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# *Propofol Attenuates β-Adrenoreceptor–mediated Signal Transduction via a Protein Kinase C–dependent Pathway in Cardiomyocytes*

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***Background:*** **Activation of β adrenoreceptors by catecholamines is an important mechanism for increasing the inotropic state of the heart. The objectives of the current study were to investigate the effects of propofol on β-adrenoreceptor–mediated increases in cardiomyocyte intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), cell shortening, L-type $Ca^{2+}$ current ($I_{Ca}$) and cyclic adenosine monophosphate (cAMP) accumulation. The authors also investigated the site of action of propofol in the β-adrenoreceptor signaling pathway, as well as the role of protein kinase C (PKC), and tested the hypothesis that propofol would inhibit the β-adrenoreceptor signaling pathway *via* a PKC-dependent mechanism.**

***Methods:*** **Freshly isolated ventricular myocytes were obtained from adult rat and guinea pig hearts. Myocyte shortening (video edge detection) and $[Ca^{2+}]_i$ (fura-2, 340/380 ratio) were monitored simultaneously in individual cells. Conventional whole cell patch clamp analysis was used to measure the $I_{Ca}$ in individual myocytes. cAMP production was assessed in suspensions of myocytes using an enzyme immunoassay kit.**

***Results:*** **Propofol (0.1–10 μM) had no effect on steady state $[Ca^{2+}]_i$, cell shortening, $I_{Ca}$, or cAMP production. In contrast, propofol caused dose-dependent decreases in isoproterenol-stimulated increases in $[Ca^{2+}]_i$, shortening, $I_{Ca}$, and cAMP. Forskolin-induced increases in $[Ca^{2+}]_i$, shortening, and cAMP production were not altered by propofol. PKC activation with phorbol myristate acetate attenuated isoproterenol-stimulated cAMP production. Inhibition of PKC with bisindolylmaleimide (broad range inhibitor) or Gö 6976 (inhibitor of $Ca^{2+}$-dependent PKC isoforms) abolished propofol-induced inhibition of isoproterenol-stimulated increases in $[Ca^{2+}]_i$, shortening, and cAMP production.**

***Conclusions:*** **Clinically relevant concentrations of propofol attenuate β-adrenergic signal transduction in cardiac myocytes *via* inhibition of cAMP production. The inhibitory site of action of propofol is upstream of adenylyl cyclase and involves activation of PKCα.**

INDUCTION of anesthesia with propofol is known to cause cardiovascular depression in patients with and without cardiac disease. This effect is mainly attributed to a decrease in sympathetic activity and systemic vasodilation.[1,2] Many *in vitro* studies using normal cardiac tissue have reported that propofol has either no direct effect on contractile function[3–6] or has a modest negative inotropic effect at supraclinical concentrations.[7–11] Catecholamine-induced activation *via* β-adrenoreceptor stimulation is a primary mechanism for increasing the inotropic state of the myocardium. Because propofol is also widely used for the sedation of critically ill patients receiving catecholamines for hemodynamic support, the extent to which propofol alters catecholamine-induced cardiac inotropy is of clinical interest.

Recent evidence suggests that propofol may exert direct inhibitory effects on β-adrenoreceptor signal transduction in cardiac muscle.[12–15] However, the cellular mechanisms mediating these effects have not been identified. Activation of cardiac β-adrenoreceptors increases cyclic adenosine monophosphate (cAMP) production, which increases myocardial contraction (inotropy) and accelerates the rate of myocardial relaxation (lusitropy). The inotropic and lusitropic effects of β-adrenoreceptor stimulation are mediated primarily *via* an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and a decrease in myofilament $Ca^{2+}$ sensitivity, respectively.[16,17] In addition, the positive lusitropic effect is also partially mediated by increased rate of $Ca^{2+}$ uptake back into the sarcoplasmic reticulum in response to phospholamban phosphorylation.[18] Using freshly dispersed ventricular myocytes, our first objective was to identify the extent to which propofol alters $[Ca^{2+}]_i$, cell shortening, L-type $Ca^{2+}$ current ($I_{Ca}$), and cAMP production in response to β-adrenoreceptor stimulation with isoproterenol. A second objective was to identify a potential site of action and cellular mechanism for propofol-induced changes in cardiac function during β-adrenoreceptor activation. Our results demonstrate that clinically relevant concentrations of propofol attenuate β-adrenoreceptor signal transduction at a site upstream of adenylyl cyclase, resulting in a decrease in cAMP production and $I_{Ca}$. The cellular mechanism of action involves a PKC-dependent pathway.

## Materials and Methods

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

### *Ventricular Myocyte Preparation*

Ventricular myocytes were freshly isolated from adult male Sprague-Dawley rat hearts as previously described.[7]

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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% $O_2$–5% $CO_2$) Krebs-Henseleit buffer (KHB; 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 $Ca^{2+}$-free KHB containing collagenase type II (347 U/ml; Worthington Biochemical Corp., Freehold, NJ). After digestion with collagenase (20 min), the ventricles were minced and shaken in KHB, and the resulting cellular digest was washed, filtered, and resuspended in phosphate-free HEPES-buffered saline (HBS; 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.

Ventricular myocytes were also obtained from adult male Hartley guinea pigs as previously described.[19] Briefly, excised hearts were subjected to coronary perfusion *via* the aorta with KHB containing 120 mM NaCl, 4.8 mM KCl, 1.5 mM $CaCl_2$, 2.2 mM $MgSO_4$, 1.2 mM $NaH_2PO_4$, 25 mM $NaHCO_3$, and 11 mM glucose. The buffer's pH was maintained at 7.35 by bubbling with 95% $O_2$–5% $CO_2$ at 37°C. Immediately after removal, the heart was perfused with normal $Ca^{2+}$-containing KHB for 5 min, followed by $Ca^{2+}$-free KHB for an additional 5 min, and then a $Ca^{2+}$-free KHB containing collagenase B (0.5–0.7 mg/ml; Boehringer Mannheim, Indianapolis, IN) for 45 min. After perfusion, the atria were removed and the ventricles minced, rinsed free of collagenase, and reintroduced to $Ca^{2+}$-containing KHB. Gentle trituration freed individual cells for use in patch clamp experiments.

### *Measurement of Intracellular $Ca^{2+}$ Concentration and Shortening*

Simultaneous measurement of $[Ca^{2+}]_i$ and cell shortening was performed as previously described.[7] Ventricular myocytes ($0.5 \times 10^6$ cells/ml) were incubated in HBS containing 2 μM fura-2–acetoxy methylester at room temperature for 15 min. Fura-2–loaded ventricular myocytes were placed in a temperature-regulated (28°C) 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 HBS at a flow rate of 2 ml/min and field-stimulated *via* bipolar platinum electrodes at a frequency of 0.3 Hz with a 5-ms pulse using a Grass SD9 stimulator (Grass Instruments, West Warwick, RI). Myocytes showing a rod-shaped appearance with clear striations were chosen for study.

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 Intracellular $Ca^{2+}$ Concentration and Shortening Data*

The following parameters were calculated for each individual contraction: diastolic $[Ca^{2+}]_i$ and cell length, systolic $[Ca^{2+}]_i$ and cell length, change in $[Ca^{2+}]_i$ (systolic $[Ca^{2+}]_i$ minus diastolic $[Ca^{2+}]_i$) and twitch amplitude, time to peak (Tp) for $[Ca^{2+}]_i$ and peak shortening, and time to 50% and 90% (T50r and T90r) diastolic $[Ca^{2+}]_i$ and 50% and 90% relengthening. Parameters from 10 contractions were averaged to obtain mean values at baseline and in response to the various interventions. Averaging the parameters over time minimizes beat-to-beat variation.

### *Measurement of L-type $Ca^{2+}$ Current*

Microelectrodes were pulled from borosilicate glass capillary tubing (Corning 8161; Warner Instrument Corp., Hamden, CT) and had resistances between 0.5 and 1.5 MΩ when filled with the following intracellular solution: 125 mM CsCl, 20 mM TEA-Cl, 10 mM HEPES, 10 mM EGTA, 5 mM MgATP, and 3.6 mM creatine phosphate, pH 7.2. The control extracellular solution contained 140 mM NaCl, 5.4 mM CsCl, 2.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5.5 mM HEPES, and 11 mM glucose; pH was adjusted to 7.4 with NaOH. Because of technical difficulties in obtaining a good seal with the patch pipette lasting the entire duration of the protocol, electrophysiologic studies in rat cardiomyocytes were not successful. Myocytes isolated from guinea pig hearts were placed in a 0.5-ml chamber (28°C) and superfused by gravity with experimental solutions. Currents were recorded with an Axopatch 200B voltage clamp amplifier (Axon Instruments, Union City, CA) and an IBM-compatible computer using pCLAMP software (Axon Instruments). A 3 M KCl-agar bridge was used to ground the bath. Cells were

voltage clamped at −40 mV to inactivate the $Na^+$ current. The time course of changes in $Ca^{2+}$ conductance was monitored by applying a 75-ms test pulse to 0 mV once every 10 s. The magnitude of $I_{Ca}$ was determined by measuring the peak inward current recorded during the step to 0 mV. The peak amplitude of $I_{Ca}$ was normalized for cell membrane capacitance and the current density (in picoamperes per picofarad) was used to assess the effects of propofol. When the pulses were applied at 0.1 Hz, the rundown of $I_{Ca}$ in most myocytes occurred during the initial 5–10 min after the patch was broken. To minimize the influence of rundown on the measurements and to optimize comparisons between groups, the time window between 10–20 min after the initial recording was chosen to measure $I_{Ca}$ with respect to drug effects. Only one concentration of drug was tested in each preparation. In addition, only currents that returned to at least 80% of their initial magnitude after washout were included in the study. Myocytes that showed marked or progressive rundown were excluded from the study.

*Measurement of Cyclic Adenosine Monophosphate*

Suspensions of rat ventricular myocytes were used for determining cAMP production. The experimental buffer was the same as that used in the $[Ca^{2+}]_i$ and shortening experiments (HBS). At the end of the protocol, the cells were quickly pelleted using a microfuge (500*g*, 5 s). The buffer was aspirated and the pellet was resuspended in ice-cold HBS and centrifuged again (500*g*, 5 s). The supernatant was aspirated and frozen using liquid nitrogen. Samples were then thawed on ice. Freezing and thawing was repeated three times. The preparations were homogenized in ice-cold ethanol (0.5 ml) to extract the cAMP. Homogenates were centrifuged (1,500*g*, 10 min) and the supernatants collected. The pellet was washed with 0.5 ml ethanol-water (2:1) and centrifuged again at 1,500*g* for 10 min. The supernatants were combined and dried under nitrogen. Samples were stored at −20°C. Production of cAMP was assessed using an enzyme-linked immunoassay kit (Cayman Chemical, Ann Arbor, MI) and normalized for protein content using the method of Bradford.[20]

*Experimental Protocols*

**Protocol 1: Effect of Propofol on Steady State Intracellular $Ca^{2+}$ Concentration and Shortening in Rat Ventricular Myocytes.** To determine the effect of propofol on steady state myocyte function, we examined changes in shortening and $[Ca^{2+}]_i$ during exposure to propofol. Pure propofol was used to avoid any possible effect of the intralipid emulsion diluent on cell signaling pathways. Baseline measurements were collected from individual myocytes for 2 min in the absence of propofol. Myocytes were then exposed to propofol (1, 10, 30, and 100 μM) in a cumulative fashion and allowed to equilibrate for 5 min at each concentration.

**Protocol 2: Effect of Propofol on Isoproterenol-induced Increases in Intracellular $Ca^{2+}$ Concentration and Shortening in Rat Ventricular Myocytes.** To identify the extent to which propofol alters β-adrenoreceptor–mediated increases in $[Ca^{2+}]_i$ and shortening, we activated β adrenoreceptors with isoproterenol and examined the changes in myocyte shortening and $[Ca^{2+}]_i$ during subsequent exposure to propofol. Baseline measurements were collected from individual myocytes for 2 min. Myocytes were exposed to isoproterenol (10 nM), and data were acquired after a 5-min equilibration period. Propofol (1, 10, 30, and 100 μM) was then added cumulatively to the isoproterenol-containing buffer and allowed to equilibrate. Data were acquired after a 5-min equilibration period. In other experiments, multiple doses of isoproterenol were used to assess the effects of propofol (3, 10, 30, or 100 μM) on the isoproterenol dose–response relation. When examining the role of PKC as a mediator of propofol-induced effects on isoproterenol-stimulated $[Ca^{2+}]_i$ and shortening, we pretreated the cells with the broad-range PKC inhibitor bisindolylmaleimide I (1 μM)[21] or Gö 6976 (1 μM), an inhibitor of $Ca^{2+}$-dependent PKC isoforms,[22] for 10 min before stimulation with isoproterenol and incubation with propofol (1, 10, 30, 100 μM). Neither inhibitor had an effect on steady state or isoproterenol-stimulated $[Ca^{2+}]_i$ or shortening. When examining the specificity of propofol for the β-adrenergic signaling pathway, we increased cardiac inotropy using Bay K8644 (1 μM: L-type $Ca^{2+}$ channel agonist) and then added propofol cumulatively to the Bay K8644–containing buffer. Similar experiments were performed using forskolin (adenylyl cyclase activator) to bypass the β-adrenergic receptor and increase cardiac inotropy.

**Protocol 3: Effect of Propofol on Isoproterenol-induced Increase in L-type $Ca^{2+}$ Current in Guinea Pig Ventricular Myocytes.** To determine whether propofol alters the β-adrenoreceptor–mediated increase in $[Ca^{2+}]_i$ and shortening *via* inhibition of $I_{Ca}$, we measured the extent to which propofol alters both steady state $I_{Ca}$ and the isoproterenol-induced increase in $I_{Ca}$ using conventional whole cell patch clamp analysis. After rundown of the current reached a steady state (approximately 10 min), propofol (0.1, 1, 10 μM) or isoproterenol (30 nM) was added to the perfusate, and effects on $I_{Ca}$ were assessed. In other experiments, the response to isoproterenol in the presence of propofol was measured. For these experiments, the control response to isoproterenol was first measured, followed by washout and pretreatment with propofol (10 min) before readdition of isoproterenol to the perfusate. In this fashion, each cell served as its own control. Ascorbic acid (50 μM) was added to all solutions to prevent oxidative degradation of isoproterenol.

**Protocol 4: Effect of Propofol on Cyclic Adenosine Monophosphate Production in Rat Ventricular Myocytes.** Suspensions of rat ventricular myocytes ($10^6$ cells/ml) were preincubated with 3-isobutyl-1-methylxanthine (0.5 mM; phosphodiesterase inhibitor) for 5 min at 37°C. After 3-isobutyl-1-methylxanthine pretreatment, propofol (1, 10, 100 μM), isoproterenol (100 nM), or forskolin (1 μM) was added to the cells (10 min), and effects on steady state cAMP concentrations were assessed. In other experiments, propofol (0.1–100 μM) was added for 10 min before addition of isoproterenol (100 nM) or forskolin (1 μM). When examining the role of PKC as a mediator of propofol-induced effects on cAMP production, we pretreated the cells with bisindolylmaleimide I (1 μM) or Gö 6976 (1 μM) for 10 min before incubation with propofol (10 min) and stimulation with isoproterenol. Separate experiments included a positive control for PKC activation using phorbol myristate acetate (1 μM).

### *Statistical Analysis and Data Presentation*

Each experimental protocol was performed on multiple myocytes from the same heart and repeated in at least four hearts. Results obtained from myocytes in each heart were averaged so all hearts were weighted equally. Data are reported as mean ± standard error of the mean. Statistical comparisons within groups were made by one-way analysis of variance for repeated measures coupled with Student *t* test. Two-way analysis of variance was used for between-group comparisons. A $P$ value $<$ 0.05 was considered statistically significant.

### *Materials*

Propofol, phorbol myristate acetate, and bisindolylmaleimide I were obtained from Research Biochemicals International (Natick, MA) and solubilized in dimethylsulfoxide to appropriate stock concentrations. Isoproterenol, forskolin, ascorbic acid, and 3-isobutyl-1-methylxanthine were obtained from Sigma Chemical Co. (St. Louis, MO). Gö 6976 was obtained from Calbiochem (La Jolla, CA).

## Results

### *Baseline Parameters for Intracellular $Ca^{2+}$ Concentration and Shortening*

The baseline 340/380 ratio was 0.87 ± 0.02. The change in the 340/380 ratio with shortening was 0.47 ± 0.03. Diastolic cell length was 113 ± 4 μm. Twitch height was 10.5 ± 2.3 μm (9.3 ± 1.5% resting cell length). Tp for $[Ca^{2+}]_i$ and shortening was 203 ± 5 and 245 ± 12 ms, respectively. T50r for $[Ca^{2+}]_i$ and shortening was 197 ± 8 and 224 ± 21 ms, respectively. T90r for $[Ca^{2+}]_i$ and shortening was 607 ± 52 and 579 ± 65 ms, respectively.

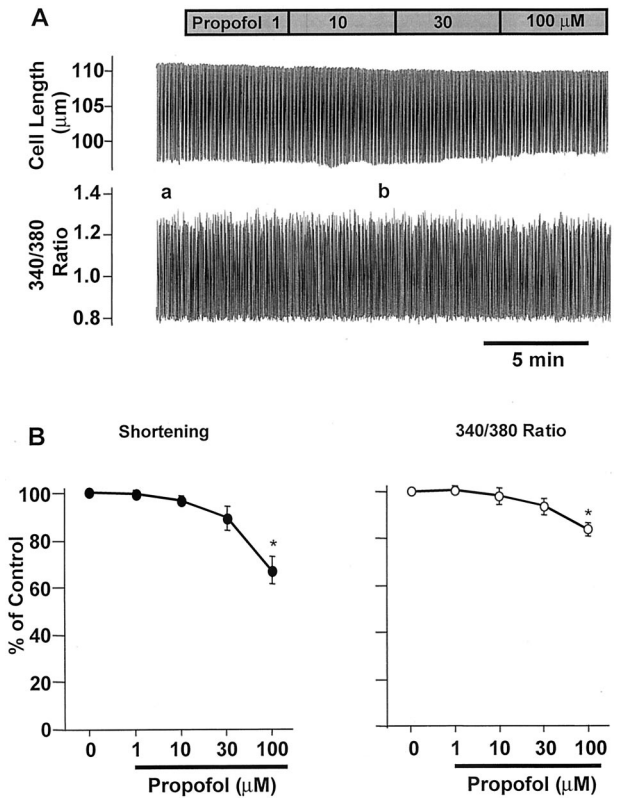


**Fig. 1. (*A*) Original traces demonstrating the effect of propofol on steady state shortening (*top*) and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) (*bottom*) in a rat ventricular myocyte. Propofol was added to individual field-stimulated myocytes at the concentrations depicted in the figure. Changes in cell length were measured in micrometers. $[Ca^{2+}]_i$ was measured as the 340/380 ratio. (*B*) Summarized data for the effects of propofol on steady state shortening and $[Ca^{2+}]_i$. Results are expressed as percent of control. Values represent mean ± standard error of the mean. *$P < 0.05$ compared with control. n = 14 cells from 6 hearts.**

### *Effect of Propofol on Steady State Intracellular $Ca^{2+}$ Concentration and Shortening*

A representative trace depicting the dose-dependent effects of propofol on shortening and $[Ca^{2+}]_i$ in an electrically stimulated ventricular myocyte is shown in figure 1A. As we previously reported,[7] low doses (1–30 μM) of propofol had no effect on shortening or peak $[Ca^{2+}]_i$. However, propofol (30 μM) reduced ($P < 0.05$) resting cell length from 113 ± 4 to 110 ± 3 μm with no effect on baseline $[Ca^{2+}]_i$. A supraclinical concentration of propofol (100 μM) attenuated shortening and peak $[Ca^{2+}]_i$. Summarized data for the dose-dependent effects of propofol on shortening and $[Ca^{2+}]_i$ are shown in figure 1B. An exploded view of the individual $[Ca^{2+}]_i$ transient and shortening is illustrated in figure 2A, and continuous $[Ca^{2+}]_i$:cell length relations are depicted as hysteresis loops in figure 2B. Propofol (10 μM) had no significant effect on peak $[Ca^{2+}]_i$ or shortening (fig. 2A, left) but caused a slight upward shift in the continuous $[Ca^{2+}]_i$:shortening relation (fig. 2B). Figure 2A (right) is an overlay of the individual cell shortening and $[Ca^{2+}]_i$ transient normalized to peak height to illustrate changes

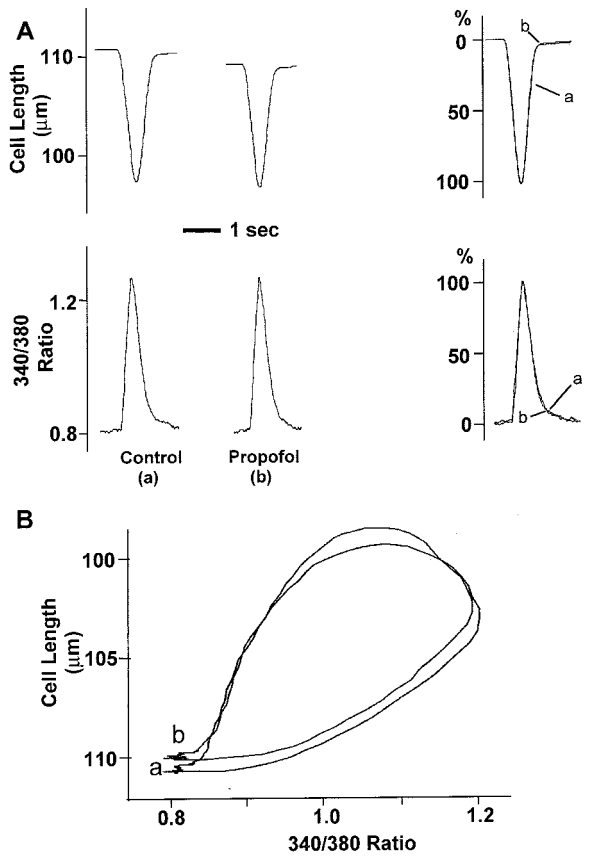


**Fig. 2. (*A*, *left*) Exploded view of individual shortening and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) transients taken from (*a*) control and (*b*) propofol (= 10 μM) in fig. 1A. (*Right*) Overlay of the individual cell shortening and $[Ca^{2+}]_i$ transient normalized to peak height to demonstrate changes in timing. (*B*) Hysteresis loops depicting the continuous $Ca^{2+}$:shortening relations.**

in timing. Propofol (10 μM) had no effect on the timing parameters (Tp, T50r, T90r) for either shortening or $[Ca^{2+}]_i$ under steady state conditions.

### *Effect of Isoproterenol on Intracellular $Ca^{2+}$ Concentration and Shortening*

A representative trace depicting cell shortening and $[Ca^{2+}]_i$ before and after exposure to isoproterenol is illustrated in figure 3A. During baseline conditions, the myocyte shortens from 120 μm down to 110 μm during contraction. After stimulation with isoproterenol (10 nM), cell shortening is increased, as demonstrated by cell length changing from 120 to 100 μm (223 ± 25%) during contraction that lasted up to 30 min. Isoproterenol also increased $[Ca^{2+}]_i$ (147 ± 9%). The effects of isoproterenol on $[Ca^{2+}]_i$ and shortening were dose-dependent (fig. 3B). An exploded view of the individual $[Ca^{2+}]_i$ transient and cell shortening is illustrated in figure 4A. Isoproterenol reduced ($P < 0.05$) Tp for shortening from 245 ± 9 to 203 ± 4 ms without any effect on Tp for $[Ca^{2+}]_i$. Isoproterenol (10 nM) reduced T50r and T90r for $[Ca^{2+}]_i$ from 197 ± 8 to 141 ± 5 ms and from 607 ± 52 to 313 ± 16 ms, respectively ($P < 0.05$). Isoproterenol also reduced T50r and T90r for shortening from 224 ± 21 to 149 ± 8 ms and from 579 ± 65 to 267 ± 9 ms, respectively ($P < 0.05$). In addition to this positive lusitropic effect, isoproterenol caused a rightward and upward shift in the $[Ca^{2+}]_i$:cell length relation (fig. 4B).

### *Effect of Propofol on Isoproterenol-stimulated Intracellular $Ca^{2+}$ Concentration and Shortening*

Figure 5A is a representative trace depicting the dose-dependent effects of propofol on shortening and $[Ca^{2+}]_i$ after exposure to isoproterenol (10 nM). The summarized data are shown in figure 5B. Propofol attenuated isoproterenol-induced increases in shortening and $[Ca^{2+}]_i$ in a dose-dependent manner. Pretreatment with propofol also caused dose-dependent decreases in isoproterenol-stimulated changes in shortening and $[Ca^{2+}]_i$ (fig. 3B). Figure 6A (left) is an exploded view of the individual $[Ca^{2+}]_i$ transients and cell shortening, demonstrating

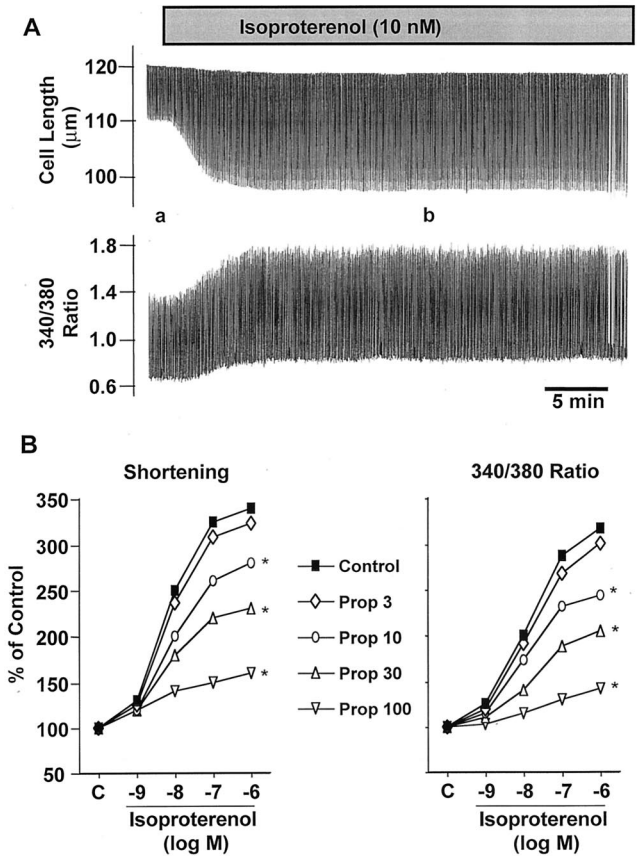


**Fig. 3. (*A*) Original traces demonstrating the effect of isoproterenol (10 nM) on shortening and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in rat ventricular myocytes. a = control; b = isoproterenol. (*B*) Summarized data for the dose-dependent effects of isoproterenol on myocyte shortening and $[Ca^{2+}]_i$. The effects of propofol (Prop) pretreatment on isoproterenol-stimulated increases in shortening and $[Ca^{2+}]_i$ are also presented. Results are expressed as percent of control (C). *$P < 0.05$ compared with control. n = 15 cells from 6 hearts.**

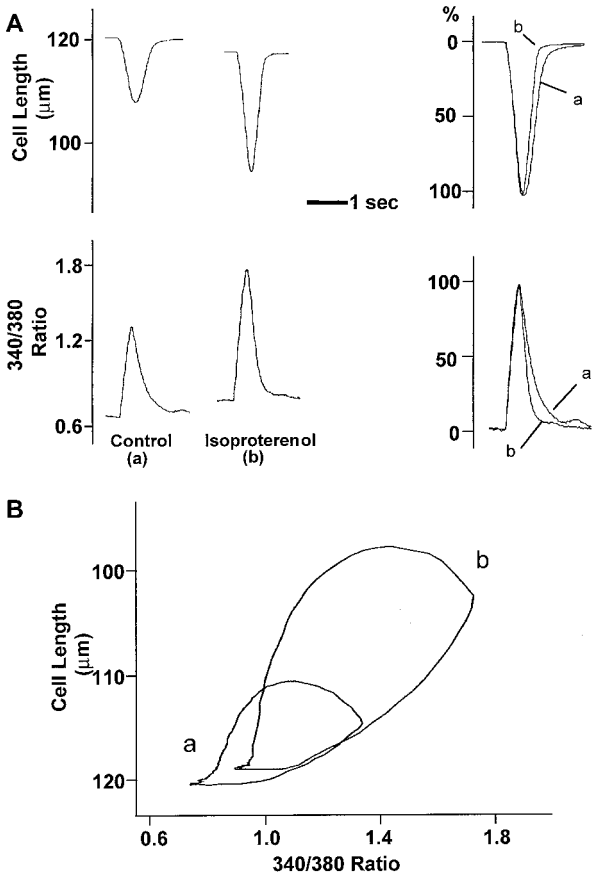


**Fig. 4. (*A*, *left*) Exploded view of individual shortening and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) transients taken from a and b in fig. 3A. (*Right*) Overlay of the individual shortening and $[Ca^{2+}]_i$ transients normalized to peak height to demonstrate changes in timing. (*B*) Hysteresis loops depicting the continuous $Ca^{2+}$:shortening relations.**

attenuation by propofol (10 μM) of the isoproterenol-induced positive inotropic effect. Figure 6A (right) illustrates that the lusitropic effect of isoproterenol on shortening was not altered by propofol. Propofol (10 μM) partially reversed the effect of isoproterenol on T50r and T90r for $[Ca^{2+}]_i$ from 142 ± 15 to 168 ± 12 ms ($P < 0.08$) and from 322 ± 12 to 388 ± 9 ms ($P < 0.07$), respectively. Propofol (100 μM) prolonged ($P < 0.05$) T50r and T90r for $[Ca^{2+}]_i$ from 137 ± 8 to 185 ± 14 ms and 312 ± 15 to 427 ± 11 ms, respectively. There was no concomitant effect of propofol (100 μM) on T50r or T90r for shortening. Figure 6B shows the continuous $[Ca^{2+}]_i$:shortening relation. Propofol attenuated the rightward and upward shift in the hysteresis loop induced by isoproterenol. In control experiments, propofol (30 μM) did not alter Bay K8644–induced increases in $[Ca^{2+}]_i$ (92 ± 7% of control) or shortening (95 ± 8% of control).

### *Effect of Propofol on Isoproterenol-stimulated L-type $Ca^{2+}$ Current*

The effect of propofol on steady state $I_{Ca}$ is shown in figure 7A. Propofol did not alter steady state $I_{Ca}$. The effect of propofol on isoproterenol-stimulated increases in $I_{Ca}$ is shown in figure 7B. Isoproterenol (30 nM) increased peak $I_{Ca}$ by 123 ± 13% compared with the steady state control. Propofol reduced the isoproterenol-stimulated increase in $I_{Ca}$ in a dose-dependent manner.

### *Effect of Propofol on Isoproterenol-stimulated Cyclic Adenosine Monophosphate Production*

Propofol alone did not alter basal cAMP accumulation (fig. 8A). Isoproterenol (100 nM) increased cAMP production by 614 ± 37%. Pretreatment with propofol caused a dose-dependent reduction in the isoproterenol-stimulated increase in cAMP (fig. 8B). In contrast, direct activation of adenylyl cyclase with forskolin (1 μM) stimulated a 494 ± 70% increase in cAMP production that was not altered by pretreatment with propofol (fig. 8C). Propofol (30 μM) also did not alter forskolin-induced increases in $[Ca^{2+}]_i$ (93 ± 7% of control) or shortening (94 ± 9% of control).

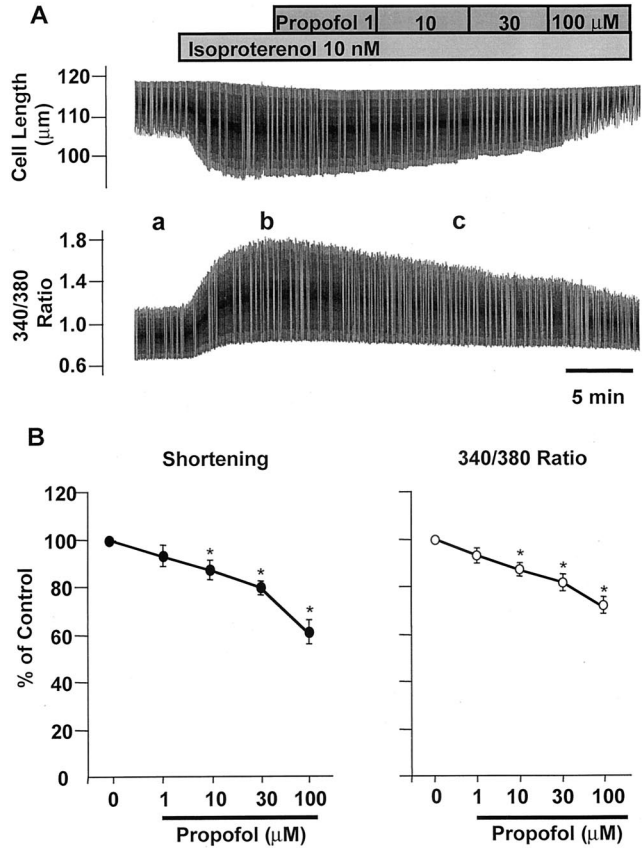


**Fig. 5. (*A*) Original traces depicting the dose-dependent effects of propofol on shortening and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) after exposure to isoproterenol (10 nM) in a rat ventricular myocyte. (*B*) Summarized data for the effects of propofol on isoproterenol-stimulated increases in shortening and $[Ca^{2+}]_i$. Results are expressed as percent of control. *$P < 0.05$ compared with control. n = 18 cells from 5 hearts.**

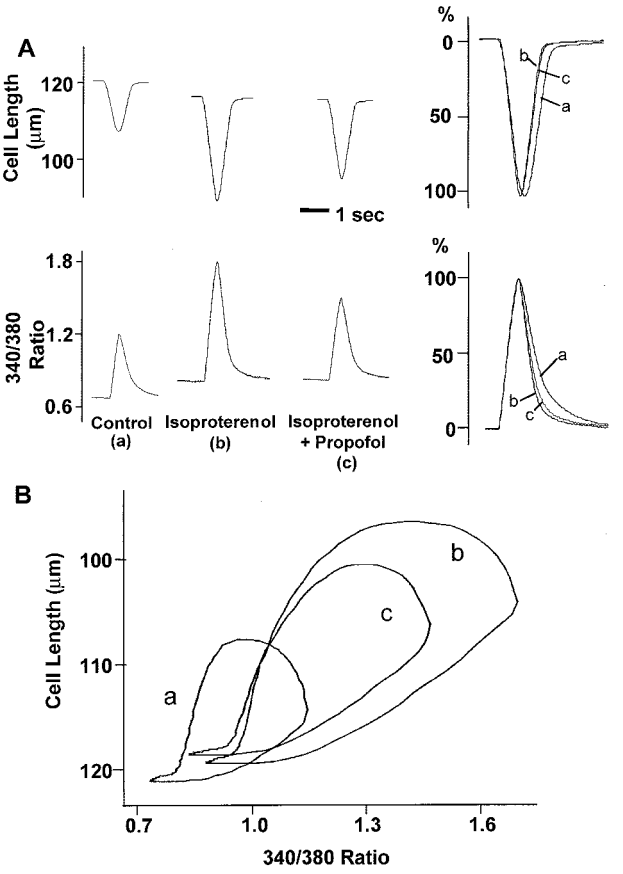


**Fig. 6. (*A*, *left*) Exploded view of individual shortening and intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) transients taken from a, b, and c in fig. 5A. (*Right*) Overlay of the individual cell shortening and $[Ca^{2+}]_i$ transients normalized to peak height to demonstrate changes in timing. (*B*) Hysteresis loops depicting the continuous $Ca^{2+}$:shortening relations.**

### *Effect of Protein Kinase C Inhibition on Propofol-induced Attenuation of Isoproterenol-stimulated Cyclic Adenosine Monophosphate Production*

Figure 9A demonstrates that PKC activation with phorbol myristate acetate caused a dose-dependent decrease in isoproterenol-stimulated cAMP production. Inhibition of PKC with the broad-range inhibitor bisindolylmaleimide I (1 μM) prevented the propofol-induced decrease in isoproterenol-stimulated cAMP production (fig. 9B). Similarly, inhibition of $Ca^{2+}$-dependent PKC isoforms with Gö 6976 abolished the propofol-induced decrease in isoproterenol-stimulated cAMP production (fig. 9C).

### *Effect of Protein Kinase C Inhibition on Propofol-induced Attenuation of Isoproterenol-stimulated Intracellular $Ca^{2+}$ Concentration and Shortening*

Figure 10A demonstrates that PKC inhibition with bisindolylmaleimide I abolished the propofol-induced reduction of isoproterenol-stimulated $[Ca^{2+}]_i$ and shortening at clinically relevant concentrations. Inhibition of $Ca^{2+}$-dependent PKC isoforms with Gö 6976 had similar effects on propofol-induced changes in isoproterenol-stimulated $[Ca^{2+}]_i$ and shortening (Fig. 10B).

## Discussion

Because neural, humoral, and local mechanisms interact to regulate myocardial contractility *in vivo*, *in vitro* preparations allow for assessment of direct actions of anesthetics on cellular mechanisms that regulate cardiac function. To our knowledge, this is the first study to directly measure $[Ca^{2+}]_i$, shortening, $I_{Ca}$, and cAMP production in cardiomyocytes during β-adrenoreceptor stimulation in the presence or absence of propofol. Our major findings are that propofol, at clinically relevant concentrations, has no effect on steady state $[Ca^{2+}]_i$, shortening, $I_{Ca}$, or cAMP levels. In contrast, propofol attenuates isoproterenol-stimulated increases in $[Ca^{2+}]_i$, shortening, $I_{Ca}$, and cAMP production. The inhibitory site of action within the β-adrenergic signal transduction pathway is upstream of adenylyl cyclase and involves activation of a PKC-dependent pathway.

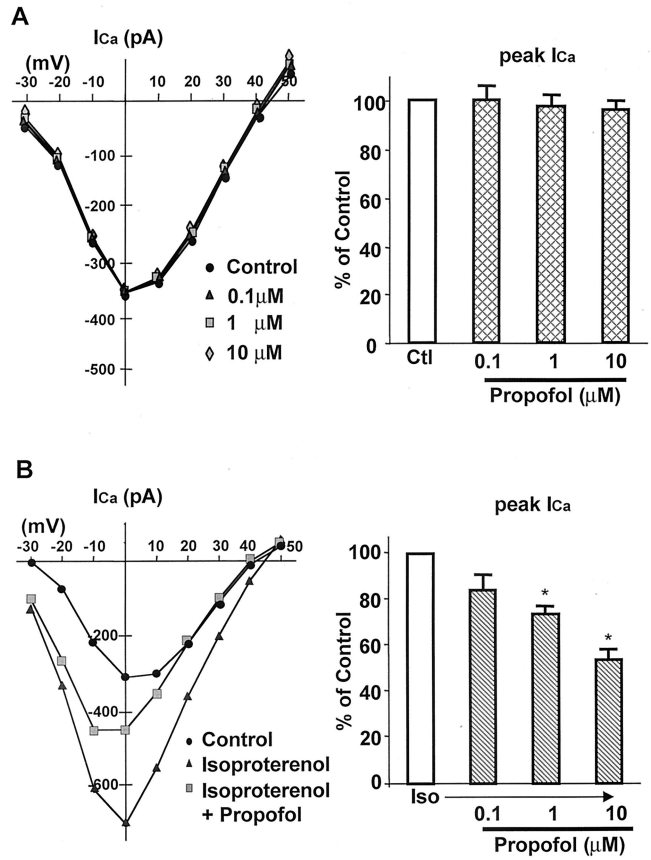


**Fig. 7. (*A*) Current–voltage relation depicting effect of propofol on steady state L-type $Ca^{2+}$ current ($I_{Ca}$; *left*) in guinea pig ventricular myocytes. Summarized data (*right*). (*B*) Current–voltage relation depicting effect of propofol on isoproterenol-induced increase in $I_{Ca}$ (*left*). Summarized data (*right*). Results are expressed as percent of control. *$P < 0.05$ compared with control. n = 10 cells from 4 hearts.**

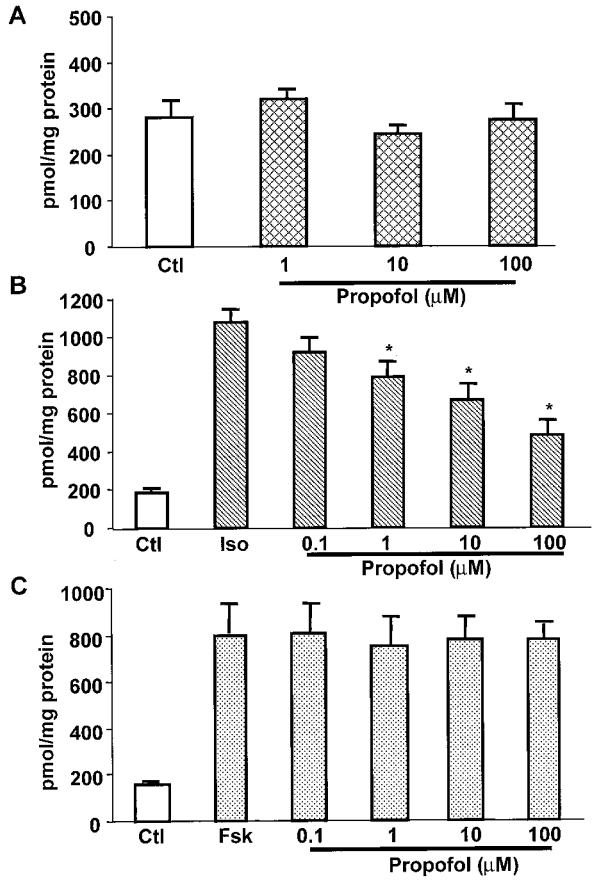


**Fig. 8. (*A*) Summarized data for the effect of propofol on steady state cyclic adenosine monophosphate (cAMP) production in rat ventricular myocytes. (*B*) Summarized data for the effect of propofol on isoproterenol-stimulated cAMP production. (*C*) Summarized data for the effect of propofol on forskolin (FSK)-stimulated cAMP production. Results are expressed as picomoles per milligram protein. *$P < 0.05$ compared with isoproterenol. n = 10 hearts. Ctl = control.**

### *Propofol and Steady State Intracellular $Ca^{2+}$ Concentration and Shortening*

The effects of propofol on myocardial contractility have previously been evaluated using a variety of experimental preparations. Results from most *in vitro* studies indicate that propofol exerts little if any direct negative inotropic effect at clinically relevant concentrations.[4,7,11] However, propofol is known to have multiple sites of action, including inhibitory effects on L-type $Ca^{2+}$ channels[23–25] and sarcoplasmic reticulum $Ca^{2+}$ handling.[26] These inhibitory effects on $Ca^{2+}$ dynamics would result in negative inotropy, but cardiac depression could be masked by a concomitant increase in myofilament $Ca^{2+}$ sensitivity[7,27,28] Our results confirm previous findings by our laboratory using the intralipid emulsion form of propofol[7] and demonstrate that pure propofol exerts no significant inhibitory effect on steady state $[Ca^{2+}]_i$ or shortening at clinically relevant concentrations. Similar to our previous findings,[7] supraclinical concentrations of propofol (100 μM) decreased peak $[Ca^{2+}]_i$ and shortening, likely *via* inhibitory effects on the $I_{Ca}$.[23,24] Propofol also caused a decrease in resting cell length with no concomitant increase in baseline $[Ca^{2+}]_i$, indirectly confirming that propofol increases myofilament $Ca^{2+}$ sensitivity.[27,28]

### *Isoproterenol-stimulated Intracellular $Ca^{2+}$ Concentration and Shortening*

Activation of β adrenoreceptors by catecholamines is an important mechanism for increasing the inotropic state of the heart. In this study, activation of β adrenoreceptors with isoproterenol resulted in positive inotropy and lusitropy, as previously reported by other investigators.[12,29] The response to β-adrenoreceptor stimulation is mediated *via* a G-protein–dependent increase in adenylyl cyclase activity and cAMP production within the myocyte, causing activation of protein kinase A. The positive inotropic effect is thought to be primarily mediated by protein kinase A–dependent phosphorylation of the L-type $Ca^{2+}$ channel, resulting in an increase

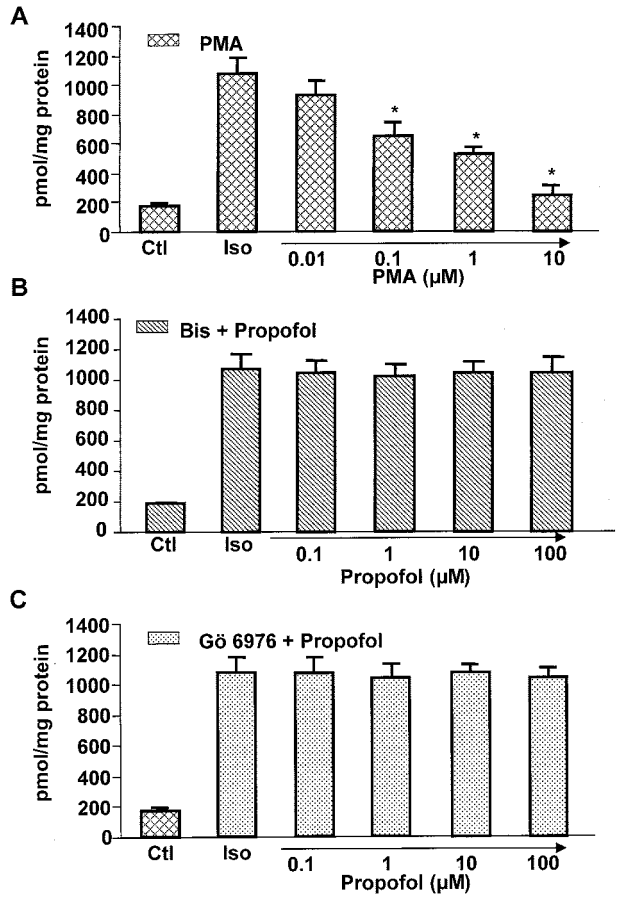


**Fig. 9. (*A*) Summarized data for the effect of phorbol myristate acetate (PMA) on isoproterenol-stimulated cyclic adenosine monophosphate (cAMP) production in rat ventricular myocytes. (*B*) Summarized data for the effect of bisindolylmaleimide I (Bis) on isoproterenol (Iso)-stimulated cAMP production. (*C*) Summarized data for the effect of Gö 6976 on isoproterenol-stimulated cAMP production. Results are expressed as picomoles per milligram protein. *$P < 0.05$ compared with isoproterenol. n = 8 hearts.**

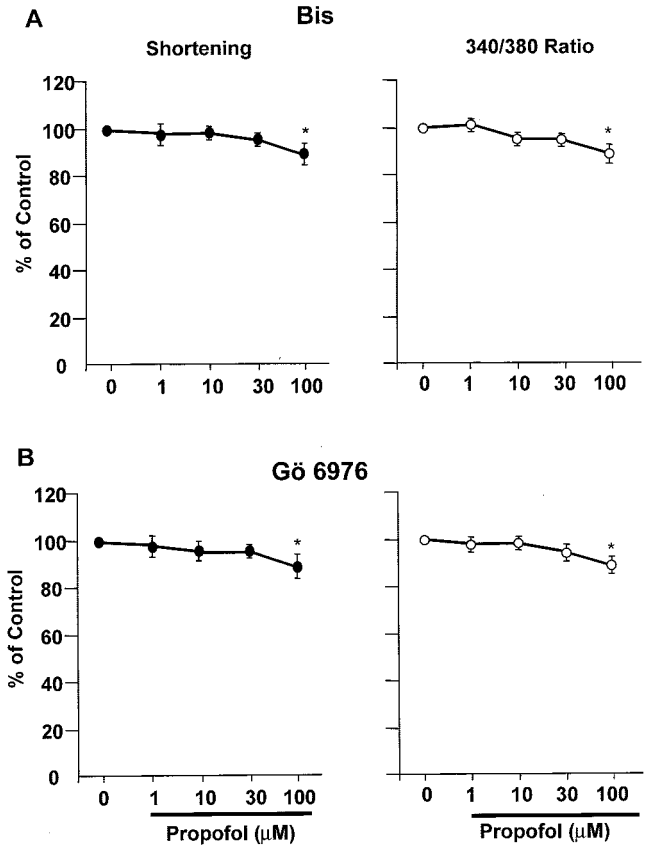


**Fig. 10. (*A*) Summarized data for the effects of bisindolylmaleimide I (Bis) on propofol-induced attenuation of isoproterenol-stimulated intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and shortening. (*B*) Summarized data for the effects of Gö 6976 on propofol-induced attenuation of isoproterenol-stimulated $[Ca^{2+}]_i$ and shortening. *$P < 0.05$ compared with control. For bisindolylmaleimide I, n = 13 cells from 7 hearts. For Gö 6976, n = 11 cells from 5 hearts.**

in $I_{Ca}$ and $[Ca^{2+}]_i$.[16] In contrast, the positive lusitropic effect is thought to be mediated primarily by protein kinase A–dependent changes in troponin I phosphorylation, causing decreased affinity of troponin C to bind $Ca^{2+}$ (*i.e.*, a decrease in myofilament $Ca^{2+}$ sensitivity).[17] Protein kinase A–dependent phosphorylation of phospholamban enhances the rate of uptake of $Ca^{2+}$ into the sarcoplasmic reticulum during relaxation and increases the amount of $Ca^{2+}$ available for release during contraction, thereby contributing to both lusitropic and inotropic effects, respectively.[18] Therefore, activation of $\beta$ adrenoreceptors induces important effects on myocardial contraction and relaxation that are mediated *via* an increase in $[Ca^{2+}]_i$ and a concomitant decrease in myofilament $Ca^{2+}$ sensitivity.

### *Propofol and Isoproterenol-stimulated Intracellular $Ca^{2+}$ Concentration and Shortening*

Propofol is widely used for the sedation of critically ill patients who are receiving catecholamines for hemodynamic support. However, the extent to which propofol alters $\beta$-adrenoreceptor signal transduction in cardiac muscle remains controversial. Hebbar *et al.*[13,14] reported that clinically relevant concentrations of propofol (1 $\mu$g/ml = 5.6 $\mu$M) reduced the inotropic response of swine cardiomyocytes to isoproterenol, although the site of action and cellular mechanism were not investigated. Propofol has been reported to reduce myocardial $\beta$-adrenoreceptor ligand binding and responsiveness in rat myocardium.[15] In contrast, Lejay *et al.*[12] reported that propofol (10 $\mu$g/ml = 56 $\mu$M) did not alter the isoproterenol-induced positive inotropic effect but enhanced the positive lusitropic effect in rat papillary muscle. In our study, propofol (10 $\mu$M) attenuated the isoproterenol-induced increase in $[Ca^{2+}]_i$ and shortening, which indicates that propofol inhibits $\beta$-adrenoreceptor–mediated cardiac inotropy at clinically relevant concentrations. This was not a nonspecific effect, because propofol did not inhibit increases in $[Ca^{2+}]_i$ and shortening in response to the L-type $Ca^{2+}$ channel agonist, Bay K8644. The lusitropic effect of isoproterenol was partially reversed by propofol at low concentrations but only reached statistical significance at 100 $\mu$M. Because the lusitropic effect is primarily mediated *via* alterations in the binding affinity of troponin C for $Ca^{2+}$ (decrease $Ca^{2+}$ sensitivity), it is possible that the partial reversal of the isoproterenol-induced lusitropic effect by propofol could be mediated by an increase in the affinity of troponin C for $Ca^{2+}$ (increased $Ca^{2+}$ sensitivity). This would be consistent with our previous study demonstrating a propofol-induced increase in myofilament $Ca^{2+}$ sensitivity mediated by a $Na^+$–$H^+$ exchange-dependent increase in intracellular pH.[28]

### *Propofol and Isoproterenol-stimulated L-type $Ca^{2+}$ Current*

The increase in $[Ca^{2+}]_i$ after $\beta$-adrenoreceptor activation is mediated primarily *via* an increase in the $I_{Ca}$. Some studies have indicated that propofol can directly inhibit $I_{Ca}$ in cardiomyocytes,[23–25] perhaps through a direct interaction with the dihydropyridine-binding site,[24] although the clinical relevance is still controversial.[25] In our study, clinically relevant doses of propofol (0.1–10 $\mu$M) had no significant effect on steady state $I_{Ca}$ but attenuated the increase in $I_{Ca}$ induced by isoproterenol. These data support the concept that the propofol-induced depression of the isoproterenol-stimulated increase in $[Ca^{2+}]_i$ and shortening is mediated by a decrease in $I_{Ca}$, although we cannot rule out an effect of propofol on sarcoplasmic reticulum $Ca^{2+}$ stores.[26,27] Again, these data further support the hypothesis that propofol interferes with the $\beta$-adrenergic signaling pathway in cardiomyocytes.

### *Propofol and Isoproterenol-stimulated Cyclic Adenosine Monophosphate Production*

If propofol is directly interacting with the $\beta$-adrenoreceptor signaling pathway, then propofol should attenu-

ate isoproterenol-induced increases in cAMP production. Propofol had no effect on steady state cAMP concentrations in cardiomyocytes. However, propofol attenuated the isoproterenol-induced increase in cAMP production in a dose-dependent manner. In contrast, propofol did not alter the increase in cAMP induced by direct activation of adenylyl cyclase with forskolin. These data indicate that propofol interacts with the β-adrenoreceptor signaling pathway at a site upstream of adenylyl cyclase. Other intravenous anesthetics have been reported to alter cAMP production in response to agonist activation. Ketamine inhibited cytokine-induced reductions in intracellular cAMP accumulation in a rat heart cell line,[30] and diazepam enhanced cAMP production induced by isoproterenol and forskolin.[31]

*Propofol and Protein Kinase C*

We recently reported that propofol increases myofilament $Ca^{2+}$ sensitivity and intracellular pH *via* a PKC-dependent activation of the $Na^+$–$H^+$ exchanger.[28] In addition, it has been demonstrated that activation of PKC attenuates β-adrenergic responsiveness in the rat heart[32] and β-adrenergic–mediated increases in $I_{Ca}$ in rat ventricular myocytes.[33] In the current study, phorbol myristate acetate attenuated isoproterenol-induced cAMP production. This indicates that PKC activation can negatively regulate β-adrenoreceptor signal transduction, resulting in a decrease in cAMP production. In addition, the propofol-induced inhibition of isoproterenol-stimulated cAMP production was attenuated by bisindolylmaleimide I and Gö 6976. Furthermore, bisindolylmaleimide I and Gö 6976 virtually abolished the propofol-induced inhibition of isoproterenol-stimulated increases in $[Ca^{2+}]_i$ and shortening, which further supports a functional role for PKC in mediating the effects of propofol. Taken together, these data suggest that the mechanism by which propofol exerts its effects on the β-adrenergic signaling pathway is *via* activation of a $Ca^{2+}$-dependent PKC isoform. The isoform involved is likely PKCα, because this is the only $Ca^{2+}$-dependent PKC isoform that exists in adult rat cardiomyocytes,[34] and its site of action appears to be upstream of adenylyl cyclase. One possibility is that propofol-induced activation of PKC may result in direct phosphorylation of the β-adrenergic receptor, leading to receptor desensitization.[35] Alternatively, PKC activation may indirectly cause β-adrenergic receptor desensitization *via* phosphorylation of β-adrenergic receptor kinases.[36,37]

**Limitations of the Study.** One limitation of the study is that the experiments were performed at 28°C with a low stimulation rate. The studies were performed during these conditions to maintain myocyte viability and reduce spontaneous contractions. These experimental conditions could alter excitation–contraction coupling or enzymatic regulatory processes compared with *in vivo* conditions. Another potential limitation is that pure propofol solubilized in dimethylsulfoxide was used instead of the commercially available 10% intralipid emulsion. This allowed us to directly assess the effects of propofol, independent of the vehicle, on cardiac inotropy. In addition, guinea pig myocytes were used for the electrophysiologic studies. It is known that there are differences between rat and guinea pig myocytes in terms of action potential characteristics, sarcoplasmic reticulum $Ca^{2+}$ handling, and the $Na^+$–$Ca^{2+}$ exchanger in the steady state regulation of excitation contraction coupling. Finally, the validity of interpreting externally unloaded myocyte shortening as an indicator of the inotropic state of the myocardium is a potential limitation, because the force developed during contraction is unknown. Even without an external load, the myocytes are shortening against an internal load composed of several components.[38]

In summary, propofol, at clinically relevant concentrations, attenuates β-adrenergic signal transduction in cardiac myocytes *via* inhibition of cAMP production. The inhibitory site of action of propofol appears to be upstream of adenylyl cyclase and involves activation of PKC.

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