Propofol Increases Myofilament Ca\(^{2+}\) Sensitivity and Intracellular pH via Activation of Na\(^+\)--H\(^+\) Exchange in Rat Ventricular Myocytes

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Background: The objectives were to determine the extent and mechanism of action by which propofol increases myofilament Ca\(^{2+}\) sensitivity and intracellular pH (pHi) in ventricular myocytes.

Methods: Freshly isolated adult rat ventricular myocytes were used for the study. Cardiac myofibrils were extracted for assessment of myofibrillar actomyosin adenosine triphosphatase (ATPase) activity. Myocyte shortening (video edge detection) and pHi (2,7'-bis-(2-carboxyethyl)-5(6')-carboxyfluorescein, 500/440 ratio) were monitored simultaneously in individual cells field-stimulated (0.3 Hz) and superfused with HEPES-buffered solution (pH 7.4, 30°C).

Results: Propofol (100 μM) reduced the Ca\(^{2+}\) concentration required for activation of myofibrillar actomyosin ATPase from pCa 5.7 ± 0.01 to 6.6 ± 0.01. Increasing pHi (7.05 ± 0.03 to 7.39 ± 0.04) with NH\(_4\)Cl increased myocyte shortening by 35 ± 12%. Washout of NH\(_4\)Cl decreased pHi to 6.82 ± 0.03 and decreased myocyte shortening to 52 ± 10% of control. Propofol caused a dose-dependent increase in pHi but reduced myocyte shortening. The propofol-induced increase in pHi was attenuated, whereas the decrease in myocyte shortening was enhanced after pretreatment with ethylisopropyl amiloride, a Na\(^+\)--H\(^+\) exchange inhibitor, or bisindolylmaleimide I, a protein kinase C inhibitor. Propofol also attenuated the NH\(_4\)Cl-induced intracellular acidosis, increased the rate of recovery from acidosis, and attenuated the associated decrease in myocyte shortening. Propofol caused a leftward shift in the extracellular Ca\(^{2+}\)–shortening relation, and this effect was attenuated by ethylisopropyl amiloride.

Conclusions: These results suggest that propofol increases the sensitivity of myofibrillar actomyosin ATPase to Ca\(^{2+}\) (i.e., increases myofilament Ca\(^{2+}\) sensitivity) at least in part by increasing pHi via protein kinase C-dependent activation of Na\(^+\)--H\(^+\) exchange.
100% O$_2$. Typically, 6–8 × 10$^6$ cells per rat heart were obtained using this procedure. Viability, as assessed by the percentage of cells retaining a rod-shaped morphology, was routinely between 80 and 90%. Myocytes were suspended in HBS (1 × 10$^6$ cells/ml) and stored in an oxygen hood until used.

**Preparation of Cardiac Myofibrils**

Two-milliliter aliquots of the freshly isolated myocyte suspension were incubated in the presence or absence of propofol (10, 30, 100 μM) for 10 min at 37°C with gentle agitation. The myocytes were immediately washed twice in ice-cold HBS containing protease and phosphatase inhibitors and pelleted at 40g for 3 min, after which an equivalent volume of extraction buffer, 50 mM Tris (pH 7.5) containing Triton-X 100 (0.1%), NaF (20 mM), dithiothreitol (0.5 mM), MgCl$_2$ (0.5 mM), EDTA (0.125 mM), antipain (5 μg/ml), leupeptin (10 μg/ml), pepstatin A (5 μg/ml), and paramethysulfonylic acid (43 μg/ml) was added to the suspension. The cells were homogenized and kept on ice for 30 min. The triton-extracted myofibrils were pelleted at 10,000g (5 min, 4°C). The detergent solubilized supernatant was set aside and the pellet was resuspended in an equivalent volume of extraction buffer and washed twice again. The resultant myofibrillar fraction was resuspended in Ca$^{2+}$-free extraction buffer and stored at −20°C. Examination of the pellet under the microscope indicated that it was enriched in myofibrils.

**Actomyosin Adenosine Triphosphatase Activity**

The Ca$^{2+}$-stimulated actomyosin ATPase activity of the myofibrillar fraction was measured from the rate of decrease of nicotinamide adenine dinucleotide (NADH) absorbance at 340 nm. The enzyme activity was monitored by the formation of adenosine diphosphate, coupled to the oxidation of NADH, and recorded by the change in absorption at 340 nm. The enzyme activity was determined from the rate of ATP hydrolysis and expressed as the percent of maximal actomyosin ATPase activity per milligram of protein. Because crude myofibrillar fractions were used for these assays, we determined whether there was a contribution to the activity measured from other ATPases present in the sample. We found no significant difference in ATPase activity measured in the presence or absence of ATPase inhibitors, including 2 mM thapsigargin, 200 mM ouabain, 2 mM rotenone, and 2 mM oligomycin.

**Contractility and Intracellular pH Measurements**

For simultaneous measurement of shortening and pH$_i$, ventricular myocytes (0.5 × 10$^6$ cells/ml) were incubated in HBS containing 2 mM 2′,7′-bis-(2-carboxyethyl)-5 (6′)-carboxyfluorescein-acetoxyethyl ester (BCECF-AM) at 37°C for 20 min. BCECF-loaded ventricular myocytes were placed in a temperature-regulated (30°C) chamber mounted on the stage of an inverted fluorescence microscope. The volume of the chamber was 1.5 ml. 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 and a duration of 5 ms using a stimulator. Fluorescence measurements were performed on single ventricular myocytes using a dual-wavelength spectrofluorometer at excitation wavelengths of 440 and 500 nm and an emission wavelength of 530 nm. The cells were also illuminated with red light at a wavelength greater than 600 nm for simultaneous video edge detection. An additional postspecimen dichroic mirror deflects light at wavelengths greater than 600 nm into a charge coupled device video camera for measurement of myocyte shortening. The fluorescence sampling frequency was 10 Hz, and data were collected using a software package. Background fluorescence was determined from the blank dish and subtracted from fluorescence at each wavelength. To estimate the pH$_i$ value from the ratio of 500/440 nm fluorescence, we used an in situ calibration procedure.$^{17,18}$ At the end of each experiment, the fluorescence ratio from each cell was calibrated in situ by exposing the cell to solutions of varying pH. Each solution contained 140 mM K$^+$, M1.0 mM gCl$_2$, 4.0 mM HEPES, 2.0 mM EGTA, 30 mM 2,3-butanediol monoxime, 50 μM 1,2bis(o-Aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, and 14 μM nigericin and was titrated to varying pH values (6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8) using 1.0 N KOH. The pH$_i$ for each cell was then determined from a linear regression of fluorescence ratio versus the pH value of the calibration buffer. We previously determined
that a linear relation exists between the 500/440 nm ratio and pHᵢ in the physiologic range (pH 6.6–7.8).¹⁷

Simultaneous measurement of cell shortening was monitored using a video edge detector with 16-ms temporal resolution. The video edge detector was calibrated using a stage micrometer so that cell lengths during shortening could be monitored. LabVIEW (National Instruments, Austin, TX) was used for data acquisition of cell shortening using a sampling rate of 100 Hz.

**Analysis of Intracellular pH and Contractile Data**

Fluorescence data for the pHᵢ measurements were imported into LabVIEW, which allowed the simultaneous analysis of pHᵢ and shortening. Myocyte length in response to field stimulation was measured (micrometers) and expressed as the change from resting cell length (twitch amplitude). Changes in twitch amplitude in response to the interventions are expressed as a percent of baseline shortening. Contractile parameters from 15 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. Changes in pHᵢ were measured as the change in the 500/440 ratio from baseline.

**Experimental Protocols**

Where applicable, protocols were designed so that each cell could serve as its own control.

**Protocol 1: Effect of Propofol on Myofibrillar Actomyosin Adenosine Triphosphatase Activity.** To determine whether propofol alters myofilament Ca²⁺ sensitivity, changes in actomyosin ATPase activity were measured in myofibrils isolated from control and propofol-treated cardiomyocytes. The myocyte suspension was divided into separate aliquots, and each aliquot was treated with one concentration of propofol (10, 30, 100 μM) or intralipid equivalent for 10 min at 37°C with gentle agitation. Propofol had no effect on extracellular pH at the concentrations used in this study and did not appear to alter the assay conditions. Activity is expressed as a percentage of the maximum rate per milligram of protein.

**Protocol 2: Effect of Alkalization and Acidification on Myocyte Shortening.** To identify the effects of changing pHᵢ on myocyte shortening, changes in myocyte shortening and pHᵢ in response to 10 mM NH₄Cl (2 min) followed by washout were determined. Baseline measurements were collected from individual myocytes for 1 min in the absence of any intervention.

**Protocol 3: Effect of Propofol on Intracellular pH.** To determine whether propofol alters pHᵢ, we measured pHᵢ for 15 min after addition of propofol. Baseline pHᵢ was collected from individual myocytes for 1 min. Propofol (10, 30, 100 μM) or intralipid equivalent was added by exchanging the superfusion buffer in the dish with new buffer containing propofol at the desired concentration. Each myocyte was exposed to only one concentration for propofol.

**Protocol 4: Effect of Propofol on Intracellular pH and Shortening—Role of Na⁺–H⁺ Exchange and Protein Kinase C.** To examine the role of the Na⁺–H⁺ exchange and PKC in modulating propofol-induced changes in pHᵢ and shortening, we assessed propofol-induced intracellular alkalinization and shortening in myocytes pretreated (5 min) with a Na⁺–H⁺ exchange inhibitor, 5-(N-ethyl-N-isopropyl)-amiloride (EIPA, 1 μM), or a PKC inhibitor, bisindolylmaleimide I (Bis, 1 μM). We have verified that propofol, EIPA, and Bis have no effect on the BCECF signal at the concentrations used in these studies.

**Protocol 5: Effect of Propofol on NH₄Cl-induced Changes in Intracellular pH and Contractility.** To determine whether propofol alters NH₄Cl-induced changes in pHᵢ and contractility, we simultaneously measured changes in pHᵢ and myocyte shortening in the presence or absence of propofol. Baseline pHᵢ and shortening were measured in individual myocytes for 1 min. In the propofol group, the cells were superfused with buffer containing propofol (30 μM) 5 min before exposure to NH₄Cl. Washout was performed with HBS containing propofol.

**Protocol 6: Effect of Propofol on Recovery from Acid Load.** To determine whether propofol alters pHᵢ recovery from acid load, we measured the changes in pHᵢ from intracellular acidosis after washout with 10 mM NH₄Cl in the presence or absence of propofol. Baseline pHᵢ was measured in individual myocytes for 1 min. At the time of NH₄Cl washout, each myocyte was superfused with buffer either in the presence or absence of propofol (10, 30, 100 μM). Rate of recovery of pHᵢ from NH₄Cl-induced acidosis was assessed by calculating the derivative of the slope of the pHᵢ time course trace for 10-s intervals through the first 120 s of recovery.

**Protocol 7: Effect of Propofol and Ethylisopropyl Amiloride on the Extracellular Ca²⁺-shortening Relation.** We previously reported that propofol caused a leftward shift in the extracellular Ca²⁺ concentration ([Ca²⁺]₀)-shortening relation, with no concomitant effect on the intracellular Ca²⁺ transient.⁵ Baseline parameters were collected from individual myocytes for 1.5 min. Dose-response curves to [Ca²⁺]₀ were performed by exchanging the buffer in the dish with a new buffer containing the desired [Ca²⁺]₀. Data were acquired for 1.5 min after establishment of a new steady state. Dose–response curves to [Ca²⁺]₀ were then performed in the presence of propofol (30 μM). Cells were allowed to stabilize for 5 min after each intervention. The relative contribution of the Na⁺–H⁺ exchanger in mediating changes in the [Ca²⁺]₀-shortening relation was assessed by pretreating the cells (5 min) with 1 μM EIPA.
Materials
Collagenase type II was obtained from Worthington Biochemical (Freehold, NJ). Propofol and intralipid were obtained from The Cleveland Clinic Pharmacy. BCECF–AM was obtained from Texas Fluorescence Labs (Austin, TX). Nigericin, EIPA, and Bis were purchased from Sigma Chemical Co. (St. Louis, MO).

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. The effects of each concentration of propofol on myocyte shortening and pH_i were assessed using one-way analysis of variance with repeated measures and the Bonferroni correction for multiple comparisons. Comparisons between groups were made by two-way analysis of variance and unpaired t test. Results are expressed as mean ± SD. P < 0.05 was considered significant.

Results

Effect of Propofol on Actomyosin ATPase Activity
To directly test the hypothesis that propofol increases myofilament Ca^{2+} sensitivity, we assessed the effects of propofol on myofibrillar actomyosin ATPase activity in myocytes pretreated with propofol (10–100 μM). Propofol caused a concentration-dependent leftward shift in the actomyosin ATPase activation curve (fig. 1A). Propofol increased the EC_{50} (pCa) value (i.e., decreased the Ca^{2+} requirement) for Ca^{2+}-activated actomyosin ATPase (fig. 1B). Propofol (30 and 100 μM) reduced (P < 0.05) the maximal activation of actomyosin ATPase (V_{max}) by 11 ± 4% and 17 ± 5%, respectively, from a control value of 175 ± 8 nmol · min^{-1} · mg^{-1}. The intralipid vehicle had no effect on actomyosin ATPase activity (EC_{50} 5.6 ± 0.1; V_{max} 169 ± 9 nmol · min^{-1} · mg^{-1}) at a concentration equivalent to 100 μM propofol.

Effect of NH_4Cl on Intracellular pH and Myocyte Shortening
Baseline pH_i and diastolic cell length were 7.05 ± 0.03 and 130 ± 15 μm, respectively. Twitch amplitude was 9 ± 1% (11.7 ± 2.8 μm) of the resting cell length. To test the hypothesis that changes in pH_i alter the contractile properties of ventricular myocytes, we measured pH_i and shortening simultaneously in response to NH_4Cl followed by washout to cause intracellular alkalinization and acidification, respectively. Figure 2 illustrates the effects of NH_4Cl (10 mM) on shortening and pH_i in an individual cardiomyocyte. Field stimulation alone had no effect on baseline pH_i. NH_4Cl initially caused intracellular alkalinization (7.39 ± 0.06), a 4.4 ± 1.7% decrease in diastolic cell length, and an increase (P < 0.05) in myocyte shortening (135 ± 10% of baseline). Washout of NH_4Cl caused intracellular acidification (6.92 ± 0.02), a return of diastolic cell length to baseline (1.0 ± 0.1%), and a decrease (P < 0.05) in myocyte shortening (48 ± 8% of baseline). Myocyte shortening gradually increased after washout of NH_4Cl and reached 95 ± 8% of the baseline value after 10 min.

Effect of Propofol on Intracellular pH and Myocyte Shortening
To test the hypothesis that the propofol-induced increase in myofilament Ca^{2+} sensitivity was caused by intracellular alkalinization, we assessed the extent to which propofol altered pH_i. Propofol at 10 μM had no significant effect on pH_i or shortening. Propofol at 30 and 100 μM caused a concentration- and time-dependent increase in pH_i of 0.03 ± 0.01 and 0.07 ± 0.02, respectively, after a 15-min exposure (fig. 3). Despite the increase in pH_i, propofol (30 and 100 μM) reduced (P < 0.05) shortening by 10 ± 3% and 33 ± 7%, respectively. The intralipid vehicle had no effect on pH_i (7.03 ± 0.03).
or shortening at a concentration equivalent to 100 μM propofol. In some cells (6 of 16), 30 μM propofol decreased resting cell length from 127 ± 10 μm to 124 ± 7 μm. Similarly, 100 μM propofol decreased resting cell length to 122 ± 9 μm in 10 of 16 cells studied.

Role of Na\(^+\)-H\(^+\) Exchange and Protein Kinase C on Propofol-induced Changes in Intracellular pH and Myocyte Shortening

To test the hypothesis that the propofol-induced increase in pH\(_i\) was mediated by an increase in Na\(^+\)-H\(^+\) exchange, we assessed the effects of propofol in cells pretreated with EIPA, a Na\(^+\)-H\(^+\) exchange inhibitor. Pretreatment with EIPA had no effect on baseline pH\(_i\) or shortening. Figure 4 summarizes changes in pH\(_i\) after 15-min exposure to 100 μM propofol. The propofol-induced intracellular alkalinization was inhibited by pretreatment with EIPA. PKC inhibition with Bis also inhibited the propofol-induced intracellular alkalinization (fig. 4). In the presence of EIPA, 30 μM propofol decreased (P < 0.05) cell shortening by an additional 17 ± 3% compared with propofol alone. In the presence of Bis, propofol (30 μM) decreased (P < 0.05) cell shortening by an additional 24 ± 4% compared with propofol alone.

Effect of Propofol on NH\(_4\)Cl-induced Changes in Intracellular pH and Myocyte Shortening

Administration of NH\(_4\)Cl results in activation of Na\(^+\)-H\(^+\) exchange without the involvement of second messengers. We used NH\(_4\)Cl to test the hypothesis that propofol-induced changes in pH\(_i\) are mediated by changes in Na\(^+\)-H\(^+\) exchange activity. Baseline pH\(_i\) and myocyte shortening were 7.03 ± 0.02 and 8 ± 1%, respectively. Pretreatment with propofol (30 μM, 10 min) increased (P < 0.05) pH\(_i\) (+0.03 ± 0.01), whereas shortening decreased (P < 0.05) to 92 ± 4% of control. As expected, pretreatment with propofol had no effect on NH\(_4\)Cl-induced increases in pH\(_i\) and cell shortening (fig. 5), because these changes are not mediated by Na\(^+\)-H\(^+\) exchange activation. However, the acidification-induced decreases in pH\(_i\) (mediated by an increase in Na\(^+\)-H\(^+\) exchange) and myocyte shortening in response to NH\(_4\)Cl were attenuated by propofol (fig. 5).

Effect of Propofol on Recovery Rate from NH\(_4\)Cl-induced Acid Load

To further test the hypothesis that propofol enhances the activity of the Na\(^+\)-H\(^+\) exchanger, we assessed the extent to which propofol altered the rate of recovery after acidosis (dpH\(_i\)/dt) induced by a 2-3-min exposure to 10 mM NH\(_4\)Cl. NH\(_4\)Cl produced an intracellular alka-
loss, whereas subsequent washout produced a transient intracellular acidosis (fig. 6A). EIPA abolished the recovery from acidosis induced by NH₄Cl (fig. 6A). The time course of pHᵢ recovery from acidosis was compared in the presence or absence of propofol. Propofol at 30 μM accelerated the rate of recovery from NH₄Cl-induced intracellular acidosis (fig. 6B). This effect of propofol on the rate of recovery is even more apparent when the data are normalized to peak acidosis (fig. 6C). As summarized in figure 7, propofol increased the recovery rate from acidosis in a concentration-dependent manner.

Fig. 5. Effect of 30 μM propofol on NH₄Cl exposure- and washout-induced changes in intracellular pH (pHᵢ) (A) and myocyte shortening (B). W/O = peak of acidosis after washout of NH₄Cl; W/O 3 = 3 min after washout of NH₄Cl. *Significant difference compared with control (P < 0.05). n = 16 cells per 4 hearts in each group.

Fig. 6. Representative traces demonstrating the effects of propofol on the rate of recovery from intracellular acidosis after washout of NH₄Cl. (A) 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) abolished the recovery from intracellular acidosis induced by washout of NH₄Cl. (B) Propofol (30 μM) attenuated the magnitude of the NH₄Cl acidosis and increased the rate of recovery. (C) Data are normalized to peak acidosis values to illustrate the effect of propofol on rate of recovery.

Fig. 7. Summarized data for the effects of propofol on the rate of recovery (dpHᵢ/dt) from acid load. *Significant difference compared with control (P < 0.05). n = 16 cells per 4 hearts in each group.

Discussion

This is the first study to directly assess the effects of propofol on myofilament Ca²⁺ sensitivity, Na⁺–H⁺ exchange, and pHᵢ in isolated cardiac muscle cells. A previous study from our laboratory using isolated rat ventricular myocytes, as well as a recent study using intact beating guinea pig hearts, provided indirect evidence that propofol may increase the sensitivity of the contractile machinery to Ca²⁺. In contrast, a recent study using phase-loop diagrams depicting the continuous relation between cell length and the fura-2 fluorescence
ratio in rat ventricular myocytes has suggested that propofol may reduce myofilament Ca$^{2+}$ sensitivity. In addition, several previous studies have indicated that propofol does not alter myofilament Ca$^{2+}$ sensitivity. In this study, we measured changes in Ca$^{2+}$-activated myofibrillar actomyosin ATPase activity in response to propofol to directly assess changes in myofilament Ca$^{2+}$ sensitivity. The key finding of this study is that propofol markedly reduced the Ca$^{2+}$ requirement for activation of myofibrillar actomyosin ATPase. Moreover, the mechanism responsible for this effect involves, at least in part, a propofol-induced increase in pH$_i$ mediated via PKC-dependent activation of the Na$^+$/H$^+$ exchanger.

**Propofol and Actomyosin ATPase Activity**

Cardiac myofilaments contain an intrinsic actomyosin ATPase that represents the molecular basis for contraction. Sensitivity of the actomyosin ATPase to Ca$^{2+}$ is mediated by the troponin complex in the thin filament. This multiprotein complex is comprised of the Ca$^{2+}$ binding TnC subunit, the ATPase inhibiting TnI subunit, and the tropomyosin binding TnT subunit. It is well established that changes in pH$_i$ and/or direct phosphorylation of the contractile proteins, TnI, TnT, and myosin light chain 2, can alter the sensitivity of the contractile machinery to Ca$^{2+}$. Although propofol had no direct effect on actomyosin ATPase activity in isolated myofibrils, it caused a leftward shift in the Ca$^{2+}$-activated actomyosin ATPase activity curve (i.e., a decrease in the amount of Ca$^{2+}$ required for activation) with a small decrease in maximal activity ($V_{max}$) in myofibrils isolated from propofol-treated cardiomyocytes. These results imply the requirement of a cytosolic mediator for the actions of propofol on actomyosin ATPase activity. Taken together, it appears that propofol may alter multiple cellular mechanisms that regulate calcium sensitivity. PKC activators have similar effects on actomyosin ATPase activity.

**Regulation of Intracellular pH in Cardiomyocytes**

Control of pH$_i$ within the physiologic range is essential for the optimal function of enzymes, cell growth, and protein synthesis. In cardiac muscle, pH$_i$ is a key factor regulating myocardial contractility, because changes in pH$_i$ alter the sensitivity of the contractile apparatus to Ca$^{2+}$. The Na$^+$/H$^+$ exchanger represents the most important mechanism for regulation of cardiomyocyte pH$_i$. Baseline pH$_i$ in this study was 7.05 ± 0.03, which is similar to that in other reports.

**Myocyte Contractility and Intracellular pH**

Both the negative and positive inotropic effects of acidosis and alkalosis, respectively, have been documented. These effects appear to result almost entirely from changes in pH$_i$ and not from changes in extracellular pH. However, the precise mechanisms underlying the changes in myocardial contractility in response to changes in pH$_i$ are not known, because the development of force on Ca$^{2+}$ activation involves many pH-dependent biochemical processes. To date, several mechanisms have been postulated to contribute to pH$_i$-mediated changes in myocardial contractility, including changes in the affinity of myofibrillar troponin C for Ca$^{2+}$, pH dependence of the myofibrillar actomyosin ATPase activity, and changes in sarcoplasmic reticulum Ca$^{2+}$ handling.

**Effects of Propofol on Intracellular pH and Myocyte Shortening**

Propofol caused a time- and dose-dependent increase in pH$_i$. The magnitude of the increase in pH$_i$ was comparable to that observed in response to α$_1$-adrenergic agonists, endothelin, and angiotensin II. Despite the increase in pH$_i$, propofol did not exert a positive inotropic effect. However, propofol caused a slight decrease in resting cell length in approximately half of the cells studied. Propofol likely alters multiple cellular mechanisms that negatively regulate contractility (e.g., sarcoplasmic reticulum Ca$^{2+}$ handling, L-type Ca$^{2+}$ channel activity), which could offset the effects of increasing myofilament Ca$^{2+}$ sensitivity. In fact, a propofol-induced depression of the intracellular Ca$^{2+}$ transient has been observed in beating guinea pig hearts with no concomitant decrease in contractility, implying enhanced myofilament Ca$^{2+}$ sensitivity. Moreover, intracellular alkalosis can cause a decrease in peak intracellular Ca$^{2+}$ concentration which could partially offset the effect of increased pH$_i$ on myofilament Ca$^{2+}$ sensitivity. Propofol may also alter myofilament Ca$^{2+}$ sensitivity via PKC-dependent phosphorylation of contractile proteins.
Role of Na\(^+\)–H\(^+\) Exchanger and Protein Kinase C on Propofol-Induced Intracellular Alkalinization

Protein kinase C activation is the primary regulator of Na\(^+\)–H\(^+\) exchange in cardiomyocytes. We hypothesized that inhibition of Na\(^+\)–H\(^+\) exchange and/or PKC should attenuate the ability of propofol to induce intracellular alkalinization. Consistent with this, the propofol-induced increase in pHi was markedly attenuated by EIPA and Bis, indicating that the effects are mediated by a PKC-dependent activation of Na\(^+\)–H\(^+\) exchange. Pretreatment with EIPA or Bis also resulted in an even greater propofol-induced reduction in shortening. Moreover, the propofol-induced decrease in shortening was greater in Bis-treated myocytes compared with EIPA-treated myocytes. Taken together, these results suggest that PKC-dependent intracellular alkalinization offsets the negative inotropic effect of propofol (likely because of a decrease in intracellular Ca\(^{2+}\) availability)\(^{15}\) via an increase in myofilament Ca\(^{2+}\) sensitivity.

Effect of Propofol on NH\(_4\)Cl-induced Changes in Intracellular pH

We used NH\(_4\)Cl as a tool to assess the effects of changing pHi on myocardial contractility in our experimental model. NH\(_4\)Cl initially causes intracellular alkalinization because of the highly permeant NH\(_3\) crossing the membrane more quickly than NH\(_4^+\). Once inside the myocyte, NH\(_3\) combines with H\(^+\) to form NH\(_4^+\), which increases pHi and shortening. With continued exposure to NH\(_4\)Cl, pHi gradually decreases as NH\(_4^+\) enters the cell. The subsequent abrupt removal of external NH\(_4\)Cl produces an intracellular acidosis. This occurs because NH\(_4^+\) ions that had accumulated in the cytosol dissociate to form NH\(_3\), which rapidly leaves the cell, resulting in intracellular retention of H\(^+\) and a decrease in shortening. We hypothesized that NH\(_4\)Cl-induced alkalinization (independent of Na\(^+\)–H\(^+\) exchange) would not be affected by propofol, whereas the acidification (dependent on Na\(^+\)–H\(^+\) exchange) would be attenuated if propofol caused intracellular alkalinization via activation of Na\(^+\)–H\(^+\) exchange. We found that propofol had no effect on NH\(_4\)Cl-induced intracellular alkalinization but attenuated the acidification. In addition, propofol attenuated the decrease in shortening induced by acidification. These data give further support to the idea that propofol activates the Na\(^+\)–H\(^+\) exchange mechanism in ventricular myocytes.

Effect of Propofol on Recovery Rate from NH\(_4\)Cl-induced Acid Load

Because these experiments were performed in the absence of HCO\(_3\) and carbon dioxide, the acid-load recovery is likely mediated by Na\(^+\)–H\(^+\) exchange.\(^{35}\) We hypothesized that if propofol activated the Na\(^+\)–H\(^+\) exchanger as a mechanism to increase pHi, propofol should stimulate a faster rate of recovery from an acid load in response to NH\(_4\)Cl washout. At concentrations greater than 10 μM, propofol enhanced the rate of recovery from an acid load. Because the recovery from an acid load is dependent on Na\(^+\)–H\(^+\) exchange activity, these data give further support to the hypothesis that propofol enhances the activity of the Na\(^+\)–H\(^+\) exchanger during acid-load conditions.

Effect of Ethylisopropyl Amiloride on the Propofol-induced Leftward Shift in the Extracellular Ca\(^{2+}\) Concentration–Shortening Relation

If intracellular alkalinization mediates the propofol-induced leftward shift in the [Ca\(^{2+}\)]\(_o\)-shortening relation, then this effect should be blocked by inhibiting Na\(^+\)–H\(^+\) exchange. Our results indicate that inhibition of Na\(^+\)–H\(^+\) exchange with EIPA attenuates the propofol-induced increase in myocardial Ca\(^{2+}\) sensitivity by approximately 50%. It is possible that phosphorylation of contractile proteins by propofol also plays a role in mediating the increase in myocardial Ca\(^{2+}\) sensitivity.\(^{52}\) Inhibition of Na\(^+\)–H\(^+\) exchange with amiloride has been reported to attenuate the increase in myocardial Ca\(^{2+}\) sensitivity in response to endothelin\(^{31}\) and phenylephrine\(^{14}\) in rat ventricular myocytes. In contrast to the present study, EIPA abolished the leftward shift in the [Ca\(^{2+}\)]\(_o\)-shortening relation induced by thiopental.\(^{17}\)

Limitations

Because propofol partitions in vivo between serum proteins, lipid microsomes, and into tissue, it is difficult to know the precise concentration of free and active propofol at the tissue level. The concentrations of propofol used in these in vitro studies are supraclinical. Because we used isolated cells rather than intact tissue, the effects of propofol may be enhanced because of optimal solute diffusion distances between the cytosol and extracellular medium. In addition, this in vitro study only deals with intrinsic myocardial contractility, whereas changes in cardiac function in vivo after propofol administration also depend on venous return, afterload, and compensatory mechanisms. We also acknowledge that the experimental conditions (temperature, stimulation frequency, unloaded cells) do not perfectly simulate the in vivo heart. However, the strength of this model is that we can directly assess the effects of propofol on the fundamental contractile unit, the individual cardiomyocyte. Finally, we now have direct evidence that propofol increases the Ca\(^{2+}\) sensitivity of the actomyosin ATPase, causes intracellular alkalinization, and stimulates both PKC-dependent phosphorylation of contractile proteins\(^{32}\) and Na\(^+\)–H\(^+\) exchange activity in rat cardiomyocytes. However, we have not directly demonstrated that intracellular alkalinization increases the Ca\(^{2+}\) sensitivity of actomyosin ATPase activity.

In summary, our results provide the first direct evidence that propofol increases the sensitivity of myofilament Ca\(^{2+}\) sensitivity.
brillar actomyosin ATPase to Ca^{2+} (i.e., increases myofilament Ca^{2+} sensitivity), at least in part by increasing pH_i via PKC-dependent activation of Na^{+}–H^{+} exchange.

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