate that could be achieved at 37°C) with propofol (1, 10, 30, or 100 μM) at 28° or 37°C. LVDP was continuously monitored before (20 min) and during administration of propofol (20 min). Summarized results are expressed as a percentage change in LVDP (left ventricular systolic pressure minus end-diastolic pressure).

Statistical Analysis

Each experimental protocol was performed on multiple myocytes from the same heart and repeated in at least five hearts. Results obtained from myocytes in each heart were averaged so that all hearts were weighted equally. The time- and frequency-dependent changes on myocyte shortening and the $[Ca^{2+}]_i$ transient were assessed using one-way analysis of variance with repeated measures and the Bonferroni *post hoc* test. Comparisons between groups were made using two-way analysis of variance. Differences were considered statistically significant at $P < 0.05$. For the Langendorff heart experiments, the protocol was repeated in five hearts, and the temperature- or frequency-dependent effects of propofol were assessed using one-way analysis of variance with repeated measures and the Bonferroni *post hoc* test. Comparisons between groups were made by two-way analysis of variance. All results are expressed as mean ± SD.

Materials

Pure propofol was purchased from Sigma Chemical Co. (St. Louis, MO) and diluted in dimethyl sulfoxide. Bisindolylmaleimide I was obtained from Calbiochem (La Jolla, CA). Collagenase was purchased from Worthington Biochemical Corp. (Lakewood, NJ). Fura-2/AM was purchased from Texas Fluorescence Labs (Austin, TX).

Results

Effects of Stimulation Frequency and Temperature on Cardiomyocyte Shortening and $[Ca^{2+}]_i$

Baseline values for myocyte shortening and $[Ca^{2+}]_i$ at 28° and 37°C are illustrated in table 1. Figure 1 shows original recordings of myocyte shortening and $[Ca^{2+}]_i$ from a representative rat ventricular myocyte at 28° (A) and 37°C (B). Summarized data for twitch amplitude and change in $[Ca^{2+}]_i$ in response to increasing stimulation frequency at 28° and 37°C are shown in figure 2. During baseline conditions at 28°C, twitch amplitude (A) was greater (14 ± 3 μm) compared with 37°C (8 ± 2 μm),

Table 1. Baseline Values for Myocyte Shortening and 340/380 Ratio at 28° and 37°C

	Temperature, °C	0.3 Hz
Myocyte shortening		
Resting cell length, μm	28	127 ± 7
	37	125 ± 9
Twitch amplitude, μm	28	9 ± 3
	37	14 ± 3*
Tp, ms	28	190 ± 22
	37	144 ± 18*
Tr, ms	28	304 ± 44
	37	226 ± 34*
340/380 ratio		
Resting 340/380 ratio	28	0.7 ± 0.2
	37	0.7 ± 0.1
Change in 340/380 ratio	28	0.9 ± 0.1
	37	1.4 ± 0.2*
Tp, ms	28	122 ± 34
	37	118 ± 30
Tr, ms	28	300 ± 26
	37	290 ± 46

Values are presented as mean ± SD. n = 15 cells/5 hearts at each temperature.

* $P < 0.05$ *vs.* 28°C.

Tp = time to peak; Tr = time to 50% recovery.

whereas the change in $[Ca^{2+}]_i$ (B) was unaltered (0.9 ± 0.2 at 28°C *vs.* 1.1 ± 0.1 at 37°C). There were no apparent differences in resting $[Ca^{2+}]_i$ or resting cell length at either temperature. Increasing stimulation frequency from 0.3 to 2 Hz augmented twitch amplitude and the change in $[Ca^{2+}]_i$ at both temperatures, although the positive shortening–frequency relation was greater at 28°C compared with 37°C. An increase in resting $[Ca^{2+}]_i$ and a decrease in resting cell length were

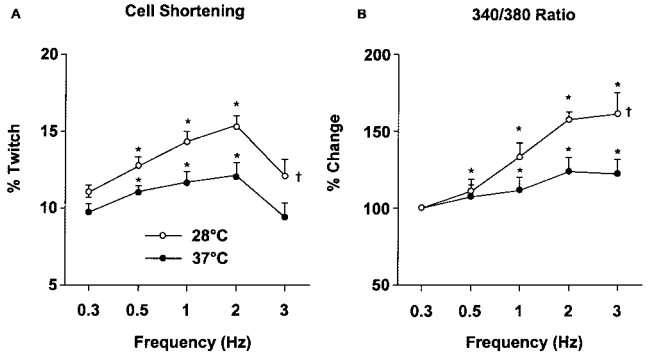

Fig. 2. Summarized data depicting the effects of increasing stimulation frequency on myocyte shortening (*A*) and intracellular free Ca^{2+} concentration (*B*) at 28° and 37°C. Values represent mean ± SD in this and all subsequent figures. * $P < 0.05$ *versus* 0.3 Hz; † $P < 0.05$ *versus* 37°C at the corresponding stimulation frequency. n = 20 cells from 5 hearts.

observed at both temperatures after increasing stimulation frequency. The changes in resting cell length and resting $[Ca^{2+}]_i$ increased gradually throughout the stimulation period and did not fully return to the prestimulation values during the rest period. Spontaneous contractions were sometimes observed during the rest periods after high stimulation frequencies, but less often at 28°C compared with 37°C. At 3 Hz, twitch amplitude was markedly decreased, whereas the change in $[Ca^{2+}]_i$ was relatively unaltered compared with 2 Hz. After the stimulation frequency returned to 0.3 Hz, twitch amplitude and change in $[Ca^{2+}]_i$ and resting $[Ca^{2+}]_i$ returned to baseline values at both temperatures, whereas resting cell length did not completely return to baseline values at either temperature.

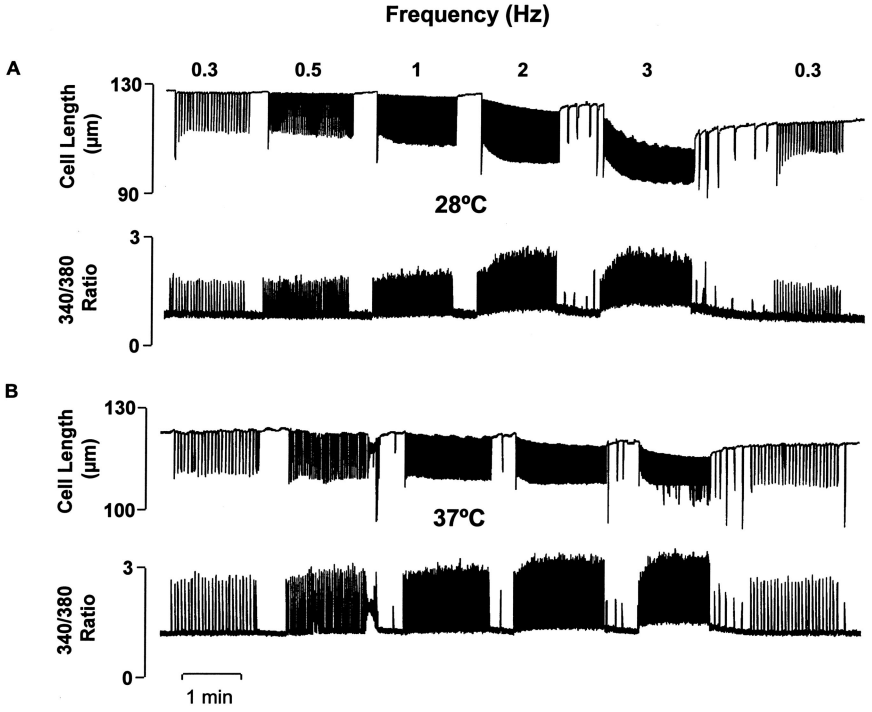

Fig. 1. Effects of changing stimulation frequency on shortening and intracellular free Ca^{2+} concentration in a representative field-stimulated isolated rat ventricular myocyte. (*A*) Original records depicting changes in cell length and intracellular free Ca^{2+} concentration in response to an increase in stimulation frequency at 28°C. (*B*) Same as *A* except at 37°C.

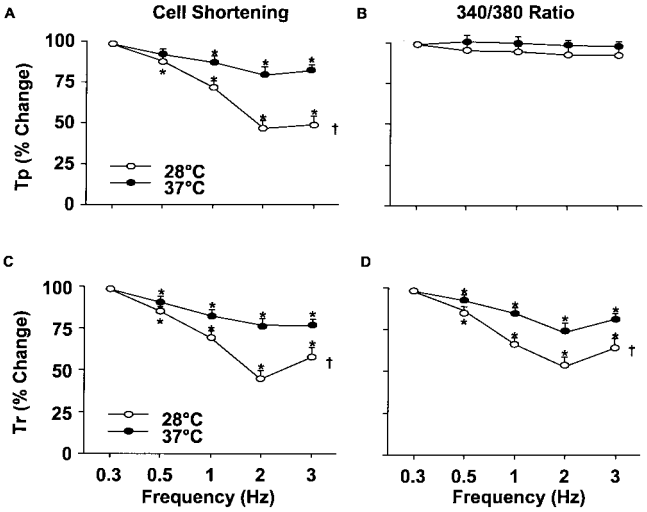

Fig. 3. Summarized data depicting the effects of increasing stimulation frequency on time to peak (Tp) intracellular free Ca^{2+} concentration and myocyte shortening (*A* and *B*) as well as time to 50% resting (Tr) intracellular free Ca^{2+} concentration and relengthening (*C* and *D*) at 28° and 37°C. * $P < 0.05$ *versus* 0.3 Hz; † $P < 0.05$ *versus* 37°C at the corresponding stimulation frequency. n = 20 cells from 5 hearts.

Effects of Stimulation Frequency and Temperature on Timing Characteristics of Cardiomyocyte Shortening and $[Ca^{2+}]_i$

Summarized data showing the effects of increasing stimulation frequency on Tp for shortening and $[Ca^{2+}]_i$ are shown in figure 3. At 28°C, increasing stimulation frequency accelerated Tp shortening (A), but had no effect on Tp $[Ca^{2+}]_i$ (B). During baseline conditions at 28°C (0.3 Hz), Tp shortening (190 ± 18 ms) was prolonged compared with 37°C (144 ± 18 ms), whereas Tp $[Ca^{2+}]_i$ was unaltered (122 ± 34 ms at 28°C *vs.* 118 ± 30 ms at 37°C). Increasing stimulation frequency prolonged Tp shortening to a greater extent at 28°C compared with 37°C.

Changes in Tr were also observed during baseline conditions at 28° and 37°C and with increasing stimulation frequency. Summarized data showing the effects of increasing stimulation frequency on Tr for shortening (C) and $[Ca^{2+}]_i$ (D) are also shown in figure 3. During baseline conditions at 28°C (0.3 Hz), Tr shortening (304 ± 31 ms) was prolonged compared with 37°C (226 ± 24 ms), whereas Tr $[Ca^{2+}]_i$ was unaltered (300 ± 26 ms at 28°C *vs.* 298 ± 46 ms at 37°C). At 28°C, increasing stimulation frequency accelerated Tr shortening and Tr $[Ca^{2+}]_i$. Increasing stimulation frequency accelerated Tr shortening and $[Ca^{2+}]_i$ to a greater extent at 28°C compared with 37°C.

Effect of Temperature and Stimulation Frequency on Propofol-induced Alterations in Cardiomyocyte Shortening and $[Ca^{2+}]_i$

Propofol caused dose-dependent decreases in myocyte shortening and peak $[Ca^{2+}]_i$ at 0.3 Hz (A) and 2 Hz (B) at both temperatures (fig. 4). The negative inotropic effect of propofol and the reduction in $[Ca^{2+}]_i$ were more pronounced at 28°C compared with 37°C. At both temperatures, the negative inotropic effect of propofol was more pronounced at 2 Hz compared with 0.3 Hz.

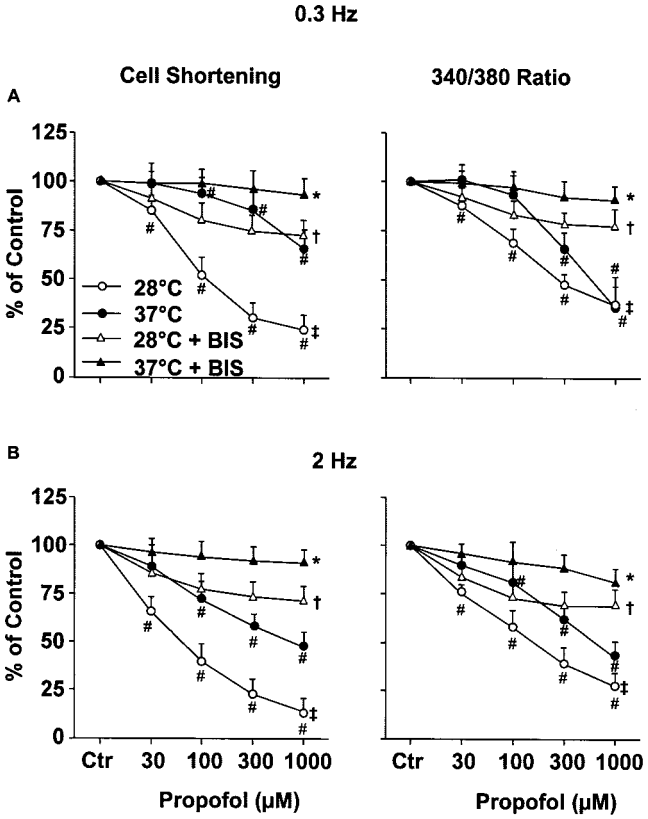

Fig. 4. Summarized data for the effects of 0.3 Hz (*A*) and 2 Hz (*B*) stimulation frequency on propofol-induced decreases in twitch amplitude and peak intracellular free Ca^{2+} concentration in the presence or absence of protein kinase C inhibition with bisindolylmaleimide (BIS; 1 μM) at 28° and 37°C. # $P < 0.05$ *versus* control (Ctr); ‡ $P < 0.05$ *versus* 37°C at the corresponding propofol concentration. n = 23 cells from 7 hearts. * $P < 0.05$ *versus* 37°C without bisindolylmaleimide; † $P < 0.05$ *versus* 28°C without bisindolylmaleimide. n = 18 cells from 5 hearts.

Effect of PKC Inhibition on Propofol-induced Decreases in Shortening and $[Ca^{2+}]_i$

The broad-range PKC inhibitor bisindolylmaleimide (1 μM) was used to assess the involvement of PKC in mediating the propofol-induced decrease in shortening and $[Ca^{2+}]_i$. Exposure of cardiomyocytes to bisindolylmaleimide alone (0.5 Hz) increased peak $[Ca^{2+}]_i$ and shortening by 10 ± 3% ($P = 0.015$) and 19 ± 5% ($P = 0.008$), respectively, at 28°C and by 15 ± 4% ($P = 0.013$) and 24 ± 7% ($P = 0.017$) at 37°C. In the presence of bisindolylmaleimide, the propofol-induced decrease in shortening and $[Ca^{2+}]_i$ at 0.3 Hz (A) and 2 Hz (B) was attenuated at both temperatures (fig. 4).

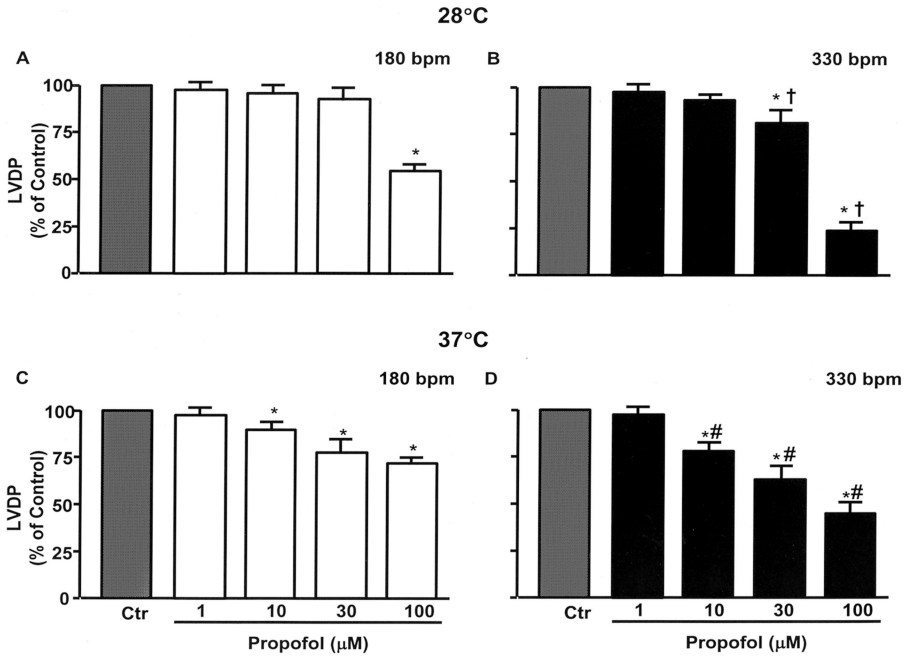

Fig. 5. Summarized data for the effect of propofol (1, 10, 30, 100 μM) on left ventricular developed pressure (LVDP) in isolated perfused Langendorff hearts at 28°C at 180 or 330 beats/min (bpm) (*A* and *B*, respectively) as well as 37°C and 180 or 330 beats/min (*C* and *D*, respectively). * *P* < 0.05 *versus* control (Ctr); † *P* < 0.05 *versus* 180 beats/min; # *P* < 0.05 *versus* 28°C. n = 6 hearts.

Effect of Stimulation Frequency and Temperature on Propofol-induced Alterations in LVDP in Langendorff Perfused Hearts

At 28°C and 180 beats/min, only a supraclinical concentration of propofol (100 μM) decreased LVDP (fig. 5A). In contrast, increasing stimulation frequency from 180 to 330 beats/min increased the efficacy of propofol at clinically relevant concentrations to cause a dose-dependent decrease in LVDP (fig. 5B). At 37°C, clinically relevant concentrations of propofol caused dose-dependent decreases in LVDP at 0.3 Hz (fig. 5C) and 2 Hz (fig. 5D), with the effect of propofol being greater at 330 beats/min. At 28°C, coronary flow was significantly less than that observed at 37°C regardless of the stimulation frequency (table 2). Propofol had no effect on coronary flow at either stimulation frequency or temperature.

Discussion

This is the first study to examine the extent to which temperature and stimulation frequency alter propofol-induced changes in $[Ca^{2+}]_i$ and shortening in individual cardiomyocytes, as well as myocardial depression in isolated perfused Langendorff hearts. The key findings are that stimulation frequency and temperature play an important role in mediating the inotropic effects of propofol. Decreasing temperature resulted in an increase in baseline cardiomyocyte twitch amplitude. Supraclinical concentrations of propofol were required to decrease cardiomyocyte $[Ca^{2+}]_i$ and shortening, and the decrease was greater during hypothermic conditions compared with normothermic conditions. The negative inotropic effect of propofol was greater at higher pacing frequencies and involved activation of PKC. In contrast, clinically relevant concentrations of propofol reduced LVDP to a greater extent during normothermic conditions compared with hypothermic conditions in isolated perfused hearts, and the effect was greater at physiologic pacing frequencies.

Frequency-dependent Changes in Cardiomyocyte $[Ca^{2+}]_i$ and Shortening

Most *in vitro* cardiac muscle studies are conducted at stimulation frequencies and temperatures lower than those observed *in vivo* to limit central ischemia, maintain metabolic stability, and reduce arrhythmic behavior. The use of different stimulation frequencies and temperatures may have contributed to contradictory findings in previous studies demonstrating an increase,[24] a decrease,[19,24–28] or no change[29,30] in inotropic status of the heart in response to propofol. Increasing stimulation frequency typically results in a positive force–frequency relation.[2,3] In the current study, we observed a positive force–frequency relation in cardiomyocytes at stimulation frequencies ranging from 0.3 to 2 Hz at both temperatures. However, a negative force–frequency relation was observed at a stimulation frequency of 3 Hz, which is likely due to a frequency-dependent partial contracture of the cardiomyocyte. Our findings also indicate a rate-dependent acceleration in Tp shortening with no concomitant abbreviation in Tp $[Ca^{2+}]_i$. Our findings of a rate-dependent acceleration in Tr shortening and $[Ca^{2+}]_i$ have been demonstrated in previous studies[7,8,11] and are likely mediated by a rate-dependent abbreviation in cellular mechanisms regulating cross-bridge cycling or myofilament Ca^{2+} sensitivity or both.[8,11]

Table 2. Baseline Values for Coronary Flow before and after Addition of Propofol

	28°C		37°C	
Propofol, μm	180 beats/min	330 beats/min	180 beats/min	330 beats/min
0	12.2 ± 2.3*	12.5 ± 1.3*	16.8 ± 2.3	17.6 ± 1.5
1	11.9 ± 2.8	13.1 ± 0.8	17.5 ± 2.8	16.0 ± 1.7
10	11.0 ± 2.3	11.4 ± 1.3	16.8 ± 2.1	16.5 ± 2.1
30	12.4 ± 2.3	11.4 ± 2.8	16.9 ± 2.5	18.5 ± 2.0
100	12.1 ± 2.3	12.1 ± 1.3	16.4 ± 1.3	17.9 ± 2.3

Values are presented as mean ± SD and reported in ml/min. n = 5 hearts/group.

* $P < 0.05$ *vs.* corresponding stimulation frequency at 37°C without propofol.

Effect of Temperature on Frequency-dependent Changes in Cardiomyocyte $[Ca^{2+}]_i$ and Shortening

Changes in temperature dramatically influence myocardial contractility. In isolated mammalian cardiac muscle, twitch amplitude and duration are both attenuated with increasing temperature.[12–15] The time to half-relaxation also accelerates in a temperature-dependent manner.[13] We observed a positive force–frequency relation at both temperatures, although this relation was more evident at 28°C compared with 37°C. Increasing temperature decreased twitch amplitude with no change in $[Ca^{2+}]_i$, and accelerated Tp and Tr for myocyte shortening with no concomitant alterations in the change in $[Ca^{2+}]_i$ from baseline, Tp, or Tr for $[Ca^{2+}]_i$. These data suggest that an increase in temperature alters cellular mechanisms primarily involved in the regulation of myocardial contractility, resulting in a decrease in myofilament Ca^{2+} sensitivity. Increasing stimulation frequency also accelerated Tp shortening with no concomitant acceleration in Tp $[Ca^{2+}]_i$ at either temperature. These data could be explained by a frequency-dependent alteration in cross-bridge cycling. In contrast, both Tr shortening and Tr $[Ca^{2+}]_i$ were accelerated with increasing stimulation frequency at both temperatures. Moreover, the accelerations in Tp and Tr shortening and $[Ca^{2+}]_i$ were greater at 28°C compared with 37°C. These data indicate that compared with normothermic conditions (37°C), an increase in stimulation frequency during hypothermic conditions (28°C) has a more pronounced effect on cellular mechanisms regulating removal of $[Ca^{2+}]_i$ from the cytoplasm, such as SR Ca^{2+} uptake and Ca^{2+} removal *via* Na^+–Ca^{2+} exchange.

Effect of Stimulation Frequency and Temperature on Propofol-induced Decreases in Cardiomyocyte $[Ca^{2+}]_i$ and Shortening

Propofol has been shown to alter action potential duration,[19] sarcolemmal L-type Ca^{2+} channels and K^+ channels,[31–33] SR Ca^{2+} handling,[21,26,34] intracellular pH,[22] and myofilament Ca^{2+} sensitivity,[22,30] suggesting that propofol should markedly alter cardiac function. However, most *in vitro* studies have reported that propofol, at concentrations relevant to clinical practice, either has no direct effect on cardiac contractile function[19,25,26,29] or has a modest negative inotropic effect.[27,28,35] However, one recent study demonstrated that the effects of propofol on the inotropic state of isolated rat cardiac trabeculae depend on stimulation frequency and extracellular Ca^{2+} concentration, and can result in either a positive or a negative inotropic effect depending on the experimental conditions.[24] In the current study, the dose-dependent negative inotropic effect of propofol and the reduction in $[Ca^{2+}]_i$ were more pronounced at 28°C compared with 37°C at a stimulation frequency of 0.5 Hz. At 2 Hz, the negative inotropic effect of propofol was potentiated at both temperatures, indicating that pacing frequency is a major determinant of propofol-induced negative inotropy when temperature is kept constant. Therefore, our data indicate that the extent to which propofol alters cardiomyocyte $[Ca^{2+}]_i$ and shortening depends on both stimulation frequency and temperature. Moreover, temperature itself alters the frequency-dependent changes in timing conditions. The negative inotropic properties of propofol are more pronounced at lower temperatures and higher stimulation frequencies. At 0.3 Hz, we also noted that high concentrations of propofol have a marked inhibitory effect on $[Ca^{2+}]_i$ at 37°C, whereas the same was not the case for shortening. This suggested that increasing temperature alters the propofol-induced decrease in cardiomyocyte shortening differently than that for regulation of $[Ca^{2+}]_i$. We previously reported that propofol caused an increase in myofilament Ca^{2+} sensitivity by increasing intracellular pH *via* activation of the Na^+–H^+ exchanger in rat ventricular myocytes.[21,22] If this mechanism is accelerated by an increase in temperature, an attenuation in the propofol-induced decrease in shortening would be predicted due to an already increased sensitivity of the myofilaments to Ca^{2+}, thereby offsetting the marked decrease in $[Ca^{2+}]_i$. A temperature-dependent increase in myofilament Ca^{2+} sensitivity has previously been reported[16,17] and, as a consequence, could result in an attenuation of propofol-induced myocardial depression.

Effect of PKC Inhibition on Propofol-induced Decreases in $[Ca^{2+}]_i$ and Shortening

The effects of propofol on $[Ca^{2+}]_i$ and shortening in isolated cardiomyocytes occurs at supraclinical concentrations.[21] However, the current study demonstrates that clinically relevant concentrations of propofol can depress cardiac function in isolated perfused hearts when the experiments are performed at physiologic temperatures and physiologic stimulation frequencies. The isolated cardiomyocytes are useful for studying the effects of propofol on $[Ca^{2+}]_i$ and excitation–contraction coupling at the cellular level, and determining cellular mech-

anisms by which propofol alters cardiomyocyte function. We previously demonstrated that clinically relevant concentrations of propofol attenuated the β-adrenoreceptor–mediated increase in $[Ca^{2+}]_i$ and shortening *via* a PKC dependent pathway.[36] We hypothesized that the propofol induced decrease in $[Ca^{2+}]_i$ and shortening may also be mediated *via* a PKC-dependent pathway. PKC inhibition resulted in an increase in $[Ca^{2+}]_i$ and shortening, suggesting that a steady state level of PKC activity exists in cardiomyocytes that negatively regulates $[Ca^{2+}]_i$ and shortening. Our studies suggest that a propofol-induced activation of one or more PKC isoforms is involved in mediating the decrease in $[Ca^{2+}]_i$ and shortening. Although we did not assess the potential role of individual PKC isoforms in mediating the decrease in $[Ca^{2+}]_i$ and shortening, we suspect that PKC-α, PKC-ε, or both are likely involved, because these isoforms have been implicated in mediating inhibitory effects on regulation of $[Ca^{2+}]_i$ in isolated cardiomyocytes.[37,38] In addition, our data also support a recent study indicating that the inotropic effect of propofol on cardiac muscle function depends on the frequency of stimulation as well as extracellular Ca^{2+} concentration.[24]

Effect of Stimulation Frequency and Temperature on Propofol-induced Myocardial Depression in Isolated Perfused Langendorff Hearts

The effects of propofol in isolated perfused hearts has previously been examined, although data obtained using this model also do not entirely agree.[25,30,35] Perfusion buffers have been shown to have a major influence on the propofol-induced myocardial depression in isolated rabbit hearts, although the stimulation frequency rate was subphysiologic for this preparation.[25] Propofol has also been shown to inhibit intracellular Ca^{2+} transients, but not contraction, in beating guinea pig hearts, suggesting that propofol increases myofilament Ca^{2+} sensitivity despite depression of $[Ca^{2+}]_i$.[30] Using an isolated heart model, we sought to identify whether temperature and frequency play a role in propofol-induced myocardial depression. Perfusion of isolated rat hearts (330 or 180 beats/min) with propofol (1, 10, 30, 100 μM) resulted in dose-dependent cardiac depression at both temperatures. The cardiodepressant effect of propofol was less at 28°C compared with 37°C in the presence of clinically relevant concentrations of propofol (1, 10 μM). Moreover, the cardiodepressant effect was less pronounced at 180 beats/min, compared with the *in vivo* stimulation frequency of 330 beats/min. With high concentrations of propofol (100 μM), the extent of depression was greater in hearts perfused at 28°C compared with those perfused at 37°C. These results support our data obtained in isolated cardiomyocytes. In contrast, propofol at clinically relevant concentrations (10 μM) had no effect on LVDP at 28°C and 180 beats/min, whereas a decrease in LVDP was observed at 330 beats/min. At 37°C, propofol (10 μM) decreased LVDP at both stimulation frequencies, with the effect being greater at a stimulation frequency of 330 beats/min. Therefore, it seems that temperature and stimulation frequency play important roles in regulating steady state contractile properties of the myocardium as well as in response to propofol. Differences in these experimental conditions may have led to the uncertainty and controversy about the direct effects of propofol on the heart. The reduced coronary flow at 28°C compared with 37°C may be due to the nitric oxide synthase–guanylyl cyclase vasodilator and ion gradient stabilization systems not being fully operational during hypothermia.[39]

Clinical Relevance

Propofol is a widely used intravenous anesthetic agent with advantageous properties such as rapid emergence after cessation of infusion.[40] However, it also has cardiovascular effects such as bradycardia, decreased systemic vascular resistance, and negative inotropic effects on cardiac muscle. Although difficulties arise when attempting to extrapolate results from *in vitro* studies to the *in vivo* situation, we believe that the results from this study support observations that occur in the clinical setting. Our goal was to assess conditions that may contribute to the highly variable depressant effects of propofol on cardiac function, by examining the role of stimulation frequency and temperature on propofol-induced inotropy. In the isolated myocytes, the effects of propofol only occur at supraclinical concentrations, whereas in the isolated perfused Langendorff hearts, cardiac depression is observed at clinically relevant concentrations of propofol. In light of these findings, we propose that the hypothermia and slower heart rate observed in the clinical setting may decrease the negative inotropic effect of propofol. Moreover, the hypothermia and slower heart rate observed in clinical practice may be an advantage when using propofol for anesthesia in patients presenting for surgery by reducing anesthesia-induced hemodynamic instability and cardiac dysfunction.

Summary

Propofol-induced decreases in cardiomyocyte $[Ca^{2+}]_i$ and shortening were greater at high stimulation frequencies and when temperatures were hypothermic. Regardless of temperature or stimulation frequency, the cellular mechanism seems to involve a propofol-induced activation of PKC. In light of these findings, an overestimation of the negative inotropic effects of propofol in cardiomyocytes is likely if the study is performed at hypothermic conditions to preserve cardiomyocyte function, whereas an underestimation of the effects is likely if the stimulation frequency is less than that observed *in vivo*. In fact, when stimulation frequency and temperature are maintained at *in vivo* levels, propofol-induced myocar-

dial depression in isolated hearts becomes more obvious at concentrations that are relevant to the clinical setting. However, simulating the *in vivo* situation is not always possible, and care must be taken when assessing the effects of anesthetic agents on cardiac function when using *in vitro* models that require different experimental conditions to maintain cellular function.

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