Negative and Selective Effects of Propofol on Isolated Swine Myocyte Contractile Function in Pacing-induced Congestive Heart Failure

Latha Hebbah, F.R.C.A., F.F.A.R.C.S.I.; B. Hugh Dorman, M.D., Ph.D.,† Mark J. Clair, B.S.,‡ Raymond C. Roy, M.D., Ph.D.,§ Francis G. Spinale, M.D., Ph.D.||

Background: Although propofol (2,6-di-isopropylphenol) is commonly used to induce and maintain anesthesia and sedation for surgery, systematic hypotension and reduced cardiac output can occur in patients with or without intrinsic cardiac disease. The effect of propofol on myocyte contractility after the development of congestive heart failure (CHF) remains unknown. This study tested the hypothesis that propofol would have direct effects on myocyte contractile function in both healthy and CHF cardiac myocyte preparations.

Methods: Isolated left ventricular (LV) myocyte contractile function (shortening velocity, μm/s) was examined in myocytes from five control pigs and in five pigs with pacing-induced CHF (240 beats/min, for 3 weeks) in the presence of propofol concentrations ranging from 1–6 μg/ml. In addition, myocyte contractility in response to β-adrenergic receptor stimulation (isoproterenol, 10–50 nM) in the presence of propofol (3 μg/ml) was examined.

Results: Three weeks of pacing caused LV dysfunction consistent with CHF as evidenced by increased LV end-diastolic diameter (control 3.5 ± 0.1 cm vs. CHF 5.6 ± 0.2 cm; P < 0.05) and reduced LV fractional shortening (control 34 ± 3% vs. CHF 12 ± 2%; P < 0.05). Propofol (6 μg/ml) caused a concentration-dependent negative effect on velocity of shortening from baseline in both control (67 ± 2 μm/s vs. 27 ± 3 μm/s; P < 0.05) and CHF myocytes (29 ± 1 μm/s vs. 15 ± 1 μm/s; P < 0.05). Importantly, CHF myocytes were more sensitive than control myocytes to the negative effects of propofol on velocity of shortening at the lower concentration (1 μg/ml). β-adrenergic responsiveness was reduced by propofol (3 μg/ml) in control myocytes only.

Conclusions: Propofol has a direct and negative effect on basal myocyte contractile processes in the setting of CHF, which is more pronounced than that on healthy myocytes at reduced propofol concentrations. (Key words: Anesthetics, intravenous; propofol. Heart: heart failure; myocyte contractility.)

CONGESTIVE heart failure (CHF) is a progressive condition afflicting more than 3 million persons in the United States.1 Many patients having surgery present with CHF, and a substantial number of persons having cardiovascular surgery experience left ventricular (LV) pump dysfunction after cardiopulmonary bypass, which further exacerbates any preoperative reductions in LV function.2-4 In patients having surgery who have compromised LV performance, it is important to choose an anesthetic agent that does not further worsen LV contractile function.

Propofol (2,6-di-isopropylphenol) is an intravenous anesthetic agent with favorable pharmacokinetic properties including a short elimination half-life and rapid systemic clearance, which permits early emergence after the drug is discontinued.5 There is also evidence that propofol reduces the incidence of postoperative nausea.6 Therefore propofol is being used with increasing frequency in the perioperative setting. Furthermore, during cardiovascular surgery propofol has been used after cardiopulmonary bypass to achieve early extubation.7 However, propofol administration also has been shown to cause systemic hypotension.8,9 The precise mechanism of this hemodynamic response remains unclear despite several clinical and experimental studies.8-15 Various postulated mechanisms for propofol-induced hemodynamic instability include alterations in loading conditions, changes in neurohormonal status, and re-

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ductions in myocardial pump function. The mechanism for the hemodynamic compromise that can occur with propofol is clinically important if propofol is to be administered during surgery or in the intensive care unit, particularly to persons with preexisting LV dysfunction. Therefore, the objectives of the present study were threefold. The first objective was to examine the direct effects of propofol on myocyte contractile function in the setting of CHF. The second objective was to determine whether myocyte contractile performance was more sensitive to the negative effects of propofol with the development of CHF. Past reports have shown alterations in myocyte β-adrenergic responsiveness with the onset of CHF, and β-adrenergic receptor agonists are commonly used in patients with decreased LV function. Accordingly, the third objective was to examine the effect of propofol on the capacity of the myocyte to respond to an inotropic stimulus in the setting of CHF.

**Materials and Methods**

The present study used a model of pacing-induced CHF in pigs that has been well described previously to cause LV dilatation and contractile dysfunction. The direct effects of increasing concentrations of propofol on isolated myocyte contractile function was examined in control animals and after the development of pacing-induced CHF. The methods used to isolate myocytes and measure indices of myocyte contractile performance have been described previously.

**Experimental Model of Congestive Heart Failure**

After Animal Review Committee approval, ten age- and weight-matched pigs (Yorkshire strain, age 6 months, weighing 25 - 30 kg) were randomly assigned to undergo sham-operation procedures (control group, n = 5) or supraventricular pacing-induced CHF (CHF group, n = 5; 240 beats/min, 3 weeks). Animals were anesthetized with isoflurane (2%) in 50% oxygen and 50% nitrous oxide. After thoracotomy, a stimulating electrode was sutured onto the left atrium and a modified pacemaker (model 8329; Medtronic Inc., Minneapolis, MN) was buried in a subcutaneous pocket. In addition, at the time pacemaker were implanted, animals in both groups had a catheter with a vascular access port (model GPV, 9 Fr; Access Technologies, Skokie, IL) placed in the right internal carotid artery and advanced to the aortic arch for subsequent measurement of arterial blood pressure when the animals were conscious. The access port was buried in a subcutaneous pocket over the thoracolumbar fascia. Seven to 10 days after recovery from the surgical procedure, atrial pacing at 240 beats/min was initiated in the CHF group. Electrocardiograms were obtained at 5-day intervals during the pacing protocol to ensure the presence of 1:1 conduction. Past studies in our laboratory have shown that chronic pacing-induced tachycardia causes LV pump dysfunction and neurohormonal activation similar to the clinical spectrum of CHF. All animals were treated and cared for in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

**Left Ventricular Function Studies**

On the day of study, an electrocardiogram was established and the pacemaker was deactivated (CHF group). Systemic blood pressure measurements were performed in both control and CHF animals when the animals were conscious. The vascular access port was entered using a 12-gauge Huber needle (Access Technologies, Skokie, IL) and basal resting arterial pressure was recorded. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID; Gould, Oxnard, CA). The electrocardiographic data and pressure waveform were recorded using a multichannel recorder (Linearorder FW1 3701; Graphtec, Irvine, CA). Two-dimensional and M mode echocardiographic studies (2.25 MHz transducer; ATL Ultramark VI, Bothell, WA) were used to visualize the left ventricle from a right parasternal approach. Echocardiographic measurements were performed using the American Society of Echocardiography criteria and included end-diastolic and end-systolic diameter and LV wall thickness. Left ventricular fractional shortening was computed as [(end diastolic diameter – end systolic diameter)/(end diastolic diameter)] and was expressed as a percent. After collection of echocardiographic and arterial pressure measurements, animals were anesthetized with isoflurane (1.5% in 50% oxygen/50% nitrous oxide) and their lungs were ventilated through a nonrebreathing anesthesia circuit. A sternotomy was performed and the heart was quickly extirpated and placed in cold oxygenated Krebs solution. A region of the LV free wall perfused by the left circumflex coronary artery (5 × 5 cm) was dissected free, the artery cannulated, and the tissue prepared for myocyte isolation.
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Myocyte Isolation

Using myocyte isolation methods described in this laboratory, 35 ml of an oxygenated modified Kraft-Bruhe solution (80 mM KCl, 30 mM K₂HPO₄, 5 mM MgSO₄, 10 mM glucose, 5 mM Na₂ATP, 20 mM taurine, 5 mM creatine, 5 mM succinate, and 5 mM HEPES) supplemented with 5 mM nitritotriacetic acid and 0.1% salt-free bovine serum albumin (BSA) with collagenase (0.5 mg/ml, type II; 146 U/mg, Worthington Biochemical Corp., Freehold, NJ) was perfused through the cannulated circumflex artery for 20 min.²² The tissue was then minced into 2-mm sections and added to an oxygenated solution containing 2% BSA, deoxyribonuclease II (DNase, 51 Kunitz units/ml, type IV; Sigma, St. Louis, MO), 300 μM CaCl₂ and collagenase (0.5 mg/ml). The tissue was transferred to a centrifuge tube and gently agitated. All procedures were performed at 37°C and the tissue continuously aerated with 95% oxygen and 5% carbon dioxide. At 15-min intervals, the supernatant was removed, filtered, and the cells were allowed to settle. This process was repeated for ten triturations. The isolated myocytes were then resuspended in Dulbecco’s modified Eagle medium (Media 199; Gibco Laboratories, Grand Island, NY). A 2-ml (5 × 10⁴ cells/ml) aliquot of the isolated myocyte suspension was plated onto coverslips previously coated with a laminin/fibronectin matrix (Matrigel; Collaborative Research, Bedford, MA) and incubated at 37°C for 1 h in the presence of 95% oxygen and 5% carbon dioxide to ensure adequate oxygenation of myocytes. Previous studies using electron microscopy have shown that this myocyte preparation has a normal sarcolemma, an intact basal lamina, interacts with basement membrane components, and retains normal cytoarchitecture.²³ The yield of viable myocytes was greater than 80% in all preparations with no difference in yields between control and CHF groups. Viable myocytes were defined as those cells that maintained a rod shape, were calcium tolerant, remained quiescent in culture, excluded trypan blue, and responded to electrical field stimulation.

Isolated Myocyte Function

Myocyte contractile function was examined using computer-assisted video microscopy techniques.²²²⁵ Isolated myocytes obtained from control animals (control group) and pacing-induced CHF animals (CHF group) were placed in thermostatically controlled chambers at 37°C for imaging on an inverted microscope (Televal 3; Jena, Germany). Each chamber volume was 2.5 ml and contained two stimulating platinum electrodes and a thermo-regulator to maintain the media temperature at 37°C. The myocytes were visualized using an x20 Hoffmann modulation contrast objective (Modulation Optics, Greenvale, NJ). Myocyte contractions were elicited by field stimulation at 1 Hz (S11; Grass Instruments, Quincy, MA) using current pulses lasting 5 ms and voltages 10% above the contraction threshold. The polarity of the stimulating electrodes was alternated at every pulse to prevent the buildup of electrochemical by-products. Myocyte contractions were visualized using a charge-coupled device (GPCD60; Panasonic, Secaucus, NJ) with a noninterlaced scan rate of 240 Hz. Images of the contracting myocytes were displayed on a video monitor (CS-13RXL; Mitsubishi, Electronic, Cypress, CA) and recorded on a videotape using a standard video recorder (HSU 32; Mitsubishi Electronics, Cypress, CA).²³²⁷ Myocyte motion signals were captured with the cell parallel to the video raster lines, and this video signal input were captured through an edge-detector system (Crescent Electronics, Sandy, UT). The edge-detection system used the changes in light intensity at the edges of the myocytes to track myocyte motion and was calibrated by recording the image of a stage micrometer (Zeiss, Oberkochen, Germany) with grating spaced 10 μm apart using the two objectives.²³²⁷ The distances between the left and right myocyte edges was converted into a voltage signal, digitized, and entered into a computer (80486; Zeiss International, Minneapolis, MN) for subsequent analysis. For the reconstruction of a digitized signal, the Nyquist criterion states that the sampling frequency must be at least twice the maximum band-limited frequency contained in that signal. Specifically, the highest frequency component that could be reconstructed in the current series of experiments was 120 Hz. Furthermore, Sato et al.²⁸ reported that a temporal resolution of 16.7 ms adequately captured changes in sarcomere length. In the current study, the on-camera magnification of the myocyte images was 9.25 pixels/μm. This pixel resolution provided an adequate spatial resolution to determine myocyte motion by video-based edge detection. In a previous study, Fourier analysis of myocyte contractile data revealed that 99% of the frequency power spectrum was less than 4 Hz when the myocytes were stimulated at 0.5 Hz, implying that all the contractile information was contained in the first eight harmonics of the principal frequency.²⁷ The current study obtained myocyte contractile motion signals that far exceeded these computed minimum requirements. The velocity of myocyte motion was computed from the contraction profile using the central difference algorithm. Noise spikes were eliminated by ex-

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minating the velocity profile for transient double-zero crossings.

Stimulated myocytes were allowed a 5-min stabilization period after electrical stimulation, and contraction data for each myocyte was recorded from a minimum of 20 consecutive contractions. Parameters computed from the digitized contraction profiles included percentage shortening (%) and velocity of shortening (μm/s). Contractile measurements were obtained only on those myocytes that maintained a long axis orientation perpendicular to the microscope objective throughout the contraction profile. Myocyte percentage shortening was determined as the percentage difference between maximum and minimum cell length for each contraction. Myocyte velocity computations were obtained by differentiating the digitized contraction profiles.

**Isolated Myocyte Function: Effects of Propofol**

Isolated myocardial contractile function was examined in both the control and CHF groups in the presence of increasing concentrations of propofol using the BSA-supplemented cell media, as described in the previous section. Isolated myocardial contractile function for control and CHF myocytes was first measured without propofol, as described in the previous section. Propofol (vehicle 10% Intralipid; Zeneca Inc. Ltd., Wilmington, DE) was then diluted in cell culture media containing 2% BSA and added to myocytes in the control and CHF groups to achieve final concentrations of 1 μg/ml (5.6 μM) and 6 μg/ml (35.6 μM). Control and CHF myocytes were incubated with propofol in cell culture media with 2% BSA at 37°C in the presence of 95% oxygen and 5% carbon dioxide for 15 min, and myocyte contractile function was measured. After measurements of contractile function were taken in the presence of propofol, myocytes were exposed to the β-adrenergic receptor agonist isoproterenol (25 nM; Sigma Chemical Co., St. Louis, MO), and contractile function measurements repeated. This concentration of isoproterenol was previously determined to be the effective dose for maximal response in this isolated myocyte preparation. To further characterize the effect of propofol on the myocyte contractile response to β-adrenergic stimulation, an isoproterenol concentration-response curve was constructed. Control and CHF myocytes were exposed to isoproterenol concentrations ranging from 10 nM to 50 nM, and the velocity of shortening was measured with and without propofol, at 3 μg/ml.

Because propofol is highly protein bound, to determine whether the concentration of protein in the cell culture media (2% BSA) had an effect on myocyte contractile function with propofol, a separate series of experiments was performed in animal plasma. Blood was obtained from the same animals in which myocytes were isolated and the plasma separated by centrifugation. Protein assay of the plasma was performed by colorimetric analysis (Bio-Rad Laboratories Inc.). Myocyte contractile function was measured in pig plasma in control and CHF myocytes at baseline and in the presence of propofol at concentrations of 1 and 6 μg/ml. To ensure that the vehicle for propofol (10% Intralipid) did not affect contractile performance, isolated myocyte contractile function was compared between myocytes under control conditions (Control group) and after the development of CHF (CHF group) in the presence and absence of propofol vehicle at baseline and after β-adrenergic stimulation. Control and CHF myocyte contractile function in the presence of propofol vehicle alone (10% Intralipid) was not different from myocytes in cell culture media with 2% BSA. Thus only values for vehicle alone are reported.

**Data Analysis**

Indices of LV function were compared between the control and CHF pigs using the Student’s t test. Indices of myocyte contractile function for the two treatment groups were compared using two-way analysis of variance. If this method identified significant differences, pairwise comparisons were performed on all measured indices by constructing Bonferroni probabilities. The limits of the Bonferroni probabilities were established using the pooled variances for each set of paired comparisons. In a similar manner, for the β-adrenergic response studies, myocyte contractile function at baseline and after β-adrenergic stimulation was directly compared using analysis of variance. All statistical procedures were performed using the BMDP statistical software package (BMDP Statistical Software Inc., Los Angeles, CA). Results are presented as means ± SEM. Probability values less than 0.05 were considered statistically significant.

**Results**

**Left Ventricular Function**

Indices of LV function and hemodynamic parameters were measured in vivo after either 21 days of pacing (CHF group) or 21 days without pacing (control group)
and are summarized in Figure 1 and Table 1. After 3 weeks of supraventricular tachycardia, there was a significant increase in LV end-diastolic diameter (45%) and resting heart rate (46%) in CHF pigs compared with controls. Left ventricular fractional shortening, LV wall thickness, and mean arterial pressure were all significantly reduced in the CHF group compared with the control group. Furthermore, symptoms of CHF developed in all of the pigs undergoing pacing-induced supraventricular tachycardia, as evidenced by ascites, peripheral edema, and respiratory distress after 21 days of pacing.

Isolated Myocyte Contractile Function

Myocyte contractile performance of myocytes isolated from control and CHF pigs are shown in Table 2. Indices of myocyte contractile function for the control group obtained in the current study were similar to previously reported values for this isolated myocyte preparation. Baseline myocyte contractile function was significantly decreased after the development of CHF compared with control values; myocyte percentage and velocity of shortening were significantly decreased in CHF myocytes compared with control myocytes at baseline (P < 0.05). Thus LV myocytes in the pacing-induced CHF group had reduced contractile function relative to control myocytes.

In the control group, myocyte contractile function was reduced in the presence of propofol in a concentration-dependent manner (Table 2). In the presence of 1 µg/ml propofol, myocyte percentage and velocity of shortening of control myocytes were significantly decreased. Both percentage and velocity of shortening were further reduced in control myocytes exposed to 6 µg/ml propofol. Similarly, in the CHF group, propofol caused a concentration-dependent reduction in indices of myocyte contractile function. Propofol at a concentration of 1 µg/ml resulted in a significant decrease in both myocyte percentage and velocity of shortening. Percentage shortening was further reduced in CHF myocytes with 6 µg/ml propofol when compared with 1 µg/ml propofol. Given the significant differences in baseline contractile function between control and CHF myocytes, the absolute change in the velocity of shortening with increasing concentrations of propofol from no propofol values was computed. The results from this analysis are shown in Figure 2. Increasing concentrations of propofol caused reductions in the velocity of shortening in both the control and CHF groups, as assessed by the absolute change in velocity of shortening. Interestingly, CHF myocytes were significantly more sensitive to the negative ef-

Table 1. Hemodynamic and Left Ventricular (LV) Function Parameters with Pacing-induced Congestive Heart Failure (CHF)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Resting heart rate (bpm)</td>
<td>114 ± 11</td>
<td>166 ± 7 *</td>
</tr>
<tr>
<td>Mean aortic blood pressure (mmHg)</td>
<td>92 ± 5</td>
<td>79 ± 6 *</td>
</tr>
<tr>
<td>LV end-diastolic diameter (cm)</td>
<td>3.3 ± 0.1</td>
<td>5.6 ± 0.2 *</td>
</tr>
<tr>
<td>LV fractional shortening (%)</td>
<td>34 ± 3</td>
<td>12 ± 2 *</td>
</tr>
<tr>
<td>LV end-diastolic wall thickness (cm)</td>
<td>0.77 ± 0.02</td>
<td>0.44 ± 0.02 *</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

CHF = congestive heart failure (induced by pacing at 240 bpm for 3 weeks).

*P < 0.05 versus control.
Table 2. Dose-dependent Effects of Propofol on Myocyte Contractile Function after Pacing-induced Congestive Heart Failure (CHF)

<table>
<thead>
<tr>
<th></th>
<th>No Propofol</th>
<th>Propofol 1 μg/ml</th>
<th>Propofol 6 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>β-AR</td>
<td>Baseline</td>
</tr>
<tr>
<td>Resting length (μM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>134 ± 1</td>
<td>129 ± 1</td>
<td>134 ± 4</td>
</tr>
<tr>
<td>CHF</td>
<td>165 ± 2*</td>
<td>162 ± 3*</td>
<td>161 ± 4*</td>
</tr>
<tr>
<td>Percent shortening (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.0 ± 0.1</td>
<td>12.2 ± 0.3†</td>
<td>4.3 ± 0.3‡</td>
</tr>
<tr>
<td>CHF</td>
<td>2.2 ± 0.1*</td>
<td>4.1 ± 0.4*,†</td>
<td>1.3 ± 0.1*‡</td>
</tr>
<tr>
<td>Velocity of shortening (μm/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66.8 ± 1.7</td>
<td>219.2 ± 8.3†</td>
<td>51.1 ± 4.3‡</td>
</tr>
<tr>
<td>CHF</td>
<td>29.1 ± 1.0*</td>
<td>78.2 ± 9.4*,†</td>
<td>17.6 ± 1.2*,‡</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, with a minimum of 20 cells analyzed in each group.

Propofol = 2.6 di-isopropylphenol; β-AR = β-adrenergic stimulation with 25 nm isoproterenol; CHF = congestive heart failure (induced by pacing at 240 bpm for 3 weeks).

*P < 0.05 versus control.
†P < 0.05 versus baseline (no isoproterenol).
‡P < 0.05 versus no propofol.
§P < 0.05 versus propofol 1 μg/ml.

Effects of 1 μg/ml propofol on velocity of shortening than were control myocytes (fig. 2).

Because propofol is highly protein bound, to ensure that the protein content of the standard cell culture media (2% BSA) did not influence the effect of propofol on contractile processes, myocyte velocity of shortening was measured in plasma obtained from control and CHF animals at baseline and with propofol at concentrations of 1 and 6 μg/ml. The protein content of the plasma was in the physiologic range in both groups (control, 4.2 g/dl; CHF, 3.6 g/dl). There was no difference in myocyte velocity of shortening at baseline between myocytes exposed to standard cell culture media or plasma. As illustrated in figure 3, there was also no difference between plasma and standard cell culture media with respect to the effect of propofol on myocyte contractile function in both control and CHF myocytes. Propofol caused a similar concentration-dependent reduction in myocyte contractile function in control myocytes in standard cell culture media and plasma. Similar to the findings with propofol in cell culture media, velocity of shortening of CHF myocytes in plasma was significantly more sensitive to the negative effects of 1 μg/ml propofol than were control myocytes.

Isoproterenol (25 nm) was added to control and CHF myocytes in the presence of propofol to determine the effects of propofol on the capacity of the myocyte to respond to an inotropic stimulus after the development of CHF. Myocyte contractile function measurements in the presence of isoproterenol are provided in table 2. Isoproterenol caused a significant increase (>100%) in all indices of myocyte contractile function in control myocytes. Propofol caused a concentration-dependent reduction of contractile function in the presence of isoproterenol in control myocytes. Increasing concentrations of propofol caused a further reduction in control myocyte contractile function with isoproterenol; at 6 μg/ml propofol, percentage and velocity of myocyte shortening was further reduced (table 2). Isoproterenol also caused significant increases in contractile function in CHF myocytes, resulting in a nearly twofold improvement in percentage and velocity of myocyte shortening. However, indices of contractile function in CHF myocytes remained lower than control myocytes after isoproterenol administration. In contrast to control myocytes, propofol did not cause reductions in contractile function in the presence of isoproterenol in CHF myocytes (table 2, fig. 2). Percentage and velocity of shortening did not decrease with 1 μg/ml or 6 μg/ml propofol in CHF myocytes exposed to isoproterenol.

To better characterize the effect of propofol on the myocyte contractile response to β-adrenergic stimulation, an isoproterenol concentration-response curve was constructed. As illustrated in figure 4, velocity of shortening was increased in control myocytes without propofol at 10-50 nm isoproterenol. A maximal in-
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Fig. 2. The absolute change in velocity of shortening for each myocyte analyzed in the control and congestive heart failure (CHF) groups with increasing concentrations of propofol at baseline and after β-adrenergic receptor stimulation. At baseline (upper, no isoproterenol), CHF myocytes were more sensitive to the negative effects of 1 μg/ml propofol on velocity of shortening compared with control myocytes. In contrast, propofol caused dose-dependent reductions in myocyte contractile function in the presence of isoproterenol (lower, 25 nm isoproterenol) only in control myocytes. *P < 0.05 versus control; †P < 0.05 versus 1 μg/ml propofol.

An increase in velocity of shortening was observed at isoproterenol concentrations of 25 and 50 nm (190% from baseline value). However, in the presence of 3 μg/ml propofol, although 10–50 nm isoproterenol resulted in an increase in velocity of shortening, it was lower than values with no propofol at all concentrations of isoproterenol considered. Isoproterenol also resulted in an increase in velocity of shortening in CHF myocytes at 10–50 nm (155% from baseline value), with no difference in contractile function at any concentration of isoproterenol examined. Propofol at 3 μg/ml did not result in any decrease in velocity of shortening in CHF myocytes at any of the isoproterenol concentrations (fig. 4).

Discussion

The significant findings from this study were three-fold: (1) propofol caused a direct, dose-dependent negative effect on isolated myocyte baseline contractile function under control conditions and after the development of CHF; (2) there was an increased sensitivity to the negative effects of propofol on myocyte contractile performance with the development of CHF; and (3) propofol caused reductions in the capacity of control myocytes to respond to an inotropic stimulus but did not alter responsiveness of failing myocytes. Therefore, a potential contributory mechanism for changes in LV function after administration of propofol is a direct negative effect on myocyte contractile function. Furthermore, with the development of CHF, myocyte contractile performance appears more sensitive to the negative effects of propofol.

Although several clinical and experimental animal reports and in vitro studies have shown a negative effect of propofol on LV contractile function, contradictory reports exist.10–12, 29–34 These contradictory findings may be attributed to confounding factors present in the various studies, such as different plasma propofol concentrations, changes in LV loading states, altered neurohormonal status, and varying experimental conditions in which the hemodynamic measurements were recorded. For example, alterations in the type of perfusate have been shown to have a major effect on the inotropic effects of propofol in an isolated rabbit heart model.32 Furthermore, there are significant species differences in the effect of propofol on contractile function of myocardium.33 Such factors make assessment of the direct effect of propofol on myocardial contractile function difficult to interpret. It is noteworthy that a recent study revealed no inhibition of myocardial contractility by propofol in isolated human atrial muscle.34 The present study revealed that propofol had a direct, negative effect on myocyte contractile function in an isolated myocyte system. Furthermore, an understanding of the effects of propofol on contractile function was expanded by the results with isolated CHF myocytes. An examination of the direct effect of propofol on myocyte contractile function in the setting of CHF may be especially useful, because any negative effects on contractility may
have more significant hemodynamic consequences. The present study showed that propofol had a direct negative effect on myocyte contractile function with the development of CHF. More importantly, with the development of CHF there was an increased sensitivity to the negative effects of propofol at low concentrations. This result contrasts with a recent study that examined the myocardial effects of propofol in isolated papillary muscle obtained from hamsters with hypertrophic cardiomyopathy. Differences among species and in the model of cardiac disease may account for the discrepancies observed in the two studies.

In the present study, a concentration-dependent effect of propofol on contractile function was primarily observed in control myocytes. In CHF myocytes, contractile function was significantly decreased at low concentrations of propofol, but it reached a plateau at higher concentrations. This apparent disparity between
control and CHF myocytes with respect to the negative inotropic effect of increasing concentrations of propofol probably is due to the preexisting defects in contractile processes in CHF myocytes. Thus the threshold for the negative effect of propofol may be reached at lower concentrations in the CHF myocyte preparation.

In an attempt to better define the mechanisms responsible for the effects of propofol on contractile function, several previous studies that examined the interactions of propofol with specific sarcolemmal systems have shown that propofol interferes with trans-sarcolemmal Ca\(^{2+}\) flux, which may contribute to a reduction in myocyte contractile function.\(^{36-38}\) Past reports from this laboratory have shown that the development of CHF is associated with fundamental abnormalities in myocyte L-type Ca\(^{2+}\) channel function, including reductions in both the peak inward Ca\(^{2+}\) current and the duration that the L-type Ca\(^{2+}\) channels remain open, which may contribute to the reduction in myocardial contractile function observed with CHF.\(^{39}\) Because the present study showed that myocyte contractile function was more sensitive to the negative effects of propofol in the setting of CHF, it is possible that the changes in L-type Ca\(^{2+}\) channel function seen with CHF were further compounded by adding propofol. Further studies are warranted to define potential ionic changes within the CHF myocyte after the administration of propofol to identify contributory mechanisms for these changes in myocyte contractile function.

The reduction in LV contractile function that occurs with CHF often results in the requirement for \(\beta\)-adrenergic stimulation in this subset of patients in the perioperative setting. Furthermore, patients with CHF have chronically elevated concentrations of circulating catecholamines. Therefore, we examined the contractile response to an inotropic stimulus after propofol administration in isolated myocytes with the development of CHF. Corresponding to past reports from this laboratory, we found a reduction in myocyte contractile function with \(\beta\)-adrenergic stimulation in CHF myocytes compared with control myocytes.\(^{17,18}\) However, propofol did not further diminish myocyte contractile function in the presence of \(\beta\)-adrenergic stimulation after the development of CHF. This corresponds with a recent study that showed that propofol did not modify the \(\beta\)-adrenergic inotropic response in rat myocardium.\(^{40}\) The results from the present study suggest that differential interactions between propofol and sarcolemmal transduction systems may occur with the development of CHF. Alterations in the \(\beta\)-adrenergic transduction system occur with CHF and include a significant reduction in \(\beta\)-adrenergic receptor density, diminished sarcolemmal content of the stimulatory component of the G protein complex, and increased content of the inhibitory component of the G protein complex.\(^{17}\) Therefore, while remaining speculative, these preexisting defects in \(\beta\)-adrenergic transduction probably are contributory factors for the minimal effect of propofol in CHF myocytes exposed to \(\beta\)-adrenergic agonists.

The present study used a model of chronic rapid pacing that produces time-dependent and progressive changes in LV functional and neurohormonal characteristics similar to that of the clinical spectrum of CHF.\(^{17-21,41-45}\) Specifically, this laboratory has shown that chronic rapid pacing in pigs causes progressive LV dilatation and pump dysfunction, as evidenced by reduced LV fractional shortening.\(^{17-21}\) Consistent with clinical CHF, there is also a significant increase in plasma concentration of catecholamines, atrial natriuretic factor, renin, and endothelin-1 with the development of CHF.\(^{19}\) Thus the pacing-induced model of CHF that we used in this study shares several characteristics with clinical CHF and appears to be a useful and representative model.

The isolated in vitro model allows us to assess the direct effects of propofol on myocyte contractile function with the development of CHF. In addition, this system has other advantages of excluding confounding influences such as loading conditions, neurohormonal status, and alterations in coronary perfusion, which all occur with both CHF and propofol administration and could affect LV pump function in vivo, making it difficult to interpret the direct effects of propofol. However, a study of isolated myocytes not subject to preload, afterload, or circulating hormones does not reflect normal physiologic conditions and, therefore, may not be strictly applicable to the clinical setting. Other limitations to this isolated model must be recognized. Propofol is lipophilic and highly bound to serum proteins. Because propofol partitions in vivo between serum proteins, lipid microsomes, and into tissue, there is difficulty in knowing the precise concentration of the free and probably active component of propofol in vivo. Therefore, concentrations in experimental solution may or may not reflect effective plasma concentrations. In addition, osmotic influences of the extracellular environment that may be important in vivo have been removed. Optimal solute diffusion between the cytosol and extracellular milieu that is present in the isolated myocyte system does not exist in vivo, where coronary
artery disease and hypertrophy alter capillary diffusion distances. Furthermore, many drugs are more potent in isolated cell preparations than in intact tissue, which may result in exaggerated effects. However, such limitations also define the strength of this system, because an assessment can be made of the direct effects of propofol on the function of the fundamental contractile unit of the heart, the myocyte. Future studies that correlate propofol-induced alterations in contractile function in isolated myocytes with measurements of contractility in vitro should provide a more useful assessment of alterations in contractile function than either model alone.

In summary, chronic pacing tachycardia caused LV pump dysfunction similar to the clinical spectrum of CHF and was associated with concomitant isolated myocyte contractile dysfunction. Propofol had a direct, concentration-dependent, negative effect on isolated myocyte contractile function in normal myocytes and with the development of CHF. Importantly, CHF myocytes were more sensitive to the negative effects of propofol on baseline contractile function at low concentrations.

References

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