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Droperidol Inhibits Intracellular Ca^{2+}, Myofilament Ca^{2+} Sensitivity, and Contraction in Rat Ventricular Myocytes

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Background: **Droperidol has recently been associated with cardiac arrhythmias and sudden cardiac death. Changes in action potential duration seem to be the cause of the arrhythmic behavior, which can lead to alterations in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). Because $[Ca^{2+}]_i$ and myofilament Ca^{2+} sensitivity are key regulators of myocardial contractility, the authors' objective was to identify whether droperidol alters $[Ca^{2+}]_i$ or myofilament Ca^{2+} sensitivity in rat ventricular myocytes and to identify the cellular mechanisms responsible for these effects.**

Methods: **Freshly isolated rat ventricular myocytes were obtained from adult rat hearts. Myocyte shortening, $[Ca^{2+}]_i$, nitric oxide production, intracellular pH, and action potentials were monitored in cardiomyocytes exposed to droperidol. Langendorff perfused hearts were used to assess overall cardiac function.**

Results: **Droperidol (0.03–1 μM) caused concentration-dependent decreases in peak $[Ca^{2+}]_i$ and shortening. Droperidol inhibited 35 mM KCl–induced increase in $[Ca^{2+}]_i$, with little direct effect on sarcoplasmic reticulum Ca^{2+} stores. Droperidol had no effect on action potential duration but caused a rightward shift in the concentration–response curve to extracellular Ca^{2+} for shortening, with no concomitant effect on peak $[Ca^{2+}]_i$. Droperidol decreased pH_i and increased nitric oxide production. Droperidol exerted a negative inotropic effect in Langendorff perfused hearts.**

Conclusion: **These data demonstrate that droperidol decreases cardiomyocyte function, which is mediated by a decrease in $[Ca^{2+}]_i$ and a decrease in myofilament Ca^{2+} sensitivity. The decrease in $[Ca^{2+}]_i$ is mediated by decreased sarcolemmal Ca^{2+} influx. The decrease in myofilament Ca^{2+} sensitivity is likely mediated by a decrease in pH_i and an increase in nitric oxide production.**

DROPERIDOL is a butyrophenone derivative that was approved for clinical use in 1970 for the treatment of postoperative nausea and vomiting. In 2001, the US Food and Drug Administration released an alert on the use of droperidol because of its possible cardiotoxicity associated with ventricular arrhythmia (torsade de pointes) and sudden cardiac death. High concentrations of droperidol have been shown to cause a concentration-dependent prolongation in the QT interval in surgical patients, suggesting that droperidol may lead to polymorphic ventricular arrhythmias.[1] Similarly, large concentrations of droperidol used to treat psychiatric patients have been associated with significant QT prolongation, contributing to serious cardiac arrhythmias and even death in some susceptible patients.[2,3] In addition, droperidol has been shown to decrease left ventricular performance in humans,[4] although the mechanisms by which droperidol exerts these effects have not been clearly identified. Despite the potential for these adverse events, many physicians question the validity of warnings that have been imposed regarding the safety of droperidol, alone or in combination with other antiemetic drugs (serotonin receptor antagonists), for the treatment of postoperative nausea and vomiting.[5,6]

Cardiac contractility is regulated by changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), myofilament Ca^{2+} sensitivity, or both. We tested the hypothesis that droperidol exerts a direct negative inotropic effect on individual freshly isolated rat ventricular myocytes. We assessed the effects of droperidol on Ca^{2+} influx *via* voltage-gated Ca^{2+} channels, action potential duration, and Ca^{2+} release from the sarcoplasmic reticulum (SR). We also investigated the effect of droperidol on myofilament Ca^{2+} sensitivity and measured droperidol-induced changes in two important mechanisms that regulate myofilament Ca^{2+} sensitivity, intracellular pH (pH_i) and nitric oxide production. Finally, we assessed the functional significance of our results in isolated myocytes by examining the effect of droperidol in a Langendorff perfused heart preparation.

Materials and Methods

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

Ventricular Myocyte Preparation

Ventricular myocytes were freshly isolated from adult male Sprague-Dawley rat hearts, as previously described.[7] Immediately after the animals were killed, 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, at a pH of 7.35. After a 5-min equilibration period, the perfusion

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buffer was changed to a Ca^{2+}-free KHB buffer containing collagenase type II (309 U/ml; Worthington Biochemical Corp., Freehold, NJ). After digestion with collagenase (20 min), the ventricles were minced and shaken in 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, at a pH of 7.35.

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

Simultaneous measurement of $[Ca^{2+}]_i$ and cell shortening was performed, as previously described by our laboratory.[7] Ventricular myocytes exhibiting a rod-shaped appearance with clear striations were chosen for study. Myocytes (0.5×10^6 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 (30°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).

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.

Intracellular pH Measurements

Measurement of pH_i was performed, as previously described.[8] Ventricular myocytes were incubated in HBS containing 2 μM of the acetoxy methylester form of 2',7'-bis(2-carboxy-ethyl)-5,6-carboxyfluorescein (BCECF/AM) at 23°C for 20 min. Similar to the procedure described for fura-2–loaded myocytes, BCECF-loaded ventricular myocytes were placed in a temperature-regulated (30°C) chamber mounted on the stage of an Olympus IX-70 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 (30°C). Fluorescence measurements were performed on single ventricular myocytes as described above for $[Ca^{2+}]_i$; however, excitation wavelengths of 440 and 500 nm and an emission wavelength of 530 nm were used, as previously described.[8] The fluorescence sampling frequency was 10 Hz, and background fluorescence was determined as described above. To estimate the pH_i value from the ratio of 500/440 nm fluorescence, we used an *in situ* calibration procedure.[8–10] 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 KCl, 1.0 mM $MgCl_2$, 4.0 mM HEPES, 2.0 mM EGTA, 30 mM 2,3-butanedione monoxime, 50 μM BAPTA-AM, 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 KOH (1.0 N). The pH_i for each cell was then determined from a linear regression of the 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 pH_i in the physiologic range (pH 6.6–7.8).[8]

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.

Electrophysiologic Recordings

Only rod-shaped, quiescent cells with smooth striations were selected. The whole cell configuration was achieved with fire-polished and Sylgard-coated tipped glass pipettes (Corning G86165T-4; World Precision Instruments, Sarasota, FL) and a resistance of 2–3 mV when filled with pipette solution containing 135 mM KCl, 1 mM $MgCl_2$, 10 mM EGTA, 10 mM HEPES, and 5 mM glucose, at a pH of 7.3. The bathing solution contained the following: 140 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, at a pH of 7.3. Using the Axopatch 1C (Axon Instruments, Foster City, CA), series resistance was 4–8 mV and was 30–80%

electronically compensated. Action potentials were evoked by repetitive square pulses of 3-ms duration at 1.5× stimulus threshold. Experiments were performed under constant flow conditions at 30°C using a temperature-controlled experimental chamber (Delta T Culture Dish; Bioptechs, Butler, PA). Myocytes were paced under current clamp conditions at a cycle duration of 1,000 ms. Runs of 12 steady state action potentials were recorded with filtering at 2 kHz and sampled at 10 kHz. Data were acquired using a Pentium computer that controlled data acquisition hardware and software (pClamp 6.03; Axon Instruments). Analysis of action potentials was performed by taking the average of each sweep of 12 stable and steady state records.

Measurement of Nitric Oxide

Nitric oxide production was assessed by measurement of nitrate/nitrite production using a colorimetric kit from Cayman Chemical (Ann Arbor, MI). Suspensions of cardiomyocytes were placed in wells and incubated in the presence or absence of droperidol (0.1, 0.3, 1 μM) at 30°C for 15 min. Total nitrite levels (after conversion of nitrate to nitrite) were determined with Griess reagent using a microplate reader (absorbance, 540 nm). Aliquots (150 μl) from each well (before and after addition of droperidol) were collected and mixed with an equal volume of Griess reagent (1% sulfanilic acid and 0.1% *N*-(1-napthyl) ethylenediamine dihydrochloride in 2% phosphoric acid). The mixture was incubated at 20°C for 10 min. Nitrite concentrations in the samples were determined based on standard calibration curves by using an aqueous solution of sodium nitrite. The background value from buffer alone was subtracted from the experimental value.

Isolated Perfused Langendorff Heart Preparation

Male Sprague-Dawley rats weighing 250–300 g, were given an intraperitoneal injection of heparin (200 U). After the animals were killed, hearts were excised rapidly and placed in ice-cold KHB before being mounted on a Langendorff apparatus for perfusion at 37°C with KHB at 330 beats/min and a constant pressure of 65–70 mmHg. The buffer was equilibrated with 95% O_2 and 5% CO_2 and had the following composition: 118 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 11 mM dextrose. A balloon-tipped catheter was inserted through the left atrium into the left ventricle, and the left ventricular end-diastolic pressure in all hearts was adjusted to between 8 and 12 mmHg. Left ventricular developed pressure was monitored continuously throughout the experiment. Coronary blood flow was measured by timed collection of the effluent into a graduated cylinder.

Experimental Protocols

All experimental protocols were performed at 30°C, with the exception of the perfused Langendorff heart experiments performed at 37°C.

Concentration-dependent Effects on $[Ca^{2+}]_i$ and Myocyte Shortening. A stock solution of droperidol was obtained by dissolving the drug in dimethyl sulfoxide. Baseline measurements were collected from individual myocytes for 1.5 min in the absence of any intervention. Myocytes were exposed to four concentrations of droperidol (0.03, 0.1, 0.3, and 1 μM) by exchanging the buffer in the dish with new buffer containing droperidol at the desired concentration. Data were acquired for 1.5 min after a 5-min equilibration period in the presence of droperidol. Summarized results for the concentration–response curves are expressed as percent of the control value. Dimethyl sulfoxide (0.05% vol/vol) alone has no effect on $[Ca^{2+}]_i$ or shortening.[7] We verified that droperidol 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 pCa (−log Ca^{2+} concentration) 9 to pCa 5 in the presence or absence of droperidol (data not shown).

Effect on KCl-induced Increase in $[Ca^{2+}]_i$. Droperidol was added to the superfusion medium for 15 min, and then KCl (35 mM) was applied to the cell. This concentration of KCl was chosen because it stimulates an increase in $[Ca^{2+}]_i$ approximately 50% of the maximum response. Peak $[Ca^{2+}]_i$ induced by KCl was compared with peak $[Ca^{2+}]_i$ before addition of droperidol (1 μM) and is reported as percent change from control.

Effect on Action Potential Duration. Action potentials were recorded before and after addition of droperidol (1 μM) in individual cardiomyocytes. Changes in action potential duration at 90% repolarization were determined.

Effect on SR Ca^{2+} Stores. Baseline values for $[Ca^{2+}]_i$ were measured in individual, field-stimulated myocytes for 1.5 min. Droperidol (0.1 and 1 μM) was then added to the superfusion buffer and allowed to perfuse the cell for 5 min. Field stimulation of the myocyte was then discontinued, and caffeine (20 mM) was applied to the cell 15 s later in the continued presence of droperidol. Peak $[Ca^{2+}]_i$ induced by caffeine was compared with peak $[Ca^{2+}]_i$ before addition of droperidol and as a percent increase above the peak $[Ca^{2+}]_i$ achieved during field stimulation (fractional release).

Effect on the Extracellular Ca^{2+}–Shortening Relation. Changes in the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$)–shortening relation (indirect measurement of myofilament Ca^{2+} sensitivity), were examined as previously described.[10,11] Baseline variables were collected from individual myocytes for 1.5 min. Concentration–response curves for $[Ca^{2+}]_o$ were performed by exchanging the buffer in the dish with a new buffer con-

taining the desired $[Ca^{2+}]_o$. Data were acquired for 1.5 min after establishment of a new steady state. Concentration–response curves for $[Ca^{2+}]_o$ were then performed in the presence of droperidol (1 μM). Cells were allowed to stabilize for 5 min after addition of droperidol.

Effect on pH_i. Baseline pH_i was collected from individual myocytes for 1 min. Droperidol (0.03, 0.1, 0.3, and 1 μM) was added by exchanging the superfusion buffer in the dish with new buffer containing droperidol at the desired concentration. Each myocyte was exposed to only one concentration of droperidol. Results are expressed as the change in pH_i over time with each concentration of droperidol.

Effect on Nitric Oxide Production. Suspensions of ventricular myocyte were exposed to droperidol (0.1, 0.3, and 1 μM) for 15 min at 37°C in HBS buffer. Total nitrite/nitrate production was measured as an indicator of nitric oxide production. Results are expressed as percent change in total nitrate/nitrite from baseline.

Effect on Left Ventricular Developed Pressure. Left ventricular pressure was continuously monitored in perfused Langendorff hearts paced at 330 beats/min at 37°C before (20 min) and during administration of droperidol (1 μM, 20 min). Results are expressed as percent change in pulse pressure (left ventricular end systolic pressure minus end diastolic pressure) from baseline.

Statistical Analysis

Each experimental protocol was performed on multiple myocytes from the same heart and repeated in five hearts. Results obtained from myocytes in each heart were averaged so that all hearts were weighted equally. Comparison of several means was performed using repeated measures and two-way analysis of variance.[12] The Bonferroni *post hoc* test was used when significant differences among groups were detected. Differences were considered statistically significant at $P < 0.05$. All results are expressed as mean ± SD.

Materials

Droperidol, caffeine, and dimethyl sulfoxide were purchased from Sigma Chemical Co. (St. Louis, MO). Collagenase was purchased from Worthington Biochemical Corp. (Lakewood, NJ). BCECF/AM and fura-2/AM were purchased from Texas Fluorescence Labs (Austin, TX). The nitrate/nitrite colorimetric assay kit was obtained from Cayman Chemical.

Results

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

Resting cell length was 125 ± 5 μm, and the baseline 340/380 ratio was 0.8 ± 0.1. Twitch height was 12 ± 1.5 μm (10.4 ± 1.6% of the resting cell length). The change in 340/380 ratio from baseline with shortening was 0.5 ± 0.1. Tp $[Ca^{2+}]_i$ and shortening were 151 ± 22 and 177 ± 16 ms, respectively. Times to 50% recovery (Tr) for $[Ca^{2+}]_i$ and shortening were 192 ± 19 and 228 ± 21 ms, respectively.

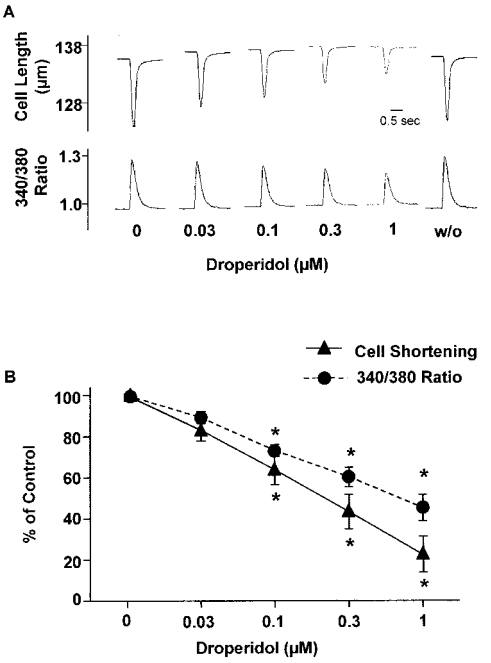

Fig. 1. (*A*) Representative trace demonstrating the concentration-dependent effect of droperidol on myocyte shortening and intracellular free Ca^{2+} concentration in a single ventricular myocyte. w/o = washout. (*B*) Summarized data for the effects of droperidol on myocyte shortening and intracellular free Ca^{2+} concentration. Results are expressed as percent of control. Values represent mean ± SD in this and all subsequent figures. * $P < 0.05$ compared with control; n = 21 cells from six hearts.

Effect on $[Ca^{2+}]_i$ and Myocyte Shortening

A representative trace depicting the concentration-dependent effects of droperidol on $[Ca^{2+}]_i$ and shortening in a single, field-stimulated ventricular myocyte is shown in figure 1A. Droperidol (1 μM) reduced peak $[Ca^{2+}]_i$ and shortening by 22 ± 4 and 43 ± 5%, respectively. An increase in resting cell length of 2 ± 0.6 μm with no change in resting $[Ca^{2+}]_i$ was observed in most cells. The myocardial depressant effects of droperidol were reversible after washout. Summarized data for the concentration-dependent effects of droperidol on $[Ca^{2+}]_i$ and shortening are also shown in figure 1B. Droperidol caused concentration-dependent decreases in $[Ca^{2+}]_i$ and shortening. Figure 2 represents an exploded overlay view of the individual $[Ca^{2+}]$ transient and shortening event before (A and B) and after being normalized to peak height (C and D) in the presence or absence of droperidol (1 μM) to illustrate changes in timing. Droperidol (1 μM) had no effect on Tp $[Ca^{2+}]_i$ (98 ± 5% of control), Tp shortening (94 ± 6% of con-

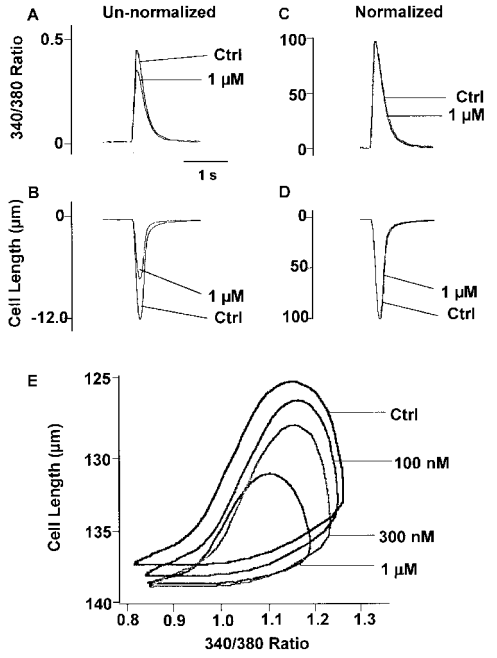

Fig. 2. (*A* and *B*) Overlay of the individual cell shortening and intracellular Ca^{2+} transients taken from control (Ctrl) and after exposure to droperidol (1 μM). Data are normalized to peak height to more easily illustrate changes in timing (*C* and *D*). (*E*) Hysteresis loops created from individual Ca^{2+} transients and shortening events in figure 1 (*top*) depicting the continuous intracellular free Ca^{2+} concentration–shortening relation in the presence or absence of droperidol.

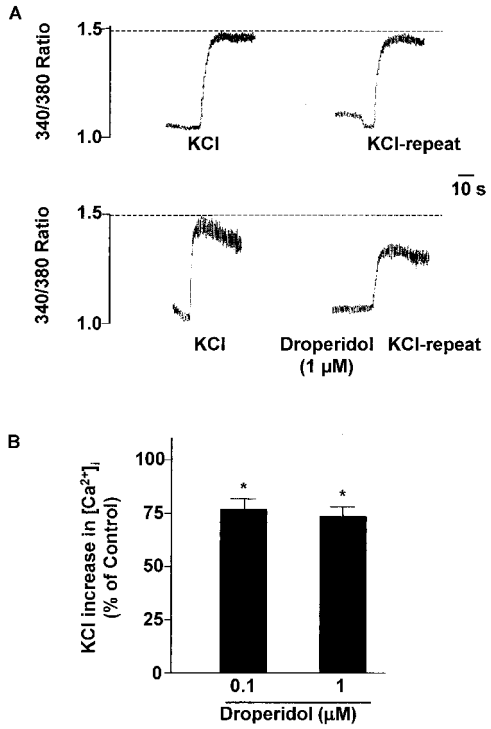

Fig. 3. (*A*) Representative trace demonstrating the effect of droperidol (1 μM) on KCl (35 mM)–induced increase in intracellular free Ca^{2+} concentration. (*B*) Summarized data for the concentration-dependent effects of droperidol on the KCl-induced increase in intracellular free Ca^{2+} concentration. Results are expressed as percent of control. * $P < 0.05$ compared with control; n = 15 cells from five hearts.

trol), Tr $[Ca^{2+}]_i$ (96 ± 8% of control), or Tr shortening (102 ± 3% of control). The continuous $[Ca^{2+}]_i$–shortening relations are also depicted as hysteresis loops in figure 2E. Droperidol caused a marked concentration-dependent downward shift in the continuous $[Ca^{2+}]_i$–shortening relation.

Effect on KCl-induced Increase in $[Ca^{2+}]_i$

Addition of KCl (35 mm) to quiescent myocytes resulted in a sustained increase in $[Ca^{2+}]_i$ (fig. 3A). Pretreatment with droperidol (0.1 and 1 μM) attenuated the KCl induced-increase in $[Ca^{2+}]_i$ by 24 ± 5 and 27 ± 5%, respectively (fig. 3B).

Effect on Action Potential Duration

Figure 4A shows that exposure of the myocyte to droperidol (1 μM) had no effect on action potential duration compared with control myocytes not exposed to droperidol. Summarized data depicting the effect of droperidol on action potential duration at 90% repolarization are also shown in figure 4B.

Effect on Caffeine-induced Release of Ca^{2+} from the SR

Although the peak $[Ca^{2+}]_i$ achieved with caffeine was reduced by 22 ± 5% in the presence of droperidol (0.1 μM) compared with control, the fractional release of Ca^{2+} from the SR was not different from that observed in the absence of droperidol (fig. 5A). Summarized data for the effects of droperidol on SR Ca^{2+} content and fractional release are depicted in figure 5B. The decrease in SR Ca^{2+} content is likely due to the inhibitory effect of droperidol on the L-type Ca^{2+} channel, which reduces the driving force for refilling the SR.

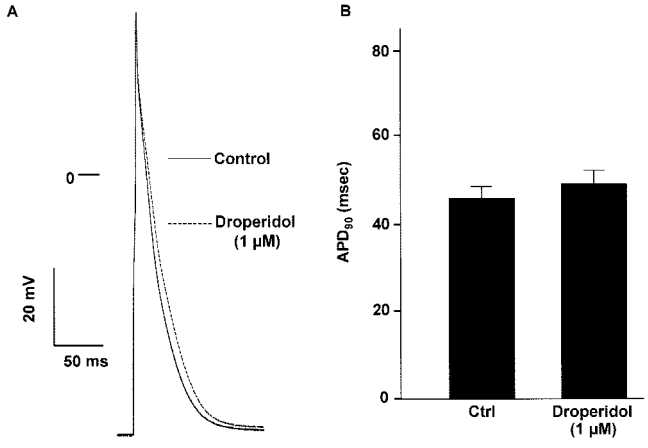

Fig. 4. (*A*) Representative trace demonstrating the effect of droperidol (1 μM) on action potential duration recorded from an individual myocyte. (*B*) Summarized data for the effect of droperidol on action potential duration. Results are expressed in milliseconds. APD_{90} = action potential duration at 90% repolarization. n = 7 cells from three hearts.

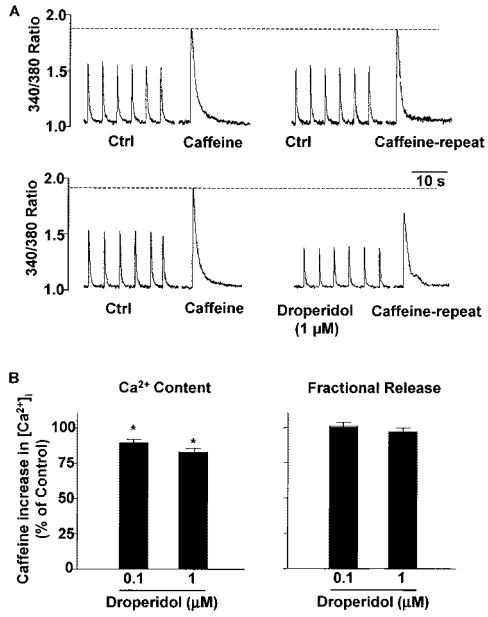

Fig. 5. (*A*) Representative trace demonstrating the effect of droperidol on caffeine-induced release of Ca^{2+} from the sarcoplasmic reticulum. (*B*) Summarized data for the concentration-dependent effects of droperidol on sarcoplasmic reticulum Ca^{2+} content and fractional release of Ca^{2+} from the sarcoplasmic reticulum. Results are expressed as percent of control. * $P <$ 0.05 compared with control (Ctrl); n = 17 cells from six hearts.

Effect on the Concentration–Response Curve to $[Ca^{2+}]_o$

Figure 6 demonstrates that increasing $[Ca^{2+}]_o$ from 1 to 4 mm (control, without droperidol) resulted in a concentration-dependent increase in shortening (A) and a concomitant increase in peak $[Ca^{2+}]_i$ (B). Droperidol (0.3 μM) caused a significant downward shift in the concentration–response curve to increasing $[Ca^{2+}]_o$ for shortening, with no concomitant effect on peak $[Ca^{2+}]_i$.

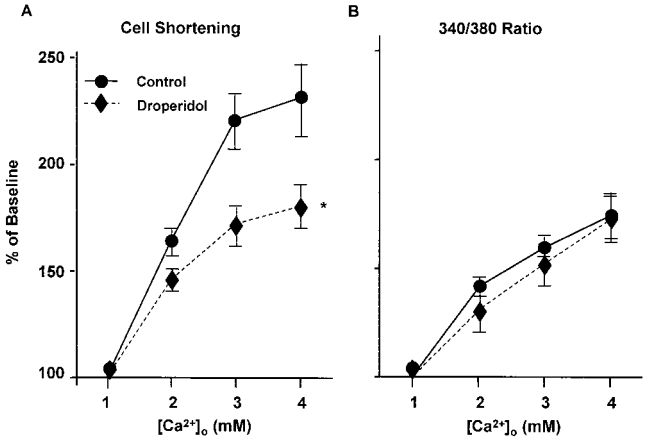

Fig. 6. Summarized data for the effect of droperidol (1 μM) on myocyte shortening (*A*) and intracellular free Ca^{2+} concentration (*B*) in response to increasing extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). Results are expressed as percent of baseline (baseline value without any droperidol at each $[Ca^{2+}]_o$). * $P <$ 0.05 compared with control; n = 15 cells from five hearts.

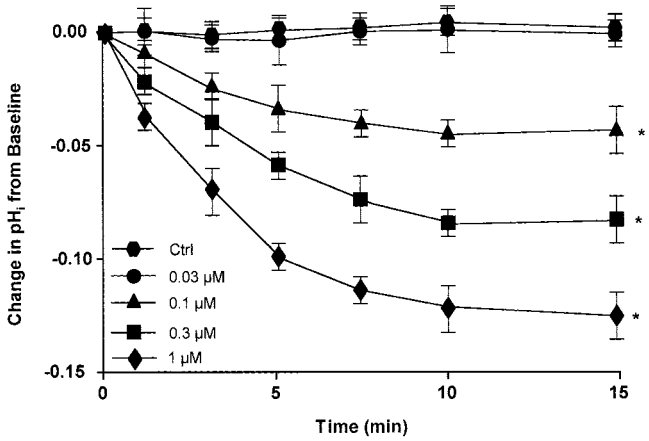

Fig. 7. Summarized data for the concentration-dependent effects of droperidol on intracellular pH (pH_i). Results are expressed as change in pH_i from baseline. * $P <$ 0.05 compared with control (Ctrl); n = at least 3 cells at each concentration of droperidol from seven hearts.

Effect on Intracellular pH_i

Baseline pH_i was 7.11 ± 0.04. Summarized data depicting the effect of droperidol on pH_i are shown in figure 7. Droperidol at 0.1, 0.3, and 1 μM caused concentration- and time-dependent decreases in pH_i. The effects of droperidol on pH_i were reversible after washout with HBS (pH_i = 7.08 ± 0.04). There was no significant change in extracellular pH in the presence of 1 μM droperidol (control: 7.35 ± 0.13, droperidol: 7.34 ± 0.11; not significant).

Effect on Nitric Oxide Production

Droperidol at concentrations of 0.1, 0.3, and 1 μM increased nitric oxide production by 22 ± 6, 53 ± 7, and 74 ± 10% compared with control. The nitric oxide donor, *S*-nitroso-*N*-acetylpenicillamine (10 μM), increased nitric oxide by 128 ± 12% compared with control.

Effect on Left Ventricular Developed Pressure

Addition of droperidol (0.1, 1 μM) to the perfusate resulted in concentration-dependent decreases in pulse pressure of 29 ± 6 and 43 ± 8%, respectively. There were no significant changes in coronary blood flow after perfusion with 1 μM droperidol (18 ± 1.4 ml/min before droperidol *vs.* 16 ± 1.8 ml/min after droperidol).

Discussion

The major findings of this study are that droperidol causes a decrease in cardiomyocyte contractility *via* a decrease in $[Ca^{2+}]_i$ and a decrease in myofilament Ca^{2+} sensitivity. The decrease in $[Ca^{2+}]_i$ is due to a decrease in sarcolemmal Ca^{2+} influx, whereas the decrease in myofilament Ca^{2+} sensitivity is likely mediated by a de-

crease in cardiomyocyte pH_i, an increase in nitric oxide production, or both.

Effect on $[Ca^{2+}]_i$ and Myocyte Shortening

There are no previous studies that have evaluated the effects of droperidol on ventricular cardiomyocyte $[Ca^{2+}]_i$ and contractility. However, droperidol has been shown to slow pacemaker activity and depress maximum velocity of contraction in guinea pig ventricular muscle, although the cellular mechanisms for these effects were not investigated.[13,14] In the current study, we observed that clinically relevant concentrations of droperidol inhibit the peak $[Ca^{2+}]_i$ achieved in response to field stimulation in a concentration-dependent manner. In addition, droperidol caused an increase in resting cell length in the absence of any change in diastolic $[Ca^{2+}]_i$, suggesting a decrease in myofilament Ca^{2+} sensitivity. Taken together, these data suggest that droperidol exerts its effects on cellular mechanisms that regulate $[Ca^{2+}]_i$ and myofilament Ca^{2+} sensitivity. Because there were no changes in the timing variables of $[Ca^{2+}]_i$, which would have been reflected in the shortening and relengthening of the myocyte, it is unlikely that droperidol has any effect on the Na^+–Ca^{2+} exchanger or the SR Ca^{2+} pump. Therefore, we investigated the effects of droperidol on Ca^{2+} influx *via* voltage-gated Ca^{2+} channels.

Effect on KCl-induced Increase in $[Ca^{2+}]_i$

The increase in cardiomyocyte $[Ca^{2+}]_i$ after addition of KCl is known to result from a depolarization-induced activation of the L-type Ca^{2+} channel. We observed a decrease in the KCl-induced increase in $[Ca^{2+}]_i$ in myocytes pretreated with droperidol. These findings suggest that droperidol may have a direct inhibitory effect on L-type Ca^{2+} channels. Alternatively, a droperidol-induced activation of the transient outward K^+ current or the delayed rectifier K^+ current could abbreviate action potential duration and thereby reduce the time in which Ca^{2+} enters the cell *via* L-type Ca^{2+} channels. Therefore, we examined the effect of droperidol on action potential duration.

Effect on Action Potential Duration

Previous studies have demonstrated a prolongation in action potential duration and the presence of early afterdepolarizations with low concentrations of droperidol (10–300 nM), whereas a shortening of action potential duration was observed with high concentrations (10–30 μM).[13,15] The prolongation in action potential duration was due to a droperidol-induced inhibition of the delayed rectifier K^+ current.[15] Droperidol has been reported to cause a concentration-dependent prolongation of the QT interval in humans[1] and subsequent torsade de pointes, which can progress to ventricular fibrillation and sudden cardiac death.[2] In the current study using rat ventricular myocytes, we did not observe a prolongation in action potential duration with any concentration of droperidol. This may be because rat ventricular myocytes lack a prominent delayed rectifier K^+ current and primarily rely on the transient outward K^+ current for repolarization. Therefore, we conclude that the inhibitory effect of droperidol on the increase in $[Ca^{2+}]_i$ induced by electrical field stimulation or KCl is not due to an indirect effect of droperidol on action potential duration.

Effect on Caffeine-induced Release of Ca^{2+} from the SR

Ca^{2+}-induced Ca^{2+} release is the process by which influx of Ca^{2+} through sarcolemmal L-type Ca^{2+} channels triggers Ca^{2+} release from the SR through an activation of the ryanodine receptor (Ca^{2+} release channel) in cardiomyocytes.[16] Rapid application of caffeine to quiescent myocytes results in direct activation of the ryanodine receptor on the SR, triggering the release of Ca^{2+} from the SR. The difference between the peak $[Ca^{2+}]_i$ induced by electrical stimulation and the caffeine-induced increase in $[Ca^{2+}]_i$ represents the fractional release of Ca^{2+} from the SR and is used to assess whether interventions have a direct effect on SR Ca^{2+} content or an indirect effect due to decreased sarcolemmal Ca^{2+} influx. Our results indicate that although the peak increase in $[Ca^{2+}]_i$ in response to caffeine is attenuated by droperidol, the fractional release of Ca^{2+} is unaltered. This is because droperidol inhibits sarcolemmal Ca^{2+} influx through the L-type Ca^{2+} channels, resulting in an overall decrease in the size of the releasable pool of Ca^{2+} in the SR. Therefore, it seems that droperidol does not exert a direct inhibitory effect on SR Ca^{2+} release but does have an indirect effect on the size of the releasable pool of Ca^{2+}.

Effect on the Concentration–Response Curve to $[Ca^{2+}]_o$

We hypothesized that a decrease in myofilament Ca^{2+} sensitivity may also play a role in the inhibitory effects of droperidol on contractility, based on our observations of a droperidol-induced increase in resting cell length and a downward shift in the $[Ca^{2+}]_i$–shortening relation. Although changes in sensitivity can also be reflected in the timing variables of contraction, we did not observe a change in the timing variables. This indicates that droperidol likely exerts its effects at multiple sites of regulation for myofilament Ca^{2+} sensitivity, which may offset or mask the effect of one another. Because alterations in myofilament Ca^{2+} sensitivity can alter contractility,[17] we examined whether droperidol altered the concentration–response relation to $[Ca^{2+}]_o$. This protocol is an indirect assessment of myofilament Ca^{2+} sensitivity that allows for a paired comparison of $[Ca^{2+}]_i$ and contractile amplitude in the same cell in the presence or

absence of droperidol over a range of values for $[Ca^{2+}]_o$. Droperidol caused a downward shift in the concentration–response curve to $[Ca^{2+}]_o$ for shortening, with no concomitant effect on $[Ca^{2+}]_i$. These data suggest that droperidol decreases the maximal response of the myofilament to Ca^{2+} as $[Ca^{2+}]_i$ increases. Therefore, it seems that in addition to a decrease in sarcolemmal Ca^{2+} influx, a droperidol-induced decrease in myofilament Ca^{2+} sensitivity contributes to the inhibitory effect of droperidol on cardiomyocyte contractility. We next assessed potential cellular mechanisms that may be responsible for the decrease in myofilament Ca^{2+} sensitivity.

Effect on pH_i

One possible mechanism for a decrease in myofilament Ca^{2+} sensitivity is intracellular acidification. It is well known that intracellular acidosis decreases the contractility of cardiac muscle,[17] although the mechanisms responsible for the decrease are complicated. Acidosis affects every step of the excitation–contraction coupling pathway, including the availability and delivery of Ca^{2+} to the myofilaments, as well as the response of the myofilaments to Ca^{2+}.[18–21] Droperidol decreased pH_i in a time- and concentration-dependent manner in cardiomyocytes. These data suggest that droperidol may have an inhibitory effect on the Na^+–H^+ exchanger, which would promote accumulation of H^+ in the cytoplasm, resulting in intracellular acidosis. Because these studies were conducted in a HBS buffer in the absence of carbon dioxide and bicarbonate, a possible interaction between droperidol and Na^+–HCO_3^- symport or Cl^-–HCO_3^- exchange is unlikely, because these transport systems are inactive in myocytes bathed in HCO_3^--free solution.[22,23] The magnitude of the change in pH_i in response to droperidol (0.1 μM) was similar in magnitude, although opposite in direction, to that previously reported by our laboratory for thiopental- or propofol-induced changes in pH_i and myofilament Ca^{2+} sensitivity.[8,10,24] Moreover, other studies have documented similar changes in pH_i for phenylephrine- and endothelin-induced intracellular alkalosis, respectively, resulting in a positive inotropic response.[25,26] It is also possible that a droperidol-induced decrease in pH_i contributes to the inhibitory effect of droperidol on the KCl-induced increase in $[Ca^{2+}]_i$. Further studies are required to confirm this possibility.

Effect on Nitric Oxide Production

An increase in cardiomyocyte nitric oxide has been shown to decrease myofilament Ca^{2+} sensitivity, resulting from an alteration in troponin I phosphorylation[27] and/or an alteration in actin–myosin cross-bridge cycling by modulating critical thiols on the myosin head.[28] In addition, nitric oxide is known to activate the cyclic guanosine monophosphate signaling pathway, which has been shown to reduce the myofilament response to Ca^{2+} in cardiac myocytes[29] but augment release of Ca^{2+} from the SR by caffeine.[30] Droperidol caused a concentration-dependent increase in nitric oxide production in cardiomyocytes. The increase in nitric oxide production, as well as the decrease in pH_i, are likely involved in the droperidol-induced decrease in myofilament Ca^{2+} sensitivity. However, direct confirmation of this hypothesis and the relative roles of each will require additional studies.

Effect on Left Ventricular Developed Pressure

To identify whether the changes in cardiomyocyte function could be extrapolated to the working heart, we assessed the effect of droperidol on overall cardiac function in buffer-perfused Langendorff hearts. Only one study assessing the effects of droperidol on isolated hearts has been performed, although the focus was on action potential duration and cardiac repolarization with no assessment of inotropic status.[15] An *in vivo* study in humans demonstrated a droperidol-induced decrease in left ventricular performance,[4] whereas another *in vivo* study focused on prolongation of the QT interval.[1] In the current study, droperidol caused decreases in left ventricular developed pressure that correlated with changes seen at the level of the individual cardiomyocyte. These data indicate that droperidol, at clinically relevant concentrations, exerts a negative inotropic effect in isolated perfused hearts. However, the magnitude of the negative inotropic effect of droperidol (1 μM) observed in cardiomyocytes, where the experiments were performed at 30°C, was greater than that observed in the perfused Langendorff hearts, where the experiments were performed at 37°C. The negative inotropic effect of droperidol in the cardiomyocytes is likely greater because of a reduced diffusion gradient for drug interaction with the cardiomyocytes.

Limitations of the Study and Clinical Implications

All anesthetic agents bind to plasma proteins, reducing the concentration available to bind to tissues. In the clinical setting, the use of droperidol (0.125 mg/kg) is standard for the prevention of postoperative nausea and vomiting. The peak plasma concentration with this dose has been estimated at 2 μM.[31] Taking into account that 90% of droperidol is bound to protein, the free concentration of droperidol is approximately 0.2 μM.[32] However, the microkinetic behavior of droperidol within the vascular space has not been defined. In addition, small changes in the amount or binding capacity of proteins could result in significant increases in the free plasma concentrations of droperidol. Not only is there uncertainty in calculating the *in vivo* concentration of droperidol during normal circumstances, but the concentration in free plasma would certainly be higher when the concentration of protein serum is reduced (*e.g.*, hemodilution, liver disease, hypoproteinemia). Another potential

limitation is the use of rat cardiomyocytes as a model for human cardiomyocytes, because species differences may exist. Action potentials in rodent cardiomyocytes are abbreviated compared with those recorded in human myocytes and are comprised primarily of the transient outward K^+ current, whereas in humans the delayed rectifier K^+ current predominates. This may explain our inability to observe a droperidol-induced prolongation in action potential duration. Moreover, this may also contribute to differences in the response of human and rat myocardium to anesthetic agents. It should be noted that this study deals only with intrinsic properties of the heart and that cardiac function also depends on preload, afterload, venous return, and heart rate, which are not factors in isolated cardiomyocytes. However, we have tried to overcome this limitation with the perfused Langendorff heart preparation. Despite these limitations, our results demonstrate that clinically relevant concentrations of droperidol decrease $[Ca^{2+}]_i$ and myofilament Ca^{2+} sensitivity in cardiomyocytes, resulting in a negative inotropic effect. Moreover, in addition to the α-adrenergic blocking effect of droperidol in the vasculature,[33,34] a decrease in the inotropic state of the heart will likely exacerbate the hypotensive effect of droperidol observed in the clinical setting.

Summary

Our results provide the first direct evidence that droperidol causes a negative inotropic effect in individual cardiomyocytes. This effect is mediated by both a decrease in $[Ca^{2+}]_i$ and a decrease in myofilament Ca^{2+} sensitivity. The decrease in $[Ca^{2+}]_i$ is not due to an effect on action potential duration, but rather due to an effect on the L-type Ca^{2+} channel to limit sarcolemmal Ca^{2+} influx. The decrease in myofilament Ca^{2+} sensitivity is likely mediated by a decrease in pH_i and an increase in nitric oxide production.

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