Chronic alcohol-induced changes in cardiac contractility are not due to changes in the cytosolic Ca\(^{2+}\) transient

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Figueredo, Vincent M., Kevin C. Chang, Anthony J. Baker, and S. Albert Camacho. Chronic alcohol-induced changes in cardiac contractility are not due to changes in the cytosolic Ca\(^{2+}\) transient. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H122–H130, 1998.—Long-standing heavy alcohol consumption may also be viewed as a chronic stress on the heart. As a result, alcohol abuse is the major cause of nonischemic cardiomyopathy in Western society (52). Changes in myocardial contractility due to chronic alcohol exposure are well documented in animal models [see review by Thomas et al. (47)] and mimic those seen in the majority of alcoholics; i.e., most alcoholics have mild left ventricular (LV) hypertrophy and contractile dysfunction (18, 25). Echocardiographic studies show that although some alcoholics have dilated cardiomyopathies, most have mild decreases of LV contractility and impaired LV filling (19, 30, 38, 45, 49). In contrast, echocardiographic studies in moderate drinkers demonstrate no change in or mildly reduced LV contractile function (29, 51).

The mechanisms underlying chronic alcohol-induced alterations of contractility remain unclear. Studies of acute alcohol-induced contractile changes (in clinically relevant doses) suggest that decreased peak contractile force is not due to decreased [Ca\(^{2+}\)], but to decreased myofilament responsiveness to [Ca\(^{2+}\)] (16, 22). A number of investigators (1, 3, 13, 26) have speculated that, in contrast to acute alcohol exposure, chronic alcohol exposure alters contractility by impairing myocyte Ca\(^{2+}\) handling. However, to date, no measurements of myocyte [Ca\(^{2+}\)], after chronic alcohol exposure have been performed, nor have potential changes in gene expression, resulting in changes in the content of proteins important in Ca\(^{2+}\) handling (e.g., SERCA2a and phospholamban), been explored. Furthermore, it may be that alcohol induces alterations of gene expression at the level of the myofilament, affecting myofilament responsiveness to [Ca\(^{2+}\)]. For example, changes in contractile proteins (e.g., MHC isoforms) or regulatory proteins (e.g. troponin and tropomyosin isoforms) may result in alterations of cross-bridge kinetics and/or the Ca\(^{2+}\)-force relationship.

The purpose of this study was therefore to determine whether changes in contractile function after chronic alcohol exposure are due to altered [Ca\(^{2+}\)]. We simultaneously measured [Ca\(^{2+}\)], transients using indo 1 fluorescence and isovolumic contraction and relaxation in perfused hearts from rats drinking 36% ethanol in their water for 7 mo. A second aim of these experiments was to study the effects of the acute stresses, rapid pacing, and acute alcohol administration on LV pressure and [Ca\(^{2+}\)] measurements in hearts of animals after chronic alcohol feeding. A third aim was to determine whether SERCA2a and/or phospholamban protein levels, proteins important in Ca\(^{2+}\) handling, were altered in myocytes isolated from hearts of alcohol-fed animals.
compared with controls. Finally, as a first step in determining how chronic alcohol exposure might alter contractility by inducing alterations at the level of the myofilament, we assessed the relative content of MHC isoforms.

**METHODS**

**Chronic Alcohol Exposure Model**

Male Sprague-Dawley rats weighing 200 g were divided into two groups: a chronic alcohol-exposure group (alcohol; n = 13) and an age-matched control group (control; n = 14). All animals were fed Purina Rat chow and water ad libitum. To acclimate rats to drinking ethanol, animals received 10% (vol/vol) ethanol in their drinking water for the first week, 20% ethanol for the second week, and 36% ethanol for the remainder of a 7-month treatment period. Mean blood ethanol concentration at the time that the animals were killed was 144 ± 29 mg/dl. This model of heavy chronic alcohol exposure does not result in nutritional deficiencies in alcohol-fed animals compared with controls (39). Body weights for both groups were recorded weekly. Blood pressure was measured by the tail-cuff method before death in 12 animals from each group.

**Heart Perfusion and Measurement of Contractile Function**

Rats were heparinized (1,000 U ip) and anesthetized (ketamine 100 mg ip). Hearts were excised, arrested in cold isosmotic saline (20 mM KCl), cannulated via the aorta, and perfused at a constant pressure of 71 mmHg on a nonrecirculating Langendorff perfusion apparatus using a Krebs-Henseleit (KH) perfusate (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 24.9 mM NaHCO3, 1.7 mM MgSO4, 1.2 mM KH2PO4, 5.6 mM glucose, 2.0 mM pyruvate, and 20 U/l insulin). Perfusate was bubbled using a 95% O2-5% CO2 gas mixture and maintained at 37°C. Hearts were paced at 300 beats/min using two platinum-tipped electrodes connected to a Grass Instruments SD-5 stimulus generator (Grass Instrument, Quincy, MA). Coronary flow was measured by an in-line flowmeter (Gilmont Instruments, Barrington, IL).

LV pressure was measured using a 2-Fr, high-fidelity micromanometer (Millar Instruments, Houston, TX). A compliant latex balloon was attached to a 2-cm segment of rigid polyethylene tubing connected to a Y adapter. One end of the Y adapter was used to advance the micromanometer to the latex balloon and the other to fill the LV balloon to set the end-diastolic pressure at 10 mmHg. The balloon was inserted through the left atrium into the LV. Pressure was recorded on a Gould series 8000 chart recorder (Gould Electronics, Hayward, CA) and digitized at 2-ms intervals by an SLM spectrofluorometer (model 48000S, SLM Instruments, Rochester, NY).

**[Ca2+]c Measurements**

Indo 1 fluorescence methods. Fluorescence studies were performed as previously described (34). Excitation light from a 450-W xenon arc lamp (SLM Instruments) was filtered through a 350-nm interference filter and focused onto the in-going leg of a quartz bifurcated fiber bundle. Emitted fluorescence was collected in the outgoing leg of the bundle and divided into two beams using a dichroic mirror and directed onto two photomultiplier tubes preceded by 385- and 456-nm interference filters.

Background autofluorescence (primarily NADH) measurements were obtained. Hearts were then loaded for 35 min by retrograde perfusion with KH buffer containing indo 1-AM (6 µM; dissolved in DMSO and Pluronic F-127, 10% wt/vol, Molecular Probes, Johnstown City, OR) and fetal bovine serum (6%; Sigma, St. Louis, MO). Probenecid (0.1 mM, Sigma) was added to all perfusates to slow the extrusion of indo 1 from the myocytes. Residual indo 1-AM was washed out using indo 1-free perfusate for 20 min. Loading with indo 1 resulted in a 1.5 - 2 and 1 ± 1 times increase in diastolic fluorescence at 385- and 456-nm emission wavelengths, respectively, compared with background fluorescence.

Corrections for factors affecting [Ca2+]c assessment. The major factors that can affect free [Ca2+]c determination were taken into account: 1) motion artifact; 9) 2) changes in the tissue inner filter, which is a consequence of the myoglobin oxygenation state (11); 3) changes of heart background fluorescence, which is primarily NADH (7, 8); and 4) noncytosolic contribution to the indo 1 fluorescence signal, which primarily represents mitochondrial indo 1 loading (20, 34, 41). Four hearts from alcohol-fed animals were studied to confirm that noncytosolic indo 1 loading was not different in alcohol-fed compared with control hearts. To determine the noncytosolic indo 1 contribution, hearts were perfused with manganese (3.5 mM) at a rate of 1.5% of coronary flow to selectively quench the cytosolic indo 1 fluorescence (6–8 min) as previously described (34, 41). The noncytosolic contribution was similar (59% at 385 nm; 52% at 456 nm) to that in control hearts previously reported by our laboratory (62 and 56%, Ref. 34). Calculation of [Ca2+]c, and mitochondrial [Ca2+]c were performed as previously described with the corrected fluorescence ratio at emission wavelengths of 385 and 456 nm using the standard equation for fluorescence indicators (34).

**Experimental Protocol**

Baseline data. After a 20-min equilibration period, baseline measurements of the LV pressure transient, perfusion pressure, coronary flow, and background fluorescence were performed. After 35 min of indo 1 loading and 25-min washout periods, baseline measurements of the LV pressure transient, perfusion pressure, and coronary flow were repeated, and simultaneous [Ca2+]c transients were recorded.

Rapid pacing and acute alcohol exposure. Hearts were then paced at 420 beats/min for 3 min (allowing LV pressure to equilibrate), and measurements were repeated (n = 11 alcohol; n = 13 control). After a 10-min recovery period, repeat baseline measurements were taken to document no change from initial baseline measurements. Hearts were then switched to a perfusate containing 24 mM ethanol (0.15% vol/vol), approximating blood levels obtained in humans acutely intoxicated. After a 15-min equilibration period, measurements were repeated (n = 11 alcohol; n = 10 control).

Decreased LV pressure and faster relaxation. A significant decrease of peak LV pressure, in and of itself, has been shown to speed relaxation in isolated hearts (48). To determine whether the faster relaxation rates observed in hearts from animals chronically exposed to alcohol were simply a consequence of decreased peak contractile force, additional experiments (n = 4 control) were performed in which peak systolic force was decreased to a comparable degree as that seen in alcohol hearts by 1) decreasing coronary perfusion pressure and 2) decreasing end-diastolic LV pressure (decreased LV balloon volume). Hemodynamic measurements of the LV pressure transient, perfusion pressure, and coronary flow were recorded after each intervention and during 15-min recovery periods to confirm baseline measurements were unchanged.

**Preparation of Isolated Myocytes**

Additional hearts (n = 6 alcohol; n = 5 control) were perfused at a constant pressure of 70 mmHg using pH 7.4
HEPES-KH (138 mM NaCl, 4.7 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgSO₄, 10 mM glucose, 10 mM pyruvate, 5 mM HEPES, 0.1 mM probenicid, and 20 U/l insulin). Perfusion was bubbled with 100% O₂ and maintained at 37°C. After 10 min, perfusate was changed to a nominally Ca²⁺-free pH 7.2 HEPES-KH solution with 0.5 mg/ml BSA. Hearts were perfused at a constant flow of 5 ml/min with KH solution containing 1 mg/ml collagenase B (Boehringer Mannheim, Indianapolis, IN), 25 µM CaCl₂, and 0.5 mg/ml BSA. After 30–45 min, both ventricles were minced in pH 7.2 Kraftbrühe (KB) buffer (70 mM potassium glutamate, 25 mM KCl, 10 mM KH₂PO₄, 10 mM oxalic acid, 10 mM taurine, 11 mM glucose, 2 mM pyruvate, 2 mM K-ATP, 2 mM phosphocreatine, 10 mM HEPES, and 5 mM MgCl₂). After trituration with a blunted Pasteur pipette, the cell suspension was filtered through a stainless mesh and centrifuged at 40 rpm for 5 min as previously described (14). This pellet was resuspended in 20 ml of 4% Ficoll 400-KB buffer, centrifuged again at 40 rpm for 5 min, and resuspended in 5 ml of KB buffer.

Myocyte number and volume were measured using a Coulter Multisizer (Coulter Electronics, Hialeah, FL) as previously described (14). Myocyte yield was determined using a Coulter Multisizer (Coulter Electronics, Hialeah, FL) as previously described (14). Myocyte yield was determined as 9–10×10⁶/heart, of which >90% were rod shaped. Myocyte homogenates were made by sonication in a phosphate-bufferedbuffer (pH 7.4) containing protease inhibitors (2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 2 µg/ml leupeptin, and 2 µg/ml aprotinin). Disruption of myocyte membranes was confirmed under a microscope. Homogenates were snap frozen in an ethanol-dry ice bath and stored at −70°C until use.

MHC Isoforms

MHC isoforms (α- and β-MHC) were separated by SDS-PAGE (Laemmli buffer system) using a slab gel apparatus (Protein X li, Bio-Rad, Hercules, CA) adapting the method of Esser et al. (17). Separating gels (0.75 mm thick) were prepared using 4% (wt/vol) acrylamide, and 0.1% (wt/vol) N,N′-methylene-bis-acrylamide in 100 mM Tris, 300 mM glycine, and 0.1% SDS. Protein concentrations of myocyte homogenates were determined by Bradford protein assay (6). Myocyte homogenates in 2× Laemmli buffer (Sigma) were incubated at 100°C for 5 min before being loaded into wells. Each lane was loaded with equal amounts of myocyte protein (2 µg). The electrophoresis buffer consisted of 50 mM Tris, 150 mM glycine, and 0.1% SDS. After 24 h of electrophoresis (4°C, constant voltage 75–80 V), SDS gels were fixed and stained (Bio-Rad Silver Stain Kit, Bio-Rad). Gels were dried and scanned using a densitometer (OneScanner, Apple operated by Ofoto 2.0 light source). Densities were analyzed (Scan Analysis, Biosoft, Cambridge, UK) to determine the relative quantities of α- and β-MHC.

SERCA2a and Phospholamban Immunodetection

Separating gels (1.5 mm thick, Mini-Protean II, Bio-Rad) were prepared with acrylamide (10% wt/vol for SERCA2a; 15% for phospholamban) and 0.1% (wt/vol) N,N′-methylene-bis-acrylamide in 100 mM Tris, 300 mM glycine, and 0.1% SDS. Myocyte homogenates in 2× Laemmli buffer were incubated at room temperature for 30 min before being loaded into wells (2 µg myocyte protein/lane). A constant voltage of 200 V was applied until the bromophenol blue migrated out of the gel. After electrophoresis, proteins were electrophoretically transferred to a polyvinylidene difluoride membrane (2 h, 100 V). Posttransferred gels were then stained with Coomassie stain to evaluate transfer efficiency. Membranes were incubated at 4°C overnight using blocking buffer, Tris-buffered saline (TBS, which contained 20 mM Tris·Cl, 0.9% NaCl, and 5% BSA (fraction V, Sigma), pH 7.5). Membranes were incubated at room temperature for 2 h with a polyclonal antibody (1/1,000) against rabbit cardiac SERCA2a and a monoclonal antibody (1/750) against canine cardiac phospholamban. Membranes were then washed with TBS containing 0.1% Tween and incubated with 125I-labeled secondary antibodies for 1 h at room temperature. The protein bands bound to the antibodies were visualized using autoradiography by exposing X-ray film (Kodak XOMAT) to the 125I-labeled membrane overnight at −70°C. Autoradiographic densities were analyzed (Scan Analysis, Biosoft, Cambridge, UK).

Statistical Analysis

All data are expressed as means ± SE. Data were analyzed using a repeated-measures ANOVA with one grouping factor. The repeated measures were baseline, rapid pacing, and acute alcohol exposure. Post hoc testing was performed using Bonferroni correction. A value of P < 0.05 was considered statistically significant.

RESULTS

Animal Weights and Blood Pressure

Similar to the findings of previous studies (12, 13, 43, 45), control animals had higher body weights (476 ± 8 vs. 417 ± 4 g) and wet heart weights (1.54 ± 0.06 vs. 1.29 ± 0.06 g) compared with alcohol animals. However, the wet heart weight-to-body weight ratio (3.1 ± 0.1×10⁻³ vs. 3.3 ± 0.1×10⁻³) and median myocyte volumes (33 ± 1×10⁶ vs. 31 ± 2×10⁶ μm³) were similar in alcohol-fed and control animals. Weight differences between alcohol-fed and control animals are due to decreased muscle mass and not to nutritional deficiencies (40). Similar to findings of previous reports (14, 24, 47), chronic alcohol exposure resulted in slightly higher blood pressures in alcohol-fed animals compared with control animals (128 ± 2 vs. 119 ± 2 mmHg; P < 0.05). Effects of Chronic Alcohol Exposure

Contractile parameters. Figure 1A shows representative LV pressure tracings of hearts from alcohol-fed and control animals. Group data are shown in Table 1. LV developed pressure (LVDP) was reduced 15% in alcohol compared with control hearts, before (121 ± 4 vs. 104 ± 3 mmHg; P < 0.05) and after (94 ± 2 vs. 79 ± 2 mmHg; P < 0.05) indo 1 loading.

Figure 1B shows normalized pressure tracings from the alcohol and control hearts shown in Fig. 1A. Normalizing the LV pressure transients makes it easier to see the faster contraction and relaxation in the alcohol compared with the control heart. Table 1 shows group data for several parameters of rates of contraction and relaxation. The maximal rate of pressure rise normalized to peak pressure ([dP/dt]/LVDP) was significantly increased, and the time to peak pressure (TTP_LVDP) was significantly decreased, both indicating faster contraction in alcohol hearts (Table 1). Similarly,
the maximal rate of pressure decline \((-\frac{dP}{dt})/LVDP\) was significantly increased, and the time to 50% decline of the LV pressure transient (T50 LVDP) and the time constant of monoexponential decline of the pressure transient (\(t_{LVDP}\)) were significantly decreased, all indicating faster relaxation in alcohol hearts.

A significant decrease of peak LV pressure, in and of itself, has been shown to speed relaxation in isolated hearts (48). To determine whether the faster relaxation observed in hearts from alcohol chronically exposed to alcohol were simply a consequence of the 15% decrease of LVDP, LVDP was decreased in control hearts to similar levels as seen in hearts from alcohol-treated animals by 1) decreasing coronary perfusion pressure (\(\frac{PP}{LVDP}\)) or 2) decreasing end-diastolic pressure (\(\frac{P}{EDP}\)).

Coronary flow (21.4 ± 0.4 vs. 21.0 ± 0.6 ml/min) and perfusion pressure (71.7 ± 0.5 vs. 70.7 ± 0.3 mmHg) were the same in control and alcohol hearts. This suggests that chronic alcohol exposure does not affect myocardial vascular tone during baseline conditions in isovolumic perfused hearts.

\(Ca^{2+}\). To determine whether decreased LVDP and faster rates of contraction and relaxation after chronic alcohol exposure were due to changes in \([Ca^{2+}]_c\), simultaneous indo 1 fluorescence transients were obtained. Figure 2 shows representative \([Ca^{2+}]_c\) transients from the alcohol and control hearts shown in Fig. 1. No differences were observed in peak systolic or diastolic \([Ca^{2+}]_c\), or rates of rise or decline of the \([Ca^{2+}]_c\) transient between alcohol and control hearts (Table 3). This suggests that chronic alcohol-induced changes in baseline contractile function are not due to alterations in \(Ca^{2+}\) handling. Instead, alcohol-induced contractile changes must be the result of alterations at the level of the myofilament (e.g., altered \(Ca^{2+}\)-force relationship and/or cross-bridge kinetics).

Effects of Rapid Pacing Stress

Contractile parameters. Increasing the pacing rate from 300 to 420 beats/min resulted in similar decreases

### Table 1. Contractile parameters of perfused hearts from guinea pigs drinking 36% ethanol for 7 mo and age-matched controls

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Rapid Pacing</th>
<th>Acute Alcohol</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alcohol</td>
<td>Control</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>94 ± 2</td>
<td>79 ± 2*</td>
<td>58 ± 4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>10</td>
<td>10</td>
<td>12 ± 1*</td>
</tr>
<tr>
<td>((-\frac{dP}{dt}))/LVDP, s(^{-1})</td>
<td>24 ± 0.7</td>
<td>27 ± 0.3*</td>
<td>21 ± 1*</td>
</tr>
<tr>
<td>TTP(_{LVDP}), ms</td>
<td>72 ± 1</td>
<td>64 ± 1*</td>
<td>57 ± 1*</td>
</tr>
<tr>
<td>((-\frac{dP}{dt}))/LVDP, s(^{-1})</td>
<td>19 ± 0.5</td>
<td>21.7 ± 0.3*</td>
<td>16 ± 1.2*</td>
</tr>
<tr>
<td>T50(_{LVDP}), ms</td>
<td>46 ± 1</td>
<td>39 ± 1*</td>
<td>36 ± 1*</td>
</tr>
<tr>
<td>(t_{LVDP}), ms(^{-1})</td>
<td>20 ± 1</td>
<td>16 ± 1*</td>
<td>20 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data were acquired under baseline conditions and during exposure to rapid pacing (7 Hz) and acute alcohol (0.15% vol/vol) stress. LVDP, left ventricular LVDP; LVEDP, LV end-diastolic pressure; \((-\frac{dP}{dt})\)/LVDP, maximal rate of LV pressure rise normalized to peak LV pressure; TTP\(_{LVDP}\), time to peak LV pressure; \((-\frac{dP}{dt})\)/LVDP, maximal rate of LV pressure decline normalized to peak LV pressure; T50\(_{LVDP}\), time to 50% LV pressure decline; \(t_{LVDP}\), time constant of monoexponential decline of LV pressure.

*P < 0.05, alcohol vs. control. †P < 0.05, stress vs. baseline.
of LVDP (−28%) and increases of LV end-diastolic pressure (−28%) in alcohol and control hearts (Table 1). In response to rapid pacing, both alcohol and control hearts demonstrated slower rates of contraction and relaxation (Table 1). Most parameters of contractile rates were no longer different between alcohol and control hearts ([+dP/dt]/LVDP, TTPLVDP, and (−dP/dt)/LVDP; Table 1), suggesting that alcohol hearts could not maintain faster contractile rates during acute stress. In agreement with other studies subjecting alcohol hearts to acute stresses (13, 43, 44), these data suggest that hearts from animals chronically exposed to alcohol may have a more limited ability to withstand the stress of rapid pacing compared with controls.

Ca\(^{2+}\). Diastolic [Ca\(^{2+}\)]\(_c\) and mitochondrial [Ca\(^{2+}\)]\(_m\) increased similarly during rapid pacing between alcohol and control hearts (Table 3). Time to 50% decline of [Ca\(^{2+}\)]\(_c\), transient was decreased similarly in alcohol and control hearts. Other parameters of [Ca\(^{2+}\)]\(_c\), rise and decline were unchanged between alcohol and control hearts. These data suggest that the contractile changes associated with rapid pacing stress are at least partly the result of changes in Ca\(^{2+}\) handling. However, there were no differences in Ca\(^{2+}\) handling between alcohol and control hearts. This would further suggest that alcohol-induced contractile changes are not due to impaired Ca\(^{2+}\) handling but are the result of alterations at the level of the cardiomyofilament.

Effects of Acute Alcohol Exposure

Contractile parameters. We next determined whether hearts from animals chronically exposed to alcohol were more tolerant of the stress of acute alcohol exposure than controls. The depressive effect of 24 mM ethanol on LVDP was similar between alcohol (20%) and control (17%) hearts (Table 1). Rates of contraction and relaxation were affected similarly by acute alcohol exposure, remaining faster in alcohol compared with control hearts. These data suggest that 1) chronic alcohol exposure does not produce tolerance to contractile depression with an intoxicating concentration of alcohol and 2) acute alcohol exposure is not more toxic to hearts from animals chronically exposed to alcohol.

Ca\(^{2+}\). Similar to previously reported data in papillary muscles and myocytes (16, 22), no differences were observed in peak systolic [Ca\(^{2+}\)]\(_c\), or in the rates of rise or decline of the [Ca\(^{2+}\)]\(_c\), transient during acute alcohol exposure in alcohol or control hearts. These data suggest that acute alcohol-induced contractile changes are not due to alterations in [Ca\(^{2+}\)]\(_c\).

Table 3. Cytosolic Ca\(^{2+}\) transient parameters of perfused hearts from guinea pigs drinking 36% ethanol for 7 mo and age-matched controls

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Alcohol</th>
<th>Rapid Pacing</th>
<th>Alcohol</th>
<th>Acute Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic [Ca(^{2+})](_c), nM</td>
<td>808±45</td>
<td>813±45</td>
<td>816±70</td>
<td>859±83</td>
<td>838±97</td>
</tr>
<tr>
<td>Diastolic [Ca(^{2+})](_c), nM</td>
<td>131±7</td>
<td>136±5</td>
<td>172±6*</td>
<td>197±23*</td>
<td>130±10</td>
</tr>
<tr>
<td>Mitochondrial [Ca(^{2+})](_m), nM</td>
<td>195±11</td>
<td>193±10</td>
<td>243±19*</td>
<td>280±23*</td>
<td>188±17</td>
</tr>
<tr>
<td>+dCa(^{2+})/dt, nM/s</td>
<td>6.1±0.8</td>
<td>6.1±0.6</td>
<td>6.8±0.8</td>
<td>6.8±0.8</td>
<td>6.5±1.1</td>
</tr>
<tr>
<td>TTP(_{Ca}), ms</td>
<td>18±1</td>
<td>20±1</td>
<td>17±1</td>
<td>19±1</td>
<td>18±1</td>
</tr>
<tr>
<td>−dCa(^{2+})/dt, nM/s</td>
<td>1.6±0.2</td>
<td>1.6±0.2</td>
<td>1.6±0.2</td>
<td>1.8±0.2</td>
<td>2.5±0.7</td>
</tr>
<tr>
<td>T50(_{Ca}), ms</td>
<td>41±3</td>
<td>40±1</td>
<td>32±2*</td>
<td>32±1*</td>
<td>36±3</td>
</tr>
<tr>
<td>τ(_{Ca}), ms(^{-1})</td>
<td>36±2</td>
<td>40±5</td>
<td>43±8</td>
<td>37±3</td>
<td>36±3</td>
</tr>
</tbody>
</table>

Values are means ± SE. Data were acquired under baseline conditions and during exposure to rapid pacing (7 Hz) and acute alcohol (0.15% vol/vol) stresses. [Ca\(^{2+}\)]\(_c\), cytosolic Ca\(^{2+}\); +dCa\(^{2+}\)/dt, maximal rate of [Ca\(^{2+}\)]\(_c\), rise; TTP\(_{Ca}\), time to peak [Ca\(^{2+}\)]\(_c\); −dCa\(^{2+}\)/dt, maximal rate of [Ca\(^{2+}\)]\(_c\), decline; T50\(_{Ca}\), time to 50% [Ca\(^{2+}\)]\(_c\), decline; τ\(_{Ca}\), time constant of monoexponential decline of [Ca\(^{2+}\)]\(_c\). *P < 0.05, stress vs. baseline.
18 ± 7/µg protein) were similar in control and alcohol-
exposed hearts. Representative autoradiographs are
shown in Fig. 3. Findings were not affected after
correction for myocyte number or median myocyte
volume as previously described (14). These findings are
in agreement with the aforementioned finding that Ca²⁺
handling was not altered by chronic alcohol
exposure.

MHC Isoforms

An increase of α-MHC relative to β-MHC would
explain decreased peak force despite more rapid rates
of contraction and relaxation in hearts from alcohol-fed
animals compared with controls (50). Representative
immunoblots for MHC are shown in Fig. 4. As shown in
Fig. 4, the relative content of α-MHC was decreased in
alcohol compared with control myocytes (a/a + b =
0.55 ± 0.03 vs. 0.66 ± 0.02; a/b = 1.3 ± 0.2 vs. 2.0 ± 0.2;
P < 0.02 for both). These data suggest that alcohol-
duced contractile changes are not due to an MHC
isoform shift but to other changes in the myofilament
(e.g., troponin-tropomyosin isoform shifts or change in
myofibrillar ATPase activity).

DISCUSSION

The major new finding of this study is that chronic
alcohol-induced changes of myocardial contractility do
not result from changes in the [Ca²⁺]c transient but are
due to abnormalities in excitation-contraction coupling
distal to [Ca²⁺]c. Specifically, hearts from rats drinking
36% ethanol for 7 mo showed depressed LVDP (−16%),
and faster rates of contraction (12%) and relaxation
(14–20%), despite no differences in systolic or diastolic
[Ca²⁺]c, or rates of rise or decline of the [Ca²⁺]c transient
compared with control hearts. In agreement with this
finding, myocyte content of Ca²⁺-handling proteins
SERCA2a and phospholamban were the same in hearts
from alcohol-fed and control animals. These data sug-
gest that myocardial contractile changes in the setting
of chronic alcohol exposure are the result of a change in
the force response to [Ca²⁺]c due to altered myofilament
responsive to [Ca²⁺]. Altered myofilament [Ca²⁺]
responsiveness can result from changes in cross-bridge
kinetics and/or an altered Ca²⁺-force relationship.
Alternatively, there may be changes in the cardiac interstitial
that could also alter systolic function. As a first
step in elucidating whether alcohol-induced changes at
the myofilament result in altered contractile function,
we assessed MHC isoform content and found a decrease
of α-MHC relative to β-MHC. This isoform shift cannot
account for the observed contractile changes, suggest-
ing that chronic alcohol exposure affects another compo-
nent(s) of the myofilament contractile apparatus.

There is substantial literature reporting altered con-
tractile function with the stress of chronic alcohol
exposure [see review by Thomas et al. (47)]. However,
the mechanisms underlying these alterations of peak
force and rates of contraction and relaxation are not
known. In agreement with this study, several studies
have demonstrated depressed peak force with chronic
alcohol exposure (11, 26, 46). In contrast, there are
conflicting data regarding the effects of chronic alcohol
exposure on rates of contraction and relaxation. Simi-
lar to this study, the findings of some investigators (5,
42, 46) showed faster rate parameters, whereas others
(11, 12, 26, 43) showed either no change or slower rate
parameters. These conflicting results may be due to use
of different animal models, alcohol dosages, and exposure duration. It may be that alcohol initially alters gene expression and physiology in a manner resulting in more rapid rates of contraction and relaxation, as seen in an echocardiographic study of moderate drinkers (51). However, with the continued stress of heavy exposure, alcohol begins to detrimentally affect the myocardium, causing impaired contraction and relaxation, as is seen in echocardiographic studies of alcoholics (19, 30, 38, 45, 49). The model of chronic alcohol exposure used in this study probably represents an early stage of the effects of chronic alcohol abuse on the heart. Future studies will examine the effects of exposure durations greater than the 7 mo used in this study.

A number of investigators have speculated that, in contrast to the stress of acute alcohol exposure, chronic alcohol exposure alters contractility by impairing myocyte Ca\textsuperscript{2+} handling (1, 3, 13, 26). However, no measurements of myocyte [Ca\textsuperscript{2+}] after chronic alcohol exposure have been made. Thus this hypothesis is based on indirect evidence. For example, several investigators (5, 40, 43) reported that chronic alcohol exposure decreases sarcoplasmic reticulum Ca\textsuperscript{2+} uptake and binding. In a study of rats consuming alcohol as 39% of their daily calories for 10 mo, peak sarcoplasmic reticulum Ca\textsuperscript{2+} uptake was reduced 18% and sarcoplasmic reticulum Ca\textsuperscript{2+} binding 17% (43). In all of these studies, baseline rates of contraction and relaxation showed nonsignificant trends toward being faster (not slower) in alcohol compared with control hearts. Furthermore, it was necessary to impose a stress (angiotensin or dobutamine) to show impaired contractile reserve in hearts from alcohol-fed animals (5, 40, 43). Finally, none of these studies measured [Ca\textsuperscript{2+} transient transients to assess whether these small changes of sarcoplasmic reticulum function were physiologically relevant. In the present study, despite significant alterations of contractile parameters with chronic alcohol exposure, systolic and diastolic [Ca\textsuperscript{2+}], and rates of [Ca\textsuperscript{2+}], rise and decline were not different between alcohol and control hearts. Therefore, under baseline conditions, alcohol-induced contractile changes are not due to altered Ca\textsuperscript{2+} handling by the sarcoplasmic reticulum.

Furthermore, during the stress of rapid pacing, changes in [Ca\textsuperscript{2+}] transients from baseline conditions were the same in alcohol and control hearts, again suggesting [Ca\textsuperscript{2+}] handling is not significantly affected by chronic alcohol exposure. This was despite the fact that the faster contraction and relaxation rates in alcohol hearts compared with controls under baseline conditions were, for the most part, lost during the pacing stress. Thus the more limited contractile reserve of hearts from alcohol-fed animals does not appear to be the result of impaired sarcoplasmic reticulum function. Future studies will determine whether [Ca\textsuperscript{2+}] homeostasis can be maintained with more severe stresses on hearts from alcohol-fed animals.

The findings of this study suggest that chronic alcohol-induced changes of contractility are the result of alterations at the level of the myofilament. In myocytes, altered myofilament responsiveness to [Ca\textsuperscript{2+}], can result from changes in the [Ca\textsuperscript{2+}]-force relationship or cross-bridge kinetics. For example, an increase of α-MHC relative to β-MHC can alter cross-bridge kinetics so that peak force is decreased and rates of contraction and relaxation are increased (50). Altered myofilament [Ca\textsuperscript{2+}]	extsubscript{r} responsiveness can also result from changes in content or isoforms of the regulatory proteins troponin and tropomyosin, which can alter the Ca\textsuperscript{2+}-force relationship. As a first step in elucidating the cause of alcohol-induced alterations of myofilament [Ca\textsuperscript{2+}]	extsubscript{r} responsiveness, we assessed MHC isoform content. Surprisingly, we found a decrease (not increase) of α-MHC relative to β-MHC, which cannot account for the observed alcohol-induced contractile changes. Future studies will assess whether alterations of other contractile proteins (e.g., actin) or regulatory proteins (e.g., troponin and tropomyosin) are associated with alcohol-induced contractile changes.

The stress of acute alcohol exposure, using a physiologically relevant concentration (0.15% vol/vol), also depressed LVDP but did not alter rates of contraction or relaxation. The 15% decrease of LVDP seen in whole hearts in the present study is similar to the observed decreases of peak force in papillary muscle (10%) (22) and isolated myocyte preparations (15%) (16) using the same ethanol concentration. Systolic and diastolic [Ca\textsuperscript{2+}], and rates of [Ca\textsuperscript{2+}], transient rise and decline were unchanged by the addition of this clinically relevant dose of alcohol. This finding is also in agreement with the aforementioned muscle and cell models of acute alcohol exposure (16, 22) and suggests that contractile depression with acute alcohol exposure is due to altered myofilament responsiveness to [Ca\textsuperscript{2+}]. This finding of decreased LVDP is in contrast to another perfused rat heart study (28) in which no change in LVDP was observed with ethanol concentrations up to 64 mM (0.4% vol/vol). It is unclear why no change of force was seen in the study by Kojima et al. (28), although significant differences exist between their study protocols and ours, including coronary perfusion pressure (100 vs. 71 mmHg), perfusate [Ca\textsuperscript{2+}] (1.5 vs. 2.5 mM), and animal weights (350–400 vs. 476 g).

Limitations

Despite prolonged exposure to heavy alcohol, contractile changes were relatively modest. These data are more consistent with the majority of alcoholics, who demonstrate subclinical contractile abnormalities (19, 30, 38, 45, 49). Investigators have not yet been able to produce an animal model of an alcohol-induced syndrome of congestive heart failure, suggesting that, in the minority of human alcoholics who develop cardiomyopathy (most have subclinical contractile changes as seen in this study), a genetic or environmental predisposition is necessary (21, 27, 36). A second limitation of this study is that although we have determined that alcohol-induced contractile changes are not due to altered Ca\textsuperscript{2+} handling but to an alteration at the level of the myofilament, the specific mechanism of this change remains unclear. Future work will be directed at studying content and isoform shifts of other contrac-
tile and regulatory proteins to determine how chronic alcohol exposure might affect gene expression to alter contractile physiology.

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