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# *Propofol Increases Contractility during $\alpha_{1a}$-Adrenoreceptor Activation in Adult Rat Cardiomyocytes*

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***Background:*** **The objective of this study was to identify the extent to which propofol alters intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), myofilament $Ca^{2+}$ sensitivity, and contraction of individual cardiomyocytes during activation of $\alpha_{1a}$ adrenoreceptors and to determine the cellular mechanism of action.**

***Methods:*** **Freshly isolated ventricular myocytes were obtained from adult rat hearts. Myocyte shortening and $[Ca^{2+}]_i$ were simultaneously monitored in individual cardiomyocytes exposed to phenylephrine after treatment with chloroethylclonidine ($\alpha_{1b}$-adrenoreceptor antagonist) and BMY 7378 ($\alpha_{1d}$-adrenoreceptor antagonist). Data are reported as mean ± SD.**

***Results:*** **Phenylephrine increased myocyte shortening by 124 ± 9% ($P = 0.002$), whereas peak $[Ca^{2+}]_i$ only increased by 8 ± 3% ($P = 0.110$). Inhibition of phospholipase $A_2$ and phospholipase C attenuated the phenylephrine-induced increase in shortening by 84 ± 11% ($P = 0.004$) and 15 ± 6% ($P = 0.010$), respectively. Inhibition of protein kinase C (PKC) and Rho kinase attenuated the phenylephrine-induced increase in shortening by 17 ± 8% ($P = 0.010$) and 74 ± 13% ($P = 0.006$), respectively. In the presence of phenylephrine, propofol increased shortening by 40 ± 6% ($P = 0.002$), with no concomitant increase in $[Ca^{2+}]_i$. PKC inhibition prevented the propofol-induced increase in shortening. Selective inhibition of PKCα, PKCδ, PKCε, and PKCζ reduced the propofol-induced increase in shortening by 12 ± 5% ($P = 0.011$), 36 ± 8% ($P = 0.001$), 32 ± 9% ($P = 0.007$), and 19 ± 5% ($P = 0.008$), respectively. $Na^+$–$H^+$ exchange inhibition reduced the propofol-induced increase in shortening by 56 ± 7% ($P = 0.001$).**

***Conclusion:*** **Activation of $\alpha_{1a}$ adrenoreceptors increases cardiomyocyte shortening primarily *via* a phospholipase $A_2$-dependent, Rho kinase–dependent increase in myofilament $Ca^{2+}$ sensitivity. Propofol further increases myofilament $Ca^{2+}$ sensitivity and shortening *via* a PKC-dependent pathway and an increase in $Na^+$–$H^+$ exchange activity.**

CATECHOLAMINE-INDUCED stimulation of cardiac α and β adrenoreceptors (ARs) activates multiple signal transduction pathways that act collectively to increase myocardial performance. The increase in cardiac contractile function results from increases in the availability of intracellular free $Ca^{2+}$ or myofilament $Ca^{2+}$ sensitivity in the cardiac muscle cells or both. $\beta_1$-AR–induced increases in the inotropic state of the heart are primarily mediated by increasing the availability of 3′-5′-cyclic adenosine monophosphate and intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$),[1] whereas $\beta_2$-AR activation is not coupled to changes in $Ca^{2+}$ dynamics or contraction.[1] In contrast, the mechanisms by which α-AR activation increases cardiac inotropy are more complex and still controversial.[2–6] The existence of specific α-AR subtypes (*e.g.*, $\alpha_{1a}$, $\alpha_{1b}$, $\alpha_{1d}$) on cardiomyocytes contributes to this complexity, because these receptor subtypes are coupled to divergent signaling pathways that can have opposing actions on cardiac inotropy.[5,7–9]

Because increased concentrations of circulating catecholamines occur in the perioperative period, the extent to which anesthetic agents alter catecholamine-induced cardiac inotropy are of clinical relevance. Propofol is widely used to induce anesthesia for cardiac and general surgery for postoperative sedation, and for a variety of outpatient procedures. We recently demonstrated that propofol attenuates β-AR–mediated increases in $[Ca^{2+}]_i$, cAMP, and cardiomyocyte inotropy at a site upstream of adenylyl cyclase *via* activation of protein kinase C (PKC).[10]

In the current study, freshly dispersed individual ventricular myocytes pretreated with the $\alpha_{1b}$-AR antagonist chloroethylclonidine and the $\alpha_{1d}$-AR antagonist BMY 7378 were used to specifically examine the role of $\alpha_{1a}$-AR activation on cardiomyocyte $[Ca^{2+}]_i$ and contraction. Only one previous study has addressed the relation between propofol and α-AR activation of cardiac muscle, and that study did not assess the effects of propofol *during* α-AR activation.[11] Moreover, the signal transduction pathway for $\alpha_1$-AR activation and regulation of $[Ca^{2+}]_i$ and myofilament $Ca^{2+}$ sensitivity are not clear but may involve activation of Rho kinase (ROK),[12,13] an increase in myosin light chain phosphorylation,[13] and an increase in myofilament $Ca^{2+}$ sensitivity.[14] Therefore, our first objective was to identify the extent to which phospholipase and protein kinase activation play a role in mediating $\alpha_{1a}$-AR–induced alterations in $[Ca^{2+}]_i$ and contraction. A second objective was to examine the extent to which propofol alters cardiomyocyte $[Ca^{2+}]_i$ and contraction in the setting of $\alpha_{1a}$-AR activation and to determine the cellular mechanism responsible for this effect. We tested the hypothesis that during $\alpha_{1a}$-AR activation, propofol increases myofilament $Ca^{2+}$ sensitivity *via* a PKC-dependent mechanism involving $Na^+$–$H^+$ exchange. The rationale for this experimental approach is that we previously demonstrated a propofol-induced, PKC-dependent phosphorylation of contractile proteins[15] and an increase in intracellular pH *via* PKC-dependent activation of $Na^+$–$H^+$ exchange.[16]

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## Materials and Methods

All experimental procedures and protocols were approved by the Cleveland Clinic Foundation Institutional Animal Care and Use Committee (Cleveland, Ohio) and conform to the international guidelines for the care and use of animals.

### *Ventricular Myocyte Preparation*

Ventricular myocytes were freshly isolated from adult male Sprague-Dawley rat hearts as previously described.[15,16] 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, at a pH of 7.35. After a 5-min equilibration period, the perfusion buffer was changed to $Ca^{2+}$-free Krebs-Henseleit buffer 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 Krebs-Henseleit buffer, and the resulting cellular digest was washed, filtered, and resuspended in phosphate-free 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, at a pH of 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.[15,16] Ventricular myocytes were incubated in HEPES-buffered saline containing 2 μM fura-2/AM at 37°C for 20 min. Fura-2–loaded ventricular myocytes were placed in a temperature-regulated chamber (Bioptechs Inc., Butler, PA) mounted on the stage of an Olympus IX70 inverted fluorescence microscope (Olympus America, Lake Success, NY). The cells were superfused continuously with HEPES-buffered saline throughout the experiment and field-stimulated *via* bipolar platinum electrodes using a Grass SD9 stimulator (Grass Instruments, West Warwick, RI). Fluorescence measurements were performed on single ventricular myocytes using a dual-wavelength spectrofluorometer at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The cells were also illuminated with red light at a wavelength above 600 nm for simultaneous measurement of myocyte shortening using a video-edge detector (Crescent Electronics, Sandy, UT). The video-edge detector was calibrated using a stage micrometer so that cell lengths during shortening and relengthening could be measured. 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 a software package from Photon Technology International (Lawrenceville, NJ). Fluorescence data for the $[Ca^{2+}]_i$ transients and myocyte shortening were imported into Lab View (National Instruments, Austin TX), where both the $[Ca^{2+}]_i$ transients and myocyte contractile responses were analyzed synchronously and simultaneously. We verified that propofol had no effect on fura-2 fluorescence at the concentrations tested. This was confirmed in separate cell-free experiments using fura-2 (pentapotassium salt) in buffers ranging from $10^{-9}$ M to $10^{-5}$ M in the presence or absence of propofol (data not shown).

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

The following variables 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. Variables from 10 contractions were averaged to obtain mean values at baseline and in response to the various interventions. Averaging the variables over time minimizes beat-to-beat variation.

### *Experimental Protocols*

**Protocol 1: Effect of $\alpha_{1a}$-AR Activation on $[Ca^{2+}]_i$ and Shortening** Baseline measurements were collected from individual myocytes for 5 min in the presence of chloroethylclonidine (1 μM), an inhibitor of $\alpha_{1b}$-AR, and BMY 7378 (1 μM), an inhibitor of $\alpha_{1d}$-AR, in this and all subsequent protocols. Alpha-1a ARs were subsequently activated with phenylephrine (1–100 μM), and $[Ca^{2+}]_i$ and shortening were measured until a new steady state was achieved (10 min). The new steady state values in the presence of phenylephrine are referred to as the control responses.

**Protocol 2: Effect of Phospholipase Inhibition on $\alpha_{1a}$-AR–induced Increases in $[Ca^{2+}]_i$ and Shortening** Phenylephrine (10 μM) was applied to the cardiomyocyte, and $[Ca^{2+}]_i$ and shortening were monitored. After establishment of a new steady state increase in shortening, phenylephrine was washed out (10 min), and U73122 (50 μM; $IC_{50}$ = 2 μM) or $AACOCF_3$ (50 μM; $IC_{50}$ = 15 μM) was added (10 min) to inhibit phospholipase C (PLC) or phospholipase $A_2$ ($PLA_2$), respectively. Phenylephrine (10 μM) was again added to the cardiomyocyte until the increase in shortening had achieved a new steady state. Time control experiments were also performed in the absence of the inhibitor. This approach

was used in all protocols assessing the effects of putative inhibitors on the phenylephrine response.

**Protocol 3: Effect of Protein Kinase Inhibition on $\alpha_{1a}$-AR–induced Increases in $[Ca^{2+}]_i$ and Shortening** Cardiomyocytes were pretreated (10 min) with the broad-range PKC inhibitor bisindolylmaleimide I (Bis, 10 $\mu$M; $IC_{50}$ = 10 nM) or the ROK inhibitor Y27632 (10 $\mu$M; $IC_{50}$ = 140 nM), and the changes in myocyte shortening and $[Ca^{2+}]_i$ were examined during subsequent exposure to phenylephrine (10 $\mu$M).

**Protocol 4: Effect of Propofol on $[Ca^{2+}]_i$ and Shortening after $\alpha_{1a}$-AR Activation** Alpha-1a ARs were activated with phenylephrine (10 $\mu$M) in individual cardiomyocytes, and the changes in myocyte $[Ca^{2+}]_i$ and shortening were examined during subsequent exposure to propofol (1, 10, 30 $\mu$M).

**Protocol 5: Effect of PKC Inhibition on Propofol-induced Changes in Shortening in the Presence of $\alpha_{1a}$-AR Activation** Cardiomyocytes were pretreated with the broad-range PKC inhibitor Bis (10 $\mu$M); a selective inhibitor of $Ca^{2+}$-dependent PKC isoforms, Gö 6976 (10 $\mu$M; $IC_{50}$ = 2 nM); a selective inhibitor of PKC$\delta$, rottlerin (10 $\mu$M; $IC_{50}$ = 3 $\mu$M); a selective inhibitor of PKC$\varepsilon$, myristoylated PKC$\varepsilon$ V1-2 (10 $\mu$M; $IC_{50}$ = 100 nM); or a selective inhibitor of PKC$\zeta$, myristoylated PKC$\zeta$ inhibitor peptide (10 $\mu$M; $IC_{50}$ = 100 nM) for 10 min after stimulation with phenylephrine (10 $\mu$M) and before incubation with propofol (1, 10, 100 $\mu$M). In a separate set of experiments, isoproterenol (1 $\mu$M) was added to demonstrate that a ceiling effect on cardiomyocyte shortening had not been achieved in the presence of phenylephrine and Bis.

**Protocol 6: Effect of $Na^+$–$H^+$ Exchange Inhibition on Propofol-induced Change in Shortening** Activation of $Na^+$–$H^+$ exchange results in intracellular alkalinization, which increases myofilament $Ca^{2+}$ sensitivity,[17] and propofol increases intracellular pH in cardiomyocytes *via* a PKC-dependent pathway.[16] Cardiomyocytes were pretreated (10 min) with or without the $Na^+$–$H^+$ exchange inhibitor HOE 694 (1 $\mu$M; $IC_{50}$ = 50 nM) before addition of propofol.

*Statistical Analysis*

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 that all hearts were weighted equally. Comparison of several means was performed using two-way analysis of variance and the Newman-Keuls test. The Bonferroni *post hoc* correction was used when significant differences among the groups were detected. All $P$ values are one tailed, and differences were considered significant at $P < 0.05$. All results are expressed as mean ± SD.

*Materials*

Propofol was obtained from Aldrich Chemical Co. (Milwaukee, WI). Phenylephrine, chloroethylclonidine, and BMY 7378 were obtained from Sigma Chemical Co. (St. Louis, MO). Phorbol myristic acetate, HOE 694, Bis, and Y-27632 were obtained from Calbiochem (San Diego, CA). $AACOCF_3$, U73122, Gö 6976, myristoylated PKC$\varepsilon$ V1-2 peptide, myristoylated PKC$\zeta$ inhibitor peptide, and rottlerin were obtained from BIOMOL (Plymouth Meeting, PA). Fura-2/AM was obtained from Texas Fluorescence Labs (Austin, TX).

## Results

*Baseline Values for $[Ca^{2+}]_i$ and Shortening*

Resting cell length was 132 ± 9 $\mu$m, and the baseline 340/380 ratio was 0.9 ± 0.2. Twitch height was 11.0 ± 1.8 $\mu$m (8.3 ± 1.4% of the resting cell length). The change in 340/380 ratio from baseline with shortening was 0.6 ± 0.1. Tp $[Ca^{2+}]_i$ and Tp shortening were 143 ± 18 and 171 ± 21 ms, respectively. Tr $[Ca^{2+}]_i$ and Tr shortening were 183 ± 18 and 217 ± 24 ms, respectively.

*Effect of $\alpha_{1a}$-AR Activation on $[Ca^{2+}]_i$ and Shortening*

The combined presence of chloroethylclonidine (1 $\mu$M) and BMY 7378 (1 $\mu$M) had no effect on baseline values for $[Ca^{2+}]_i$ or shortening (data not shown). A representative trace depicting the effect of phenylephrine on $[Ca^{2+}]_i$ and shortening in a single field-stimulated ventricular myocyte is shown in figure 1A. Phenylephrine (10 $\mu$M) increased peak shortening without a concomitant increase in $[Ca^{2+}]_i$. A decrease in resting cell length of 2.0 ± 0.4 $\mu$m with no change in resting $[Ca^{2+}]_i$ was observed in most cells. Summarized data for the concentration-dependent effects of phenylephrine on $[Ca^{2+}]_i$ and shortening are shown in figure 1B. Phenylephrine caused concentration-dependent increases in shortening ($P$ = 0.001), with no significant change in $[Ca^{2+}]_i$. Phenylephrine (10 $\mu$M) had no significant effect on Tp $[Ca^{2+}]_i$ (97 ± 6% of baseline; not significant [NS]), Tp shortening (95 ± 5% of baseline; NS), Tr $[Ca^{2+}]_i$ (93 ± 8% of baseline; NS), or Tr shortening (92 ± 7% of baseline; NS).

*Effect of Phospholipase Inhibition on $\alpha_{1a}$-AR–induced Increase in Shortening*

Neither U73122 nor $AACOCF_3$ alone had an effect on baseline $[Ca^{2+}]_i$ or shortening. Summarized data for the effects of U73122 or $AACOCF_3$ on the phenylephrine-induced increase in shortening are depicted in figure 2. Inhibition of PLC with U73122 reduced the phenylephrine-induced increase in shortening by 15 ± 6% ($P$ = 0.017), whereas inhibition of $PLA_2$ with $AACOCF_3$ atten-

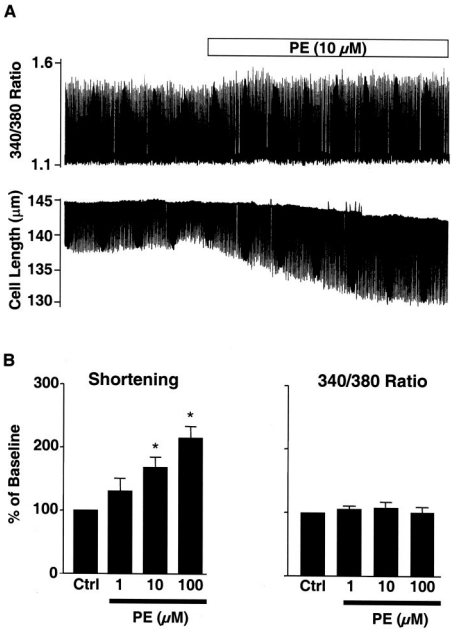


**Fig. 1. (*A*) Representative trace demonstrating the effect of $\alpha_{1a}$-adrenoreceptor activation with phenylephrine (PE) on myocyte shortening and intracellular free $Ca^{2+}$ concentration in a single ventricular myocyte. (*B*) Summarized data for the effects of phenylephrine on myocyte shortening and intracellular free $Ca^{2+}$ concentration. Results are expressed as percent of baseline. Values represent mean ± SD in this and all subsequent figures. Control (Ctrl) represents the steady state baseline value for twitch amplitude or the change in intracellular free $Ca^{2+}$ concentration, set to 100%. * $P < 0.05$ compared with control. n = 18 cells from 7 hearts.**

uated the phenylephrine-induced increase in shortening by 84 ± 11% ($P = 0.003$).

### *Effect of Protein Kinase Inhibition on $\alpha_{1a}$-AR–induced Increase in Shortening*

Summarized data for the effects of Bis (10 μM) and Y-27632 (10 μM) on the phenylephrine-induced (10 μM) increase in shortening are shown in figure 3. Inhibition of PKC with Bis reduced the phenylephrine-induced increase in shortening by 17 ± 8% ($P = 0.014$), whereas inhibition of ROK with Y-27632 inhibited the phenylephrine-induced increase in shortening by 74 ± 13% ($P = 0.001$).

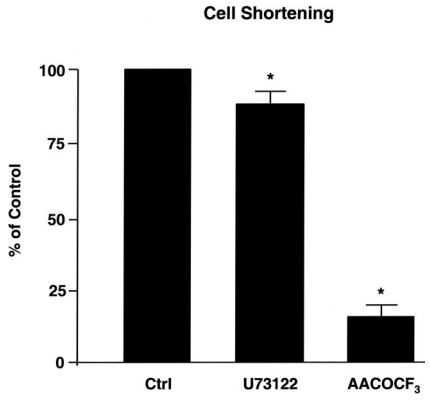


**Fig. 2. Summarized data depicting the effect of phospholipase C inhibition with U73122 (50 μM) or phospholipase $A_2$ inhibition with $AACOCF_3$ (50 μM) on the phenylephrine-induced increase in shortening. Results are expressed as percent of control (Ctrl), which represents the twitch amplitude achieved with phenylephrine in the absence of the inhibitors, set to 100%. * $P < 0.05$ compared with control. n = 14 cells from 5 hearts (U73122) and n = 12 cells from 4 hearts (Y-27632).**

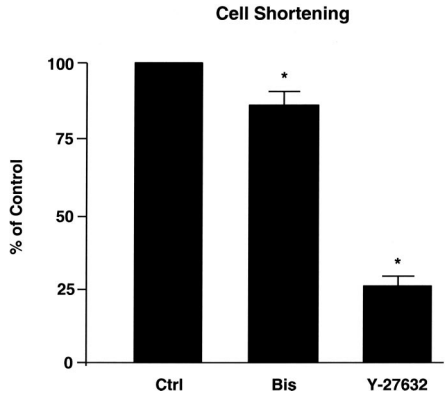


**Fig. 3. Summarized data depicting the effect of protein kinase C inhibition with bisindolylmaleimide I (Bis; 10 μM) or Rho kinase inhibition with Y-27632 (10 μM) on the phenylephrine-induced (10 μM) increase in shortening. Results are expressed as percent of control (Ctrl), which represents the twitch amplitude achieved with phenylephrine in the absence of the inhibitors, set to 100%. * $P < 0.05$ compared with control. n = 11 cells from 4 hearts (Bis) and n = 13 cells from 5 hearts (Y-27632).**

### *Effect of Propofol on $[Ca^{2+}]_i$ and Shortening During $\alpha_{1a}$-AR Activation*

A representative trace depicting the effect of propofol (30 μM) on $[Ca^{2+}]_i$ and shortening during $\alpha_{1a}$-AR activation with phenylephrine (10 μM) is shown in figure 4A. Summarized data depicting the concentration-dependent effects of propofol on $[Ca^{2+}]_i$ and shortening during $\alpha_{1a}$-AR are shown in figure 4B. Propofol caused concentration-dependent increases in shortening ($P = 0.002$), with no significant effect on $[Ca^{2+}]_i$. Propofol (30 μM) had no significant effect on Tp $[Ca^{2+}]_i$ (103 ± 7%; NS), Tp shortening (98 ± 5%; NS), Tr $[Ca^{2+}]_i$ (104 ± 5%; NS), or Tr shortening (109 ± 5%; NS) during $\alpha_{1a}$-AR activation.

### *Effect of PKC Inhibition on Propofol-induced Increase in Shortening during $\alpha_{1a}$-AR Activation*

A representative trace depicting the effect of PKC inhibition with Bis on the propofol-induced increase in shortening is shown in figure 5A. Summarized data for the concentration-dependent effects of propofol on $[Ca^{2+}]_i$ and shortening in the presence of Bis are shown in figure 5B. After $\alpha_{1a}$-AR activation and in the continued presence of phenylephrine, addition of Bis (10 μM) increased $[Ca^{2+}]_i$ and shortening by 14 ± 4% ($P = 0.021$) and 27 ± 6% ($P = 0.003$), respectively. Under these conditions, Bis completely inhibited the propofol-induced increase in shortening. However, activation of the β-AR signaling pathway with isoproterenol (10 nM) after pretreatment with phenylephrine and Bis increased

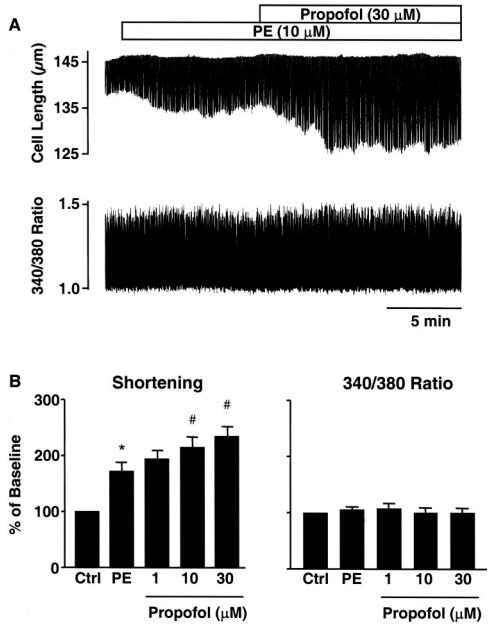


**Fig. 4. (*A*) Representative trace demonstrating the effect of propofol (30 μM) on myocyte shortening and intracellular free $Ca^{2+}$ concentration in a single ventricular myocyte during $\alpha_{1a}$-adrenoreceptor activation with phenylephrine (PE; 10 μM). (*B*) Summarized data for the concentration-dependent effects of propofol on myocyte shortening and intracellular free $Ca^{2+}$ concentration during $\alpha_{1a}$-adrenoreceptor activation. Results are expressed as percent of baseline value. Control (Ctrl) represents the steady state baseline value for twitch amplitude or the change in intracellular free $Ca^{2+}$ concentration, set to 100%. * $P < 0.05$ compared with control; #$P < 0.05$ compared with phenylephrine. n = 17 cells from 6 hearts.**

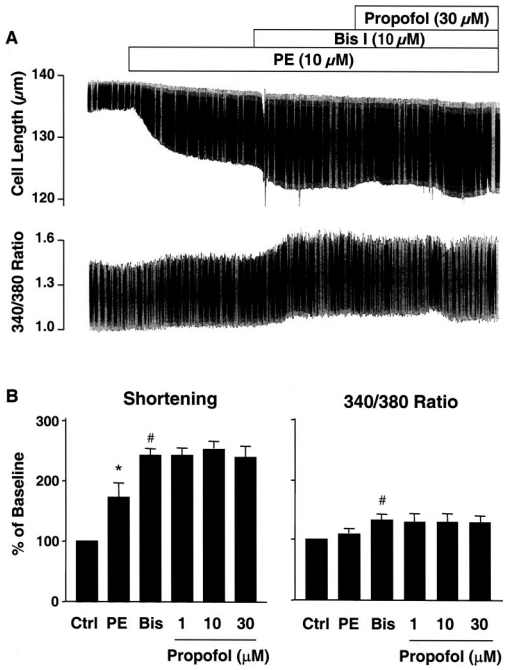


**Fig. 5. (*A*) Representative trace demonstrating the effect of bisindolylmaleimide I (Bis; 10 μM) and Bis plus propofol (30 μM) on myocyte shortening and intracellular free $Ca^{2+}$ concentration in a single ventricular myocyte during $\alpha_{1a}$-adrenoreceptor activation with phenylephrine (PE; 10 μM). (*B*) Summarized data for the concentration-dependent effects of propofol during $\alpha_{1a}$-adrenoreceptor activation on myocyte shortening and intracellular free $Ca^{2+}$ concentration in the presence of protein kinase C inhibition with Bis. Results are expressed as percent of baseline value. Control (Ctrl) represents the steady state baseline value for twitch amplitude or the change in intracellular free $Ca^{2+}$ concentration, set to 100%. * $P < 0.05$ compared with control; #$P < 0.05$ compared with phenylephrine. n = 18 cells from 7 hearts.**

$[Ca^{2+}]_i$ and shortening by 25 ± 5% ($P = 0.001$) and 48 ± 9% ($P = 0.001$), respectively, which demonstrates that the inhibitory effect of Bis on propofol-induced increases in shortening is not due to a ceiling effect. Propofol (30 μM) had no effect on Tp $[Ca^{2+}]_i$ (97 ± 6%; NS), Tp shortening (94 ± 8%; NS), Tr $[Ca^{2+}]_i$ (101 ± 7%; NS), or Tr shortening (96 ± 6%; NS) in the presence of Bis or during $\alpha_{1a}$-AR activation.

We next assessed the extent to which selective inhibitors of specific PKC isoforms were involved in mediating the propofol-induced increase in shortening. Summarized data for the effects of PKCα inhibition with Gö 6976 (10 μM), PKCδ inhibition with rottlerin (10 μM), PKCε inhibition with myristoylated PKCε V1-2 (10 μM), and PKCζ inhibition with myristoylated PKCζ inhibitor peptide (10 μM) on the propofol-induced increase in shortening are shown in figure 6. Inhibition of PKCα, PKCδ, PKCε, and PKCζ reduced the propofol-induced increase in shortening during $\alpha_{1a}$-AR activation by 12 ± 5% ($P = 0.011$), 36 ± 8% ($P = 0.001$), 32 ± 9% ($P = 0.007$), and 19 ± 5% ($P = 0.008$), respectively.

### *Effect of $Na^+$-$H^+$ Exchange Inhibition on Propofol-induced Increase in Shortening*

The $Na^+$-$H^+$ exchange inhibitor HOE 694 (10 μM) had no significant effect on cell shortening during $\alpha_{1a}$-AR activation with phenylephrine (96 ± 8% of phenylephrine response; NS). Summarized data for the effects HOE 694 on the propofol-induced increase in shortening are shown in figure 7. HOE 694 attenuated the propofol-

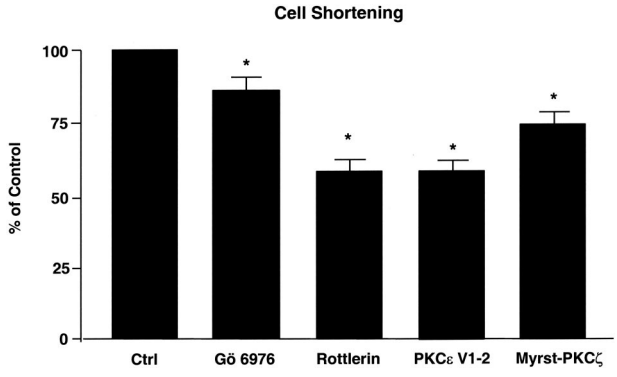


**Fig. 6. Summarized data for effects of Gö 6976 (10 μM), rottlerin (10 μM), protein kinase C (PKC) ε V1-2 (10 μM), and myristoylated (Myrst) PKCζ inhibitor peptide (10 μM) on the propofol-induced (30 μM) increase in cell shortening during $\alpha_{1a}$-adrenoreceptor activation with phenylephrine (10 μM). Results are expressed as percent of control (Ctrl), which represents the twitch amplitude achieved with phenylephrine in the absence of the inhibitors, set to 100%. * $P < 0.05$ compared with control value. n = at least 3 cells with each inhibitor from 7 hearts.**

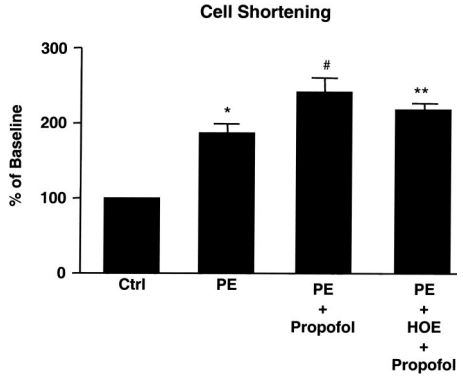


**Fig. 7. Summarized data for effects of HOE 694 (10 μM) on cell shortening during $\alpha_{1a}$-adrenoreceptor activation with phenylephrine (PE; 10 μM) and addition of propofol (30 μM). Results are expressed as percent of baseline value. Control (Ctrl) represents the steady state baseline value for twitch amplitude, set to 100%. * $P < 0.05$ compared with baseline value; #$P < 0.05$ compared with phenylephrine; ** $P < 0.05$ compared with phenylephrine plus propofol. n = 15 cells from 7 hearts.**

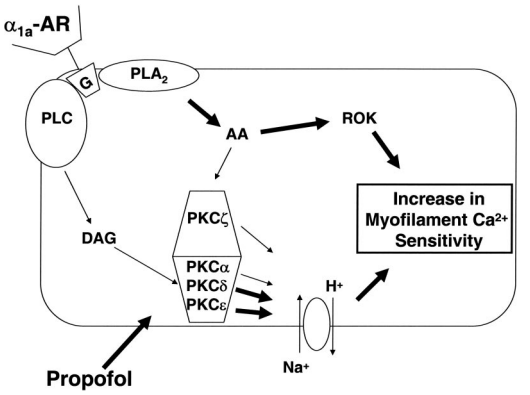


**Fig. 8. Schematic diagram illustrating the putative signaling pathways and cellular mechanisms of $\alpha_{1a}$-adrenoreceptor (AR) activation and propofol in cardiomyocytes. The thickness of the *arrow* reflects the relative contribution of the mechanism. AA = arachidonic acid; DAG = diacylglycerol; G = G protein; PKC = protein kinase C; $PLA_2$ = phospholipase $A_2$; PLC = phospholipase C; ROK = Rho kinase.**

induced increase in shortening during $\alpha_{1a}$-AR activation by 47 ± 9% ($P = 0.001$).

## Discussion

Although propofol is widely used for the sedation of critically ill patients who are receiving catecholamines for hemodynamic support, relatively little is known about the interaction of propofol with adrenoreceptor stimulation. There is only one previous study that demonstrated that propofol abolished the positive inotropic effect of phenylephrine in isolated rat ventricular papillary muscles but enhanced the positive inotropic effect of isoproterenol (β-AR activation).[11] We previously demonstrated that propofol attenuated β-AR–mediated increases in $[Ca^{2+}]_i$ and shortening *via* a PKC-dependent pathway in cardiomyocytes, at a site upstream of adenylyl cyclase.[10] This is the first *in vitro* study to directly assess the effects of propofol on $[Ca^{2+}]_i$ and contractility in isolated ventricular cardiomyocytes in the setting of $\alpha_{1a}$-AR activation. The key findings of this study are that the positive inotropic effect of $\alpha_{1a}$-AR activation is mediated primarily *via* a ROK-dependent increase in myofilament $Ca^{2+}$ sensitivity. Moreover, in the setting of $\alpha_{1a}$-AR activation, propofol increases cardiomyocyte shortening with no concomitant effect on $[Ca^{2+}]_i$, indicating a propofol-induced increase in myofilament $Ca^{2+}$ sensitivity. The increased sensitivity seems to involve PKC activation and $Na^+$–$H^+$ exchange. Figure 8 represents a schematic of the proposed signaling pathways and cellular mechanisms for $\alpha_{1a}$-AR activation and propofol in cardiomyocytes.

### *Effect of $\alpha_{1a}$-AR Activation on $[Ca^{2+}]_i$ and Shortening*

It is well established that three pharmacologically distinct α-AR subtypes ($\alpha_{1a}$, $\alpha_{1b}$, and $\alpha_{1d}$) exist in rat cardiomyocytes and in the human heart.[5,18,19] However, the signal transduction pathways associated with activation of individual α-AR subtype activation and functional consequences on myocardial contractility are still controversial, likely due to activation of multiple parallel signaling pathways by each subtype.[5] Two possible mechanisms have been proposed to explain the inotropic response to α-AR activation: an increase in myofilament $Ca^{2+}$ sensitivity or an increase in transsarcolemmal $Ca^{2+}$ influx.[6,8,20] One recent report indicated opposing effects of $\alpha_1$-adrenergic subtypes ($\alpha_{1a}$ *vs.* $\alpha_{1b}$) on $[Ca^{2+}]_i$, intracellular pH, and contractility in rat cardiac myocytes.[3] Opposing effects of the α-AR subtypes on intracellular $[Ca^{2+}]_i$ and pH regulation may explain in part the observed increase in cardiomyocyte contractility independent of an increase in $[Ca^{2+}]_i$ in response to the $\alpha_1$-AR selective agonist phenylephrine.[21] In our study, selective activation of $\alpha_{1a}$-AR resulted in an increase in shortening with no concomitant increase in $[Ca^{2+}]_i$. These results indicate that the primary mechanism by which $\alpha_{1a}$-AR activation increases cardiomyocyte shortening is *via* an increase in myofilament $Ca^{2+}$ sensitivity.

### *Effect of Phospholipase Inhibition on $\alpha_{1a}$-AR–mediated Increases in Shortening*

Alpha-1 ARs have been shown to couple to a variety of cellular phospholipases, including PLC and $PLA_2$, resulting in production of several important second messengers capable of modulating $[Ca^{2+}]_i$ or myofilament $Ca^{2+}$ sensitivity or both, including diacylglycerol, inositol trisphosphate, arachidonic acid, and RhoA.[4,5,12,13] However, the extent to which $\alpha_{1a}$-AR–mediated increases in cardiomyocyte contractility are induced by these second messengers has not been clearly defined. In our study, inhibition of PLC had a minimal effect on the $\alpha_{1a}$-AR–mediated increase in cardiomyocyte shortening, whereas $PLA_2$ inhibition attenuated the response by

more than 80%. These data indicate that the $\alpha_{1a}$-AR–mediated increase in cardiomyocyte shortening may involve the release of arachidonic acid from membrane phospholipids and that diacylglycerol release and PKC activation play a minimal role. We previously demonstrated that exogenously added arachidonic acid attenuates the transient outward $K^+$ current in cardiomyocytes,[22] resulting in an increase in cardiomyocyte $[Ca^{2+}]_i$ and shortening.[23] The lack of an increase in $[Ca^{2+}]_i$ in this study may be due to intracellular release of arachidonic acid by $PLA_2$ compared with exogenously applied arachidonic acid in our previous study.[22] We also demonstrated an arachidonic acid–dependent increase in the phosphorylation of the contractile proteins troponin I and myosin light chain 2, which can modulate myofilament $Ca^{2+}$ sensitivity.[24] Our data are also consistent with previous studies indicating that $\alpha_{1a}$-AR activation results in a positive inotropic effect independent of PLC activation and phosphoinositide hydrolysis.[25–28]

### *Effect of Protein Kinase Inhibition on $\alpha_{1a}$-AR–induced Increase and Shortening*

We investigated the extent to which protein kinases play a role in mediating the $\alpha_{1a}$-AR–induced increase in cardiomyocyte shortening. Previous studies have suggested that activation of PKC plays a role in mediating the inotropic response to $\alpha$-AR activation.[2,4,29] However, more recent studies have suggested that the $\alpha$-AR–induced positive inotropic effect is independent of PKC activation and is a result of myosin light chain phosphorylation mediated by myosin light chain kinase activation, activation of ROK, or both.[12–14] In the current study, PKC inhibition attenuated the $\alpha_{1a}$-AR–mediated increase in cardiomyocyte shortening by less than 20%, whereas ROK inhibition resulted in greater than 70% inhibition of the response. These data suggest that both PKC and ROK are mediators of the $\alpha_{1a}$-AR–induced increase in cardiomyocyte shortening, with ROK playing a predominant role. It is likely that diacylglycerol formation due to PLC activation and arachidonic acid production from $PLA_2$ activation play an important role in activating these kinases. Interestingly, diacylglycerol and arachidonic acid synergistically increase cardiomyocyte contraction *via* activation of PKC.[30] The mechanism by which PKC activation or arachidonic acid release results in an increase in myofilament $Ca^{2+}$ sensitivity may involve a direct phosphorylation of myosin light chain 2,[24,31,32] or may be due to indirect phosphorylation *via* inhibition of myosin light chain phosphatase, as previously described in smooth muscle.[33,34] Further experiments are required to identify the precise mechanisms.

Recent studies have implicated activation of RhoA, a member of the Rho family of small-molecular-weight guanosine 5′-triphosphate–binding proteins, in mediating $\alpha_1$-adrenergic signaling in cardiomyocytes[12] and in failing hearts.[14] Direct activation of RhoA by $G\alpha_q$ after $\alpha_{1a}$-AR activation,[12] G protein–coupled release of arachidonic acid as a consequence of $PLA_2$ activation,[33,35] or both are likely involved in mediating the observed increase in cardiomyocyte shortening observed in our study. The mechanism likely involves the activation of ROK by $G\alpha_q$, arachidonic acid, or both,[35,36] leading to an inhibition of the myosin light chain phosphatase resulting in an increase in myosin light chain phosphorylation.[33] It has been recently demonstrated that the $\alpha_1$-AR–induced positive inotropic response in the heart is dependent on myosin light chain phosphorylation.[13]

### *Effect of Propofol on $[Ca^{2+}]_i$ and Shortening during $\alpha_{1a}$-AR Activation*

We previously demonstrated that propofol had no effect on $[Ca^{2+}]_i$ and contraction of individual myocyte at clinically relevant concentrations[37] but attenuated the inotropic response to $\beta$-AR activation in cardiomyocytes.[10] In the current study, propofol increased cardiomyocyte shortening with no concomitant effect on $[Ca^{2+}]_i$ during activation of $\alpha_{1a}$ AR with phenylephrine. In contrast, a previous study indicated that propofol abolished the inotropic effect of phenylephrine in isolated papillary muscles.[11] There are several reasons that may explain the differences between the findings. The major difference between the two studies is that the current study examines the effects of propofol *during* $\alpha$-AR activation, whereas the study by Lejay *et al.*[11] examined the propofol-induced modification of the inotropic response to $\alpha$-AR activation (pretreatment with propofol). Moreover, the previous study[11] did not isolate a single signaling pathway ($\alpha_{1a}$, $\alpha_{1b}$, and $\alpha_{1d}$ AR are all activated by phenylephrine, which can have opposing effects on cardiomyocyte shortening[3]), whereas the current study isolates the $\alpha_{1a}$-AR signaling pathway. In addition, differences in the extracellular $Ca^{2+}$ concentration of the experimental buffers can affect the inotropic effects of propofol.[38] Finally, the isolated cardiomyocytes in the current study were not loaded, and therefore, the resting cell length and myofilament $Ca^{2+}$ sensitivity may be modified and may contribute to the differences observed between the two studies. Regardless, the results of the current study indicate that propofol increases myofilament $Ca^{2+}$ sensitivity during $\alpha_{1a}$-AR activation. Our data are the first to directly demonstrate a positive inotropic effect of propofol on cardiomyocyte shortening during $\alpha_{1a}$-AR activation mediated by an increase in myofilament $Ca^{2+}$ sensitivity.

### *Effect of PKC Inhibition on Propofol-induced Increase in Shortening after $\alpha_{1a}$-AR Activation*

The extent to which propofol-induced changes in cardiomyocyte signaling are mediated *via* activation of PKC has been actively explored by our laboratory.[10,15,16] However, isozyme specificity for specific cellular interactions has not been extensively examined. In our study,

inhibition of PKC with Bis during $\alpha_{1a}$ AR resulted in an increase in cardiomyocyte $[Ca^{2+}]_i$ and shortening. This may be explained by an inhibition in tonic activity of PKC isoforms involved in limiting the availability of $[Ca^{2+}]_i$ and hence cardiomyocyte shortening.[39] Alternatively, direct block of human *ether-a-go-go*–related gene potassium channels by Bis has recently been reported,[40] which results in action potential prolongation and would increase $[Ca^{2+}]_i$ and cardiomyocyte shortening. We observed that PKC inhibition with Bis prevented the propofol-induced increase in cardiomyocyte shortening after $\alpha_{1a}$-AR activation. Moreover, PKC$\delta$ and PKC$\varepsilon$ seem to be the predominant isoforms involved in mediating the response, with PKC$\alpha$ and PKC$\zeta$ playing a lesser role. However, it seems that all four PKC isoforms have some role in mediating the increase in cardiomyocyte shortening in response to propofol, because the sum of their inhibitory effects accounts for virtually all of the propofol effect. The inability of propofol to increase shortening after inhibition of PKC with Bis was not due to a ceiling effect on cardiomyocyte shortening, because activation of the $\beta$-AR signaling pathway further increased both $[Ca^{2+}]_i$ and shortening. It remains to be determined what specific roles each of the individual isoforms play in the propofol-induced increase in shortening. Activation of PKC has been shown to increase myofilament $Ca^{2+}$ sensitivity *via* phosphorylation of contractile proteins,[15] changes in intracellular pH, or both.[16] Little is known about the relative roles of the PKC isoforms in regulating myofibrillar protein phosphorylation,[41,42] and even less is known about isoform-specific modulation of $Na^+$–$H^+$ exchange.[43]

### *Effect of $Na^+$–$H^+$ Exchange Inhibition on Propofol-induced Increase in Shortening*

To identify a cellular mechanism to explain the propofol-induced increase in cardiomyocyte shortening, we examined whether $Na^+$–$H^+$ exchange inhibition attenuates the propofol-induced increase in shortening after $\alpha_{1a}$-AR activation. Our results indicate that $Na^+$–$H^+$ exchange inhibition had little effect on shortening during $\alpha_{1a}$-AR activation, indicating that intracellular alkalinization, which can increase myofilament $Ca^{2+}$ sensitivity, was not likely the mechanism for the increase in shortening in response to $\alpha_{1a}$-AR activation. In contrast, $Na^+$–$H^+$ exchange inhibition attenuated the propofol-induced increase in shortening during $\alpha_{1a}$-AR activation, indicating a propofol-induced activation of $Na^+$–$H^+$ exchange. These data suggest that intracellular alkalinization plays a role in mediating the propofol-induced increase in cardiomyocyte shortening during $\alpha_{1a}$-AR activation.

### *Clinical Implications*

Extrapolation of results obtained from *in vitro* studies at the cellular level to the clinical setting can be difficult. However, it is known that PKC activation is a key mediator of ischemic and anesthetic preconditioning and cardiac protection. In addition, increases in myofilament $Ca^{2+}$ sensitivity can partially offset the negative inotropic effects of certain agents, including propofol. Therefore, propofol may be beneficial in patients exhibiting end-stage heart failure (or other cardiomyopathies) where $Ca^{2+}$ overload is observed. Moreover, $\alpha$-AR are up-regulated in a variety of cardiomyopathies and may become more important than $\beta$-AR signal transduction in mediating catecholamine-induced increases in the inotropic state of the heart, particularly in ischemic heart disease. In this patient population, propofol may increase cardiac function without further altering $Ca^{2+}$ homeostasis, which could be beneficial to the patient.

### *Limitations of the Study*

As with all *in vitro* methodologies and experimental approaches used to study cardiac function, there are some limitations.[10,38] The use of putative $\alpha$-AR subtype–selective antagonists to focus on the signal transduction pathways of a particular receptor subtype prevents potential interactions among receptor subtypes. In addition, this *in vitro* study only deals with intrinsic myocardial function, whereas changes in cardiac contractility *in vivo* after propofol administration also depend on a variety of other factors, including venous return, afterload, and neurohumoral compensatory mechanisms. It is well established that species differences can contribute to the controversy surrounding specific cellular mechanisms regulating cardiac contractility. Also, there is no load on the isolated cardiomyocyte, which may be a limitation when comparing findings to studies using isometrically contracting cardiac muscle strips or Langendorff perfused hearts. Finally, the experimental conditions (temperature, stimulation frequency) used in this study do not parallel *in vivo* conditions. However, the strength of this model is that we can directly assess the effects of $\alpha_{1a}$-AR activation, propofol, and their interactions on cellular mechanisms that regulate cardiomyocyte contractile function.

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