Ethanol Acutely and Reversibly Suppresses Excitation–Contraction Coupling in Cardiac Myocytes

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We used adult rat cardiac myocytes to examine the acute effects of 0.1–5.0% (vol/vol) ethanol (ETOH) on 1) the cytosolic [Ca^{2+}] (Ca) transient measured as the change in indo 1 fluorescence at 410/490 nm and contraction elicited by electrical stimulation of single cells and 2) the sarcoplasmic reticulum (SR) Ca^{2+} content in cell suspensions. During stimulation at 1 Hz, clinically relevant ETOH correlations (0.1–0.15% [vol/vol]) caused a 10–15% decrease in the contraction amplitude, measured by myocyte edge tracking, without decreasing the Ca transient that initiates contraction. At higher ETOH concentrations (1–5% [vol/vol]), ETOH caused profound contractile depression and also reduced the magnitude of the Ca transient. These effects were reversed within minutes of ETOH washout. Addition of norepinephrine (10 μM) to the bathing solution or an increase in bathing [Ca^{2+}] in the continued presence of ETOH could also reverse its effects. The relation of the amplitude of the Ca transient to the contraction amplitude measured across a range of bathing [Ca^{2+}] was shifted by ETOH, such that for a given Ca transient a marked reduction in contraction amplitude occurred. In unstimulated myocyte suspensions, ETOH (1–5% [vol/vol]) caused a concentration-dependent depletion of SR Ca^{2+} content, manifested as a diminution in the Ca increase elicited by caffeine in the presence of extracellular EGTA and no added Ca^{2+}. Thus, in rat cardiac myocytes a reduction in the myofilament Ca^{2+} response, possibly due to a decrease in myofilament Ca^{2+} sensitivity, is a mechanism for contractile depression due to clinically relevant ETOH concentrations. Higher concentrations of ETOH cause further contractile depression, in part, by inducing SR Ca^{2+} release and depleting the SR of Ca^{2+}, leading to an attenuation of the Ca transient elicited by electrical stimulation, and by further depressing the myofilament length–Ca^{2+} relation. (Circulation Research 1991;68:1660–1668)

It has been established that ethanol (ETOH) depresses myocardial contractility in humans and experimental animals, both in situ and in isolated hearts and cardiac muscle.\textsuperscript{1–11} The primary mechanism(s) of the negative inotropic effect of ETOH is only partially understood. If ETOH induced metabolic alterations (e.g., a reduction in cytosolic pH or high-energy phosphates), it could decrease contractility on this basis. Recent studies, however, have indicated that ETOH, in concentrations up to 5%, does not deplete high-energy phosphates, decrease cytosolic pH, or increase inorganic phosphate.\textsuperscript{7}

Regulation of myocardial contractility is under the control of Ca^{2+}, which initiates contraction by binding to troponin C.\textsuperscript{12} The major source and sink of the excitation-induced cytosolic [Ca^{2+}] (Ca) transient in cardiac muscle is the sarcoplasmic reticulum (SR).\textsuperscript{13} ETOH may decrease cardiac contractility through actions at the myofilaments or by alteration of Ca^{2+} transport at the SR. The former possibility would likely involve a decrease in the myofilament sensitivity to cytoplasmic Ca^{2+}. An action at the SR could be the result of a decrease in SR Ca^{2+} store or secondary to a reduction in the effectiveness or size of the trigger for SR Ca^{2+} release. Studies in isolated subcellular organelles may provide some insight into the acute contractile depression by ETOH, because isolated canine cardiac microsomes enriched in SR acutely exhibit decreased Ca^{2+} uptake, increased passive Ca^{2+} permeability, and decreased maximal storable Ca^{2+} in response to ETOH.\textsuperscript{14} Acute addition of ETOH also reduces Ca^{2+} binding to the regulatory

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complex of isolated troponymosin and decreases actomyosin ATP hydrolysis. However, whether ETOH depletes SR Ca\(^{2+}\) in the intact cardiac myocyte (i.e., when the amount of Ca\(^{2+}\) available to SR is regulated by the sarcolemmal transport mechanisms) or whether it alters the interaction of Ca\(^{2+}\) with intact myofibrils is not known.

Advances in the isolation of Ca\(^{2+}\)-tolerant adult cardiocytes and the more recent development of Ca\(^{2+}\)-sensitive fluorescent probes, which permit the detection of changes in Ca\(_s\), provide opportunities for further study of the cellular mechanisms that underlie the cardiac depressant effect of ETOH. The present study used these Ca\(^{2+}\) probes in rat cardiac myocytes, with simultaneous measurement of contraction. In some experiments, it was determined whether the acute ETOH-mediated depression of contractility could be attributed to a decrease in SR Ca\(^{2+}\) release, measured either as diminution of the amplitude of the Ca\(_t\) transient in response to electrical stimulation or a diminution in the caffeine-induced increase in Ca\(_s\). In other experiments, it was determined whether ETOH alters the myofilament responsiveness to Ca\(^{2+}\), measured as the relation of contraction amplitude to that of the Ca\(_t\) transient that initiates contraction.

**Materials and Methods**

Enzymatically isolated rat cardiac myocytes were prepared via collagenase digestion as previously described\(^{13}\) and plated in Petri dishes perfused with HEPES-buffered solution containing (mM) NaCl 137, MgSO\(_4\) 1.2, KCl 5.0, NaH\(_2\)PO\(_4\) 1.2, HEPES 20, D-glucose 11, and CaCl\(_2\) 1.0 (pH adjusted to 7.4 with NaOH).

In the initial experiments, the effect of ETOH (0.10–5% [vol/vol]) on the contractile response to electrical stimulation of single cells not loaded with Ca\(^{2+}\) indicators was determined. Cells were in 1 mM bathing [Ca\(^{2+}\)] (Ca\(_s\)) at 37°C and were stimulated continually at 1 Hz via field stimulation (2–5-msec pulses). Contraction amplitude was measured via video edge-motion detection as described previously\(^{10}\) and was recorded 1 minute before ETOH exposure after 3–5 minutes of ETOH exposure, and 5 minutes after ETOH washout. In a subset of these cells, norepinephrine (10 \(\mu\)M) was added in the continued presence of ETOH. In another series of experiments, the Ca\(_t\) transient and the contraction in single myocytes were simultaneously measured, as recently described,\(^{15}\) before and after a broad concentration range of ETOH (0.1–3.0 [vol/vol]). Briefly, single myocytes bathed in a HEPES-buffered medium containing 1 mM Ca\(_s\) at 23°C were loaded with the membrane-permeant acetoxyethyl ester derivative (AM form) of the Ca\(^{2+}\) probe indo 1 (4 \(\mu\)M), which was dissolved in dimethyl sulfoxide mixed with fetal calf serum and pluronic F-127 (BASF Wyandotte Corp., Wyandotte, Mich.), a dispersing agent.\(^{18}\) Indo 1 fluorescence was excited by epi-illumination with 3.8-\(\mu\)sec flashes of 350±5 nm light. Paired photomultipliers collected indo 1 emission by simultaneously measuring spectral windows of 391–434 and 457–507 nm selected by band-pass interference filters. The ratio of indo 1 emission at the two wavelengths was calculated as a measure of Ca\(_t\) using a pair of fast-integrator sample-and-hold circuits under the control of a VAX 11/730 computer (Digital Equipment Corp., Maynard, Mass.). Cell length was simultaneously monitored using red light (650–750 nm) to form a bright field image of the cell, which was projected onto a photodiode array (1024 SAQ Starlight Sensor, EG&G Reticon, Inc., Sunnyvale, Calif.) with a 5-msec time scan rate. Cells were stimulated at 0.5 or 1.0 Hz. Ten to 20 twitch contractions and the associated Ca\(_t\) transients were signal-averaged before and after ETOH.

In a third series of experiments, the Ca\(_t\) of suspensions of myocytes was monitored by recording the fluorescence of cell suspensions loaded with quin 2 using a modified fluorometer (model A2, Farrand Optical Co., New York) as described previously.\(^{19}\) Briefly, the cells were loaded by exposure to 40 \(\mu\)M quin 2-AM for 20 minutes at 37°C. For these studies, the incubation medium comprised 117 mM NaCl, 1 mM MgSO\(_4\), 5.4 mM KCl, 25 mM HEPES-Tris (pH 7.4), 1.2 mM NaH\(_2\)PO\(_4\), 20 mM glucose, 1 mM CaCl\(_2\) and 5 mg/ml bovine serum albumin (Fraction 5, Miles Laboratories, Inc., Elkhart, Ind.). Creatine phosphate and creatinine kinase were present at 10 mM and 20 units/ml, respectively. Excitation of fluorescence was at 333 nm, and emission was collected at wavelengths >480 nm. Temperature was maintained at 37°C, and the cell suspension was stirred magnetically in the cuvette of the instrument and maintained under an atmosphere of 100% O\(_2\).

To validate comparisons between cuvettes of cells,\(^{19}\) the minimal fluorescence with MnCl\(_2\) (0.1 mM) and Triton X-100 (0.1%) and the maximal fluorescence achieved with a molar excess of diethylenetriaminepentaacetic acid (5 mM)/CaCl\(_2\) (6 mM) were measured.

**Materials**

Indo 1-AM and quin 2-AM were obtained from Calbiochem Corp., La Jolla, Calif. All other chemicals were of laboratory grade obtained from standard sources.

**Results**

**Contractile Measurements in Individual Myocytes**

The threshold concentration necessary for ETOH to depress contractility varied from 0.1% to 0.2% (vol/vol) (see below). Figure 1 shows a representative chart recording of reversible twitch depression by threshold and higher concentrations of ETOH in myocytes not loaded with fluorescent Ca\(^{2+}\) probes. Table 1 depicts the average effects of a 5-minute exposure to ETOH on the following parameters: resting cell length, maximum velocity of cell shortening during the twitch (VS), maximum extent of cell shortening (ES), and contraction duration, that is,
the time from stimulus to half twitch relaxation. At 0.1% (vol/vol) ETOH, ES and VS were decreased by 15% (Table 1). This result is strikingly similar to the depression of contraction by this concentration of ETOH in intact ferret cardiac muscle.20 VS and ES decreased to 68% and 66% of control, respectively, in 1% (vol/vol) and to 53% and 49%, respectively, in 3% (vol/vol) ETOH. The contraction was abolished in 5% ETOH. ETOH (1% [vol/vol] or greater) also caused a small increase in resting cell length. After washout of the higher ETOH concentration (1% or greater), VS, ES, and contraction duration not only recovered, but at 5 minutes after washout, the twitch contraction amplitude and velocity were greater than they were before ETOH exposure (Table 1). In many cells this overshoot in twitch amplitude was accompanied by the occurrence of spontaneous contractile waves in diastole (not shown). A similar overshoot in contraction strength has been observed after washout of high concentrations of ETOH in isolated perfused rat hearts.21

Because previous studies8,11 in vivo indicated that reflex sympathetic stimulation could compensate for ETOH-induced myocardial suppression in situ, a subset of cells was exposed to norepinephrine (10 μM) during exposure to ETOH (Figure 1B). Norepinephrine caused marked increases in VS and ES during 0.1% (vol/vol) ETOH exposure, reversed the effect of 1% and 3% (vol/vol) ETOH, and partially restored the contraction in 5% (vol/vol) ETOH (Table 1).

**Measurements of Cytosolic Ca^{2+} and Contraction**

Threshold concentrations of ETOH for contractile depression did not markedly reduce the Ca, transient, measured as the change in indo 1 fluorescence after electrical stimulation (Figure 2). In this regard, it is important to note that this concentration of ETOH

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**TABLE 1. Effect of Ethanol and Ethanol Plus Norepinephrine on Twitch Parameters of Single Cardiac Myocytes of Rats**

<table>
<thead>
<tr>
<th>ETOH concentration</th>
<th>n</th>
<th>VS (% control)</th>
<th>ES (% control)</th>
<th>RL (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETOH</td>
<td>6</td>
<td>88±5</td>
<td>85±2</td>
<td>100±0.2</td>
</tr>
<tr>
<td>After ETOH</td>
<td>3</td>
<td>96±7</td>
<td>103±3</td>
<td>100±0.1</td>
</tr>
<tr>
<td>ETOH+NE (10 μM)</td>
<td>3</td>
<td>308±106</td>
<td>198±62</td>
<td>97±2</td>
</tr>
<tr>
<td>1%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETOH</td>
<td>10</td>
<td>68±7</td>
<td>66±7</td>
<td>101±0.3</td>
</tr>
<tr>
<td>After ETOH</td>
<td>5</td>
<td>164±36</td>
<td>199±44</td>
<td>100±0.6</td>
</tr>
<tr>
<td>ETOH+NE (10 μM)</td>
<td>5</td>
<td>152±26</td>
<td>156±32</td>
<td>99±0.7</td>
</tr>
<tr>
<td>3%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETOH</td>
<td>10</td>
<td>53±9</td>
<td>49±9</td>
<td>101±0.4</td>
</tr>
<tr>
<td>After ETOH</td>
<td>5</td>
<td>128±33</td>
<td>154±42</td>
<td>101±0.7</td>
</tr>
<tr>
<td>ETOH+NE (10 μM)</td>
<td>5</td>
<td>127±33</td>
<td>114±36</td>
<td>97±1</td>
</tr>
<tr>
<td>5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETOH</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>101±0.5</td>
</tr>
<tr>
<td>After ETOH</td>
<td>5</td>
<td>127±15</td>
<td>135±17</td>
<td>103±3</td>
</tr>
<tr>
<td>ETOH+NE (10 μM)</td>
<td>5</td>
<td>8±8</td>
<td>6±6</td>
<td>100±1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. ETOH, ethanol; VS, maximum velocity of cell shortening during twitch; ES, maximum extent of cell shortening; RL, resting cell length; NE, norepinephrine. Cells were stimulated at 1 Hz at 37°C. In all cells measurements were made 5 minutes after exposure to ETOH and 5 minutes after washout in half the cells. In the remainder of cells, NE was added after 5 minutes of ETOH, and measurements were taken after an additional 5 minutes of ETOH plus NE. Before NE, VS, ES, and RL averaged 135±26 μm/sec; 7±0.8% of RL, and 107±8 μm, respectively.

**FIGURE 1.** Recordings showing examples of the suppression of the twitch amplitude in single ventricular myocytes of rats by 0.15% (vol/vol) ethanol (ETOH) (panel A) and 3% (vol/vol) ETOH (panel B). ETOH was present in the bath during the time indicated. Recovery of contractile function occurs on ETOH washout. Panel C illustrates reversal of ETOH twitch depression by 10 μM norepinephrine (NE). The ETOH concentration was 4% (vol/vol), and it was present during the period between the arrows.
twitch amplitudes as a function of the peak systolic indo 1 ratios measured across a range of Ca, The data points in ETOH are displaced downward to the right, and this is reversed on ETOH washout.

**ETOH Addition to Unstimulated Myocytes**

Monitoring the spontaneous focal propagated wave frequency that occurs in some resting rat myocytes allows further inferences to be made on the mechanism of the decrease in the Ca transient by ETOH. These contractile waves result from a spontaneous localized release of Ca from the SR, which is thought to propagate by diffusion, and regenerative Ca-induced Ca release at the wave front. One determinant of the frequency of contractile wave occurrence is the extent to which the SR is Ca2+-loaded. Figure 5 shows that ETOH initially increases the wave frequency by 50%. The initial concomitant decrease in wave amplitude is attributable to the increased frequency. Eventually, as the cell apparently becomes Ca2+-depleted, the amplitude decreases further. The increase in wave frequency suggests that a primary effect of ETOH on the SR is to increase Ca2+ release similar to that described previously for caffeine, although an increase in the sarcolemmal Ca permeability due to ETOH could also produce a similar result. Enhanced SR Ca2+ release subsequently leads to SR Ca2+ depletion.

The hypothesis that ETOH leads to SR Ca2+ release was tested in indo 1–loaded myocytes. Figure 6 shows that addition of 3% (vol/vol) ETOH to an unstimulated cell leads to a spontaneous rapid increase in indo 1 fluorescence followed by smaller oscillations, during which the resting fluorescence remains elevated. This is followed by a gradual decrease in resting indo 1 fluorescence. Additional experiments with suspensions of cardiac myocytes loaded with quin 2 allow further quantitative insights to be made into the ETOH-induced depletion of SR Ca2+. Figure 7A illustrates a protocol used to assess an increase in Ca by SR Ca2+ release induced by the acute addition of caffeine. Figure 7B shows the effect of the addition of ETOH on resting Ca and on the subsequent caffeine response. Figure 8 shows that the fluorescence transient induced by the addition of caffeine after ETOH (1–5% [vol/vol]) is decreased in a dependent manner. In these experiments any contribution to the Ca increase by caffeine via sarcolemmal influx is prevented by prior addition of EGTA to the cuvette. Thus, the net increase in fluorescence after the addition of caffeine is due to a release of Ca2+ from the SR.

**Discussion**

The present study shows that acute ETOH exposure reversibly depresses contraction in single cardiac myocytes, just as in intact isolated myocardium and in the heart in situ. The results indicate that the effect of ETOH in amounts routinely ingested by man, that is, those leading to clinically relevant

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Top panel: Chart recording of the electrically stimulated twitch in a single rat myocyte loaded with indo 1-AM before, during, and after exposure to a low concentration (0.1% [vol/vol]) of ethanol (ETOH). Bottom panel: Tracings depicting, on an expanded time scale, the indo 1 fluorescence transients and contractions at times A, B, and C. in upper tracing. ETOH causes a 10% reduction in contractile amplitude, but the fluorescence transients before, during, and after ETOH exposure are superimposable.

does not affect the indo 1 fluorescence ratio or alter the Ca2+ response of the indo 1 ratio. This was determined by addition to ETOH to droplets of indo 1–free acid at diastolic (100 nM) and systolic (1,000 nM) Ca2+ levels. A similar result (i.e., contractile depression at 0.15% ETOH without a reduction in the cytosolic Ca2+ transient), measured via aequorin luminescence, has been recently observed in intact, isolated ferret heart muscle.

Higher concentrations of ETOH (3% [vol/vol]) elicited a reversible decline in twitch amplitude and a reduction in the amplitude of the Ca transient that leads to myofilament shortening (Figure 3). Note that ETOH also causes a decrease in resting indo 1 fluorescence accompanied by an increase in the resting cell length; both changes are reversible on ETOH washout. Figure 4 shows that an increase in Ca, can partially reverse this ETOH-induced twitch depression. A spectrum of twitch and Ca transients produced by changing Ca (0.25–8 mM) in the absence or presence of ETOH is depicted in panels A and B. Contraction amplitudes associated with a Ca transient of the same amplitude in the presence and absence of ETOH are depicted in panel C. For a given Ca transient, the twitch is substantially depressed in the presence of ETOH. This suggests that ETOH affects the myofilament–Ca2+ interaction. This is shown clearly in panel D, which depicts the
ETOH plasma concentrations (0.1–0.15% [vol/vol]), is to depress contraction in the absence of a reduction in the Ca$^{2+}$ transient. A similar result for clinically relevant ETOH concentration (0.15%) has recently been observed in intact isolated heart. In that study, contractile depression was observed not only in the absence of a reduction in the Ca$^{2+}$ transient but also in the absence of a change in the transmembrane action potential configuration. These results suggest that a reduced myofilament response to Ca$^{2+}$ underlies the contractile depression at clinically relevant ETOH concentrations. The specific mechanism by which ETOH alters myofilament response to Ca$^{2+}$ in the intact cell cannot be determined from the present experiments. However, it may be related to decreased Ca$^{2+}$ binding to the myofilaments. Because ETOH in any concentration used in the present study does not cause a reduction of pH or an increase in inorganic phosphate, these are not likely a cause of a decreased myofilament Ca$^{2+}$ response effected by ETOH.

At higher ETOH concentrations (1–5% [vol/vol]), contractile depression is the result, in part, of both a decreased Ca$^{2+}$ transient and an altered Ca$^{2+}$–myofilament interaction during an electrically stimulated contraction. Thus, these alterations, in addition to sarcolemmal membrane effects, appear to play a role in the “general anesthetic” effects of high concentrations of ETOH. The elucidation of the mechanisms for the contractile depression by high concentrations of ETOH and the observation that profound contractile depression at the cellular level is reversible may be of significance with respect to the quest for more optimal modalities of cardioplegia. The present results suggest that even very high (5% [vol/vol]) concentrations of ETOH do not cause a generalized increase in sarcolemmal ion permeability, because if this were the case, Ca$^{2+}$ ions would flow along their electrochemical gradient and produce cytosolic Ca$^{2+}$ overload; clearly this does not occur.

The addition of high concentrations of ETOH to single unstimulated myocytes caused an increase in indo 1 fluorescence. That this ETOH effect (an increase in Ca$^{2+}$ in unstimulated cells) resulted from Ca$^{2+}$ release from SR is suggested by the diminished effect of a subsequent addition of caffeine (an increase in Ca$^{2+}$ in cell suspensions). That ETOH initially increases spontaneous focal propagated contractile wave frequency in resting cells may be a functional manifestation of its enhancement of SR Ca$^{2+}$ release. However, the possibility that the SR Ca$^{2+}$ pump is simultaneously inhibited and contributes to SR Ca$^{2+}$ depletion cannot be excluded on the
tions in single myocytes likely occurs because Ca\textsuperscript{2+} removed from the cytosol, and probably that removed from the cell via sarcolemmal efflux mechanisms, depletes the SR of Ca\textsuperscript{2+}. Thus, the present results suggest that a prominent effect of high concentrations of ethanol, like that of the myocardial depressant halothane,\textsuperscript{27} is to decrease the amount of Ca\textsuperscript{2+} stored within the SR. The reversal of ETOH contractile depression by norepinephrine (Figure 1B and Table 1) or by increased Ca\textsubscript{c} (Figure 4) suggests that, if increased Ca\textsuperscript{2+} is available to the SR, it can be pumped into the SR and that this compensates for a direct depletion of SR Ca\textsuperscript{2+} content by ETOH (even in the presence of an alteration by ETOH of the myofilament response to Ca\textsuperscript{2+}). In the case of norepinephrine, the reversal of the ETOH effect by a direct cAMP-dependent stimulation of the SR Ca\textsuperscript{2+} pump may also be involved.\textsuperscript{28–30}

The reversibility of profound contractile depression of Ca\textsuperscript{2+} ions or after ETOH washout indicates that, under the conditions of the present study, generalized membrane damage due to protein denaturation does not occur. A similar reversibility of ETOH effect in cardiac plasma membranes has previously been observed.\textsuperscript{31} On washout of high ETOH concentrations, an overshoot in contraction amplitude was observed, often associated with spontaneous contractile oscillations occurring in the interstimulus interval, a sign of cell Ca\textsuperscript{2+} overload.\textsuperscript{32} A similar overshoot in myocardial contraction amplitude occurs in intact perfused rat hearts.\textsuperscript{21} In that model the magnitude of the contractile overshoot after ETOH washout varied directly with the extent of contractile expression during ETOH exposure. This apparent Ca\textsuperscript{2+} overload after washout of high concentrations of ETOH may be related, in part, to an increase in Ca\textsuperscript{2+} binding of cardiac plasma membrane lipoproteins by ETOH.\textsuperscript{33}

Although the present study does not specifically address the effect of ETOH on sarcolemmal ion flux,
the observed decrease in $C_a$ with time after the initial ETOH-induced increase in individual resting cells in conjunction with a decrease in SR $C_a^{2+}$ stores suggests that a net cellular $C_a^{2+}$ efflux occurs; that is, either $C_a^{2+}$ influx into the cell is inhibited, or efflux is enhanced. Studies in mechanically skinned single cardiac cell fragments indicate that a large $C_a^{2+}$ release from the SR can be triggered by a small quantity of $C_a^{2+}$ in intact cells, the $C_a^{2+}$ trigger is $C_a^{2+}$ flux via L-type $C_a^{2+}$ channels. A reduction in the magnitude of L-type sarcolemmal $C_a^{2+}$ current by ETOH likely contributes to the decrease of SR $C_a^{2+}$ release and contractile amplitude elicited by electrical stimulation in the presence of a high concentration of ETOH. When the $C_a$ is increased or when norepinephrine is added to the bathing solution, an enhanced $C_a^{2+}$ trigger for $C_a^{2+}$ release would also be expected to occur. Thus, the effect of these perturbations (reversal of the ETOH effect) could occur via this mechanism, in addition to their other effects (Ca$^{2+}$ loading of the cell and stimulation of SR Ca$^{2+}$ pumping). The inexciability of most cells in 5% ETOH likely occurs because of total action potential failure. ETOH also inhibits Na-K pump activity in cultured rat heart cells. In summary, multiple effects of ETOH underlie its depressant effects on cardiac contractile function. At clinically relevant ETOH concentrations, this appears to occur as a result of a depression of the myofilament response to $C_a^{2+}$. At higher ETOH concentrations, impairments of excitation–contraction coupling mechanisms occur at both

**Figure 6.** Tracings showing that the addition of 3% (vol/vol) ethanol (ETOH) to a representative unstimulated myocyte leads to a rapid increase in indo 1 fluorescence ratio followed by fluorescence fluorescence about an elevated resting value. (The inset shows an amplified and filtered tracing of the initial ETOH-induced events). After additional time in ETOH, the average fluorescence decreases.

**Figure 7.** Tracings from experiments with suspensions of quin 2–loaded myocytes to assess whether ethanol (ETOH) depletes $C_a^{2+}$ content of the sarcoplasmic reticulum. Protocols for the introduction of quin 2 into the cells and for the study of fluorescence are given in “Materials and Methods.” Panel A: Addition of caffeine after EGTA to control, resting myocytes gives an increase in fluorescence that is used as an index of $C_a^{2+}$ content of the sarcoplasmic reticulum. DTPA, diethyl- enetriaminepentacetic acid. Panel B: Subsequent addition of caffeine after ETOH exposure leads to a reduced $C_a$, transient compared with control in panel A.

**Figure 8.** Graph showing the diminution of the fluorescence transient in response to caffeine. Ethanol (ETOH) data were previously obtained in experiments of the type shown in Figure 7, using three different preparations of cells. The response to caffeine, after ETOH addition, is presented as a percentage of the unattenuated response to caffeine in control cells.
the SR and myofilaments and probably (though not investigated in the present study) at the sarcolemma as well. In this regard, the ETOH effects resemble those of the local anesthetic, halothane. Each of these ETOH effects is reversible within minutes after washout and can be overcome in the continued presence of ETOH by an increase in Ca, or by the addition of norepinephrine. The present results, thus, also suggest a cellular mechanism by which reflex adrenergic stimulation of the heart serves to compensate for an ETOH depression of cardiac function.9–11

References


13. Fabiato A: Time and calcium dependence of activation and inactivation of calcium induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J Gen Physiol 1985;85:247–289


22. Stern MD, Capogrossi MC, Lakatta EG: Propagated contractile waves in single cardiac myocytes modeled as regenerative calcium induced calcium release from the sarcoplasmic reticulum (abstract). Biophys J 1984;45(pt 2):94a


32. Capogrossi MC, Stern MD, Spurgeon HA, Lakatta EG: Spontaneous Ca2+-release from the sarcoplasmic reticulum limits Ca2+-dependent twitch potentiation in individual cardiac myocytes: A mechanism for maximal inotropy in the myocardium. J Gen Physiol 1989;91:133–155


38. Beuckelmann DJ, Wier WG: Mechanism of release of calcium from sarcoplasmic reticulum of guinea-pig cardiac cells. J Physiol (Lond) 1988;405:233–255


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