Mechanism of Myocardial Contractile Depression by Clinical Concentrations of Ethanol

A Study in Ferret Papillary Muscles

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Abstract

Moderate alcohol intoxication in man, a ubiqitious social event, causes acute but reversible myocardial depression, the mechanism of which is unknown. We investigated whether this depression could be due to a direct effect of ethanol on the process of electromechanical coupling by simultaneously measuring the transmembrane action potential and contraction, or the cytosolic calcium transient (via aequorin photoluminescence) and contraction in isolated ferret right ventricular papillary muscle. Ethanol, in concentrations that are similar to plasma levels in man during intoxication (0.15 vol %), depressed the force of contraction ~ 10%. The step in the electromechanical process that was affected appeared to be the calcium-myofilament interaction, as there was no change in the transmembrane action potential or cytosolic calcium transient. This inhibition was quickly reversed by removal of the ethanol from the perfusate.

On the other hand, higher concentrations of ethanol produced changes in contraction, the calcium transient, and the action potential, suggesting multiple levels of inhibition of electromechanical coupling. Increasing the perfusate calcium or use of the calcium channel agonist, BAY-K 8644, increased cytosolic calcium to near maximum but had little effect on contractility, confirming that the relationship between calcium and the myofilaments had been altered.

These data suggest that the acute depression in ventricular function seen with alcohol consumption may be due to a direct effect on electromechanical coupling through inhibition of the calcium myofilament interaction. (*J. Clin. Invest.* 1990. 85:1462–1467.) alcohol • calcium • cardiomyopathy • ethanol • ventricular function

Introduction

Acute intoxication with alcohol causes reversible myocardial dysfunction, the mechanism of which has never been defined (1–6). This effect occurs in normals, alcoholics, and patients with dilated cardiomyopathy, and almost certainly has a different mechanism from the cardiomyopathy induced by long-term ethanol consumption (7–14). Most studies of isolated myocardium have sought to explain these acute and reversible changes in the force of contraction by using pharmacologic concentrations of ethanol in the 1% (by volume) range (see reference 12). These studies have demonstrated that ethanol has multiple effects on the myocardium, and as such could depress the force of contraction at several points in the process of electromechanical coupling. Contrary to the above studies, we wished to ascertain whether ethanol, in concentrations similar to the serum levels frequently achieved in man (~ 0.1%), would depress contractile force in isolated cardiac muscle, and if so, at what stage in electromechanical coupling.

To study at which stage in the contractile process this depression occurred, we attempted to analyze the effects of low concentrations (0.05–0.2 vol percentage) of ethanol on the various components of electromechanical coupling in isolated right ventricular ferret papillary muscle. First we examined the effect of ethanol on the sarcolemma by simultaneously measuring contractile force and the cardiac action potential. Next, we examined the importance of the cytosolic calcium transient by simultaneously measuring intracellular calcium ($[Ca^{2+}]_i$)[1] via the photoluminescent indicator, aequorin, and contractile force. Last, we examined the relationship between $[Ca^{2+}]_i$ and the myofilaments by analyzing the effect of ethanol on the $[Ca^{2+}]_i$/force curve.

Methods

Male ferrets (Marshall Research Animals, Inc., North Rose, NY), 4–10 wks old, were killed with anhydrous ether and intraperitoneal pentobarbital. The hearts were quickly removed and placed in room temperature Hepes-buffered Tyrode solution. Right ventricular papillary muscles were removed and placed in a fast flow bath. The muscular end was fixed to a stainless steel clamp and the tendon attached to a force tranducer by thin silk thread. The muscles were superfused by Hepes-buffered Tyrode solution of the following composition (in mM): NaCl 108; KCl 5; $CaCl_2$ 1; Hepes 4.5 (pH 7.4 with NaOH); $MgCl_2$ 1; $NaC_2H_3O_2$ 20. The temperature was 31–32°C in the bath while flow was maintained at 12 ml/min. Pacing was performed at twice diastolic threshold strength at 0.3 Hz. Muscles were allowed to equilibrate for ~ 30 min before being stretched to the peak of their length-tension curve, at which all experiments were conducted. Since we conducted these experiments in muscle at the peak of the length-tension curve, we were unable to obtain simultaneous aequorin luminescence and action potentials. Therefore, parallel experiments were performed.

The muscles selected for action potential/stress measurement underwent impalement with standard 3 M KCl-filled electrodes (10–30 M Ω). The action potentials were measured using a high input impedance electrometer (WPI FD 223; World Precision Instruments, New Haven, CT). The signals were simultaneously displayed on a standard strip chart recorder (model 2400S; Gould Inc., Cleveland, OH) and oscilloscope (model 5111A; Tektronix, Inc., Beaverton, OR) from

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1. *Abbreviations used in this paper:* APD, action potential duration; $[Ca^{2+}]_i$, intracellular calcium; ETOH, ethanol.

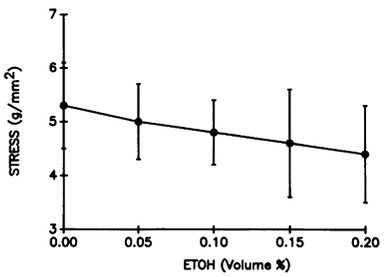

Figure 1. For 13 muscles the concentration response to threshold ethanol is displayed. The concentration that produced a ~ 10% decline in stress was 0.15±0.5%. We arbitrarily chose a 10% decrease in stress as the threshold for ethanol effect.

Table I. Action Potential at Threshold ETOH

	Control	Threshold
V_m, *mV*	−82±4	−81±3
APD50, *ms*	175±15	169±18
APD90, *ms*	203±12	210±15
dV/dT_{max}, *V/s*	130±20	142±38

Results are mean±SD; $n = 4$.

which photographs and subsequent measurements of rest potential (V_m) and action potential duration at 50 (APD50) and 90% (APD90) repolarization were made. The maximum value of the first derivative of the upstroke of the action potential (dV/dT_{max}) was obtained from a custom-built sample and hold circuit, which was linear from 10 to 1,000 V/s. Tension was measured using a linear force transducer (Kulite; Gould Inc.), while stress was calculated from the tension and estimated cross-sectional area.

The muscles selected for aequorin (purchased from J. Blinks, Mayo Clinic, Rochester, MN) measurements were impaled between 50 and 100 times for microinjection of aequorin. The luminescence passed through a lucite guide to a specially adapted photomultiplier tube (EMI 9893 B/350; Thorn EMI Gencom Inc., Fairfield, NJ). The output of the tube was displayed on the strip chart recorder and stored on tape (HP 3968A instrument recorder; Hewlett-Packard Co., Boston, MA). The data were also digitized (DT 2801A; Data Translation, Marlborough, MA) for signal averaging. The data are reported as raw luminescence or as the estimated $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$ was estimated by expressing the luminescence as the ratio of the emitted light to the maximum light emitted (L/L_{max}) by the preparation after exposure to a Triton X solution having 20 mM $CaCl_2$ (15). Ethanol (0.05–3%) had no effect on the in vitro calcium/luminescence curve (15).

After successful impalement or microinjection, we characterized the response (luminescence and stress) of the muscles to increasing superfusate calcium (0.5–5.0 mM). We used this curve to compare the relationship between $[Ca^{2+}]_i$ and stress before and after ethanol, in an attempt to analyze the $[Ca^{2+}]_i$/myofilament interaction. Separate preparations were then exposed to one of two concentration ranges of ethanol (as vol %), 0.05–0.2 or 1.0–3.0%. The first range was the major area of interest of the present study and was selected to describe the threshold for changes in contractility (at clinically encountered concentrations), while the latter was selected as representative of the range where major changes in both the electrical and contractile properties have been described in prior studies of isolated cardiac preparations. In the lower range, we called the ethanol concentration that produced a 10% decline in stress the threshold concentration. In the higher range we tested whether ethanol shifted the $[Ca^{2+}]_i$/force curve and whether the negative inotropic effects of ethanol could be reversed by addition of extra calcium to the superfusate (from 0.5 to 10 mM) or by addition of the calcium channel agonist, BAY-K 8644 (10^{-6} M).

Statistics. Data are presented as means and standard deviations. Comparison between groups was performed by one- or two-way analysis of variance.

Results

Threshold concentrations of ethanol. We established a threshold for changes in contractility in 13 muscles in the lower range of ethanol concentrations. Fig. 1 displays the changes in stress as a function of ethanol concentration. The ethanol concentration that produced a ~ 10% decrease in stress, the threshold concentration, was 0.15±0.5%. When expressed as a percent of control, at 0.15% there was an 11.4±2% decrease in force in the entire group ($P < 0.01$). It appeared, therefore, that the force of contraction was altered in isolated cardiac muscle at ethanol concentrations similar to serum levels seen in man. These changes in stress were all reversible with removal of the ethanol (Fig. 2).

The next experiments investigated whether threshold concentrations of ethanol decreased contraction by altering electromechanical coupling at the level of the sarcolemma. We examined the action potentials from four muscles at the ethanol concentration that produced a 10% decline in stress. In these experiments, the twitch force and transmembrane action potential were simultaneously measured. At threshold, we found no change in the action potential (Table I). Fig. 3 is an unretouched photograph of the simultaneous contractions and

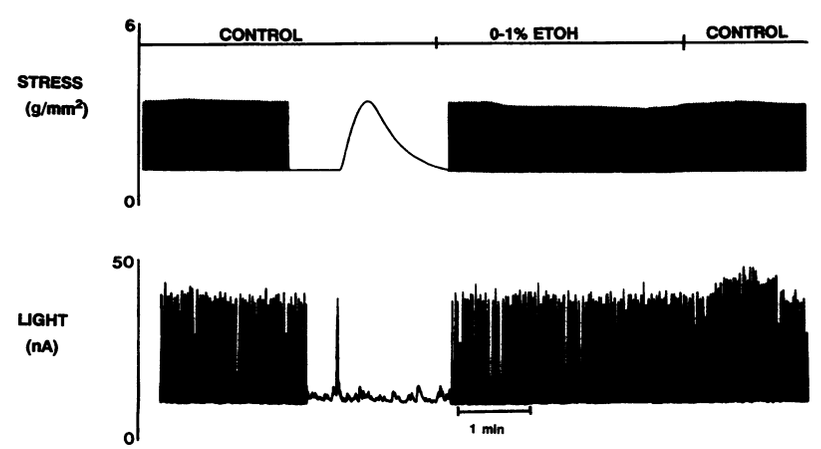

Figure 2. Stress and light during exposure to 0.1% ethanol at an initial perfusate Ca^{2+} of 2 mM. The analogue calcium transients are unchanged when contraction is depressed. When the ethanol is removed, the stress returns to its previous level. In this preparation there appears to be an "overshoot" in $[Ca^{2+}]_i$ when the ethanol is removed.

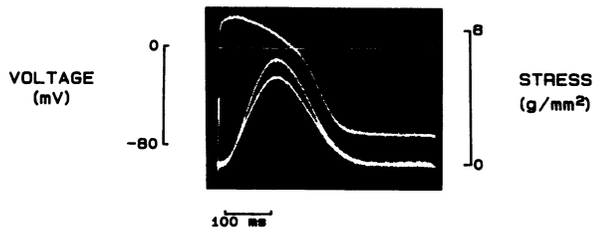

Figure 3. The action potentials and contraction during control and after exposure to threshold concentrations of alcohol. The action potential sweeps are superimposable when contraction declines.

Table II. Stress/Light at Threshold Concentrations of ETOH

	Control	Threshold
Stress, g/mM^2	5.3±0.8	4.6±0.9*
Time to peak stress, *ms*	112±10	104±10
Luminescence (L/L_{max}), $\times 10^{-5}$	5.3±0.1	5.4±0.3
Time to peak light, *ms*	46±3	52±6
Time to 75% light dissipation, *ms*	74±2	71±3

Results are mean±SD; $n = 13$.
* $P < 0.01$.

action potentials from a muscle at control and after exposure to 0.1% ethanol. As can be seen, there is a ~ 10% decrease in contraction, but the action potentials are unchanged and superimposable.

The next step in electromechanical coupling that we investigated was the cytosolic calcium transient. In individual muscles, the ethanol concentration that produced a 10% decrease in stress did not change either the shape (duration) or peak of the calcium transient. Fig. 2 displays a representative experiment where the stress is seen to decline by ~ 10%. As can be seen from the raw luminescence records, there was no change in the light transient, suggesting that the mechanism of the decrease in stress was distal to the release of cytosolic free calcium, i.e., inhibition of the calcium myofilament interaction. In some preparations (Fig. 2) there was an increase in light with the removal of ethanol. This was an inconstant finding whose mechanism was not clear. Fig. 4 demonstrates the signal averaged twitch and light transient superimposed at the same time base and gain from another threshold experiment. There is a ~ 10% decline in stress, but no change in the time course or peak of the calcium transient. Table II displays the summary data from the light and stress (50–100 signal averaged beats) in these muscles at control and at exposure to threshold concentrations of ethanol. We used time to peak light (from the stimulus) and time to 75% light dissipation (analogous to time to 75% relaxation) as a means to compare the time course and shape of the calcium transient, all of which show no change in the calcium signal at threshold concentrations of ethanol.

The effects on contraction were readily reversible, as demonstrated in Fig. 2, by simply removing the ethanol. The decrease in contractility could be easily reversed by increasing superfusate calcium (Fig. 5). With the addition of as little as 0.5 mM calcium to the superfusate, both stress and light increased.

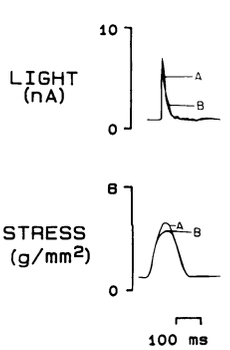

Figure 4. A superimposed trace of stress and light measurements (100 beats) before (*A*) and after (*B*) 0.15% ethanol. The stress decreases but the time course and peak luminescence are unchanged.

High ethanol concentrations. To examine the effects of ethanol at higher concentrations, we exposed muscles to 1.0–3.0% ethanol. We first investigated the effects of ethanol on the sarcolemma by measuring the action potential in four separate muscles exposed to the higher concentrations of ethanol. Fig. 6 is a photograph of the twitch and action potential after exposure to 3% ethanol. Unlike the exposure to threshold concentrations, 3% ethanol was associated with concomitant and significant changes in the action potential (Table III). The decrease in duration (APD 50) of the action potential alone would allow less time per beat for entry of calcium via the slow calcium channel. The effects of ethanol on the sarcolemma appear to be complex since the decrease in dV/dT_{max} suggests a concomitant inhibition of the fast sodium channel. In the experiments at the higher ethanol concentrations we were unable to dissociate the effects of the action potential and the twitch, as was present at the threshold. These same changes were also evident at 1 or 2% ethanol (Table IV).

Unlike the threshold exposure, there was a significant decline in the peak and duration of luminescence during the exposure to ethanol concentrations between 1 and 3%. As noted in Table IV, there appeared to be a concentration-dependent decrease in both force and $[Ca^{2+}]_i$, consistent with an effect of ethanol at multiple levels of electromechanical cou-

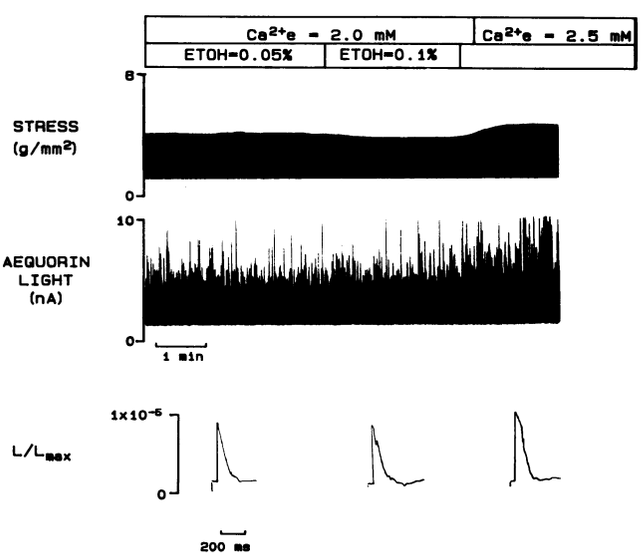

Figure 5. Stress and light during exposure to threshold ethanol at an initial perfusate Ca^{2+} of 2 mM. When the perfusate Ca^{2+} is raised to 2.5 mM, stress and light increase.

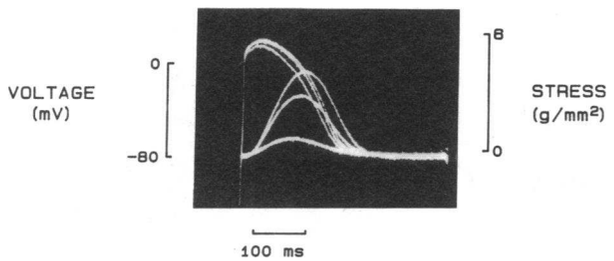

Figure 6. Action potential during exposure to 3% ethanol demonstrating reductions in both contraction and the action potential.

pling. At 3% ethanol there was a remarkable alteration in stress and $[Ca^{2+}]_i$ (Fig. 7). With extra superfusate Ca^{2+}, primarily $[Ca^{2+}]_i$ was decreased, suggesting a significant inhibition of the $[Ca^{2+}]_i$/myofilament interaction. In contrast to threshold, both the peak and time course of the calcium transient were significantly abbreviated (Table IV). In concert with the changes in the action potential, these data confirm multiple levels of inhibition of electromechanical coupling.

These data indicated that at the higher concentrations of ethanol multiple mechanisms of contractile inhibition were present. Nonetheless, we wanted to know whether the apparent change in the calcium myofilament interaction would still be evident at these high concentrations. To do this we investigated whether higher concentrations of ethanol shifted the $[Ca^{2+}]_i$/stress curve, and if so, whether increasing transsarcolemmal calcium flux (via increasing perfusate calcium or using Bay-K 8644) would increase both $[Ca^{2+}]_i$ and stress. Fig. 8 shows the relationship between peak contractile force (stress) and luminescence (L/L_{max}) generated during the Ca^{2+} concentration–response curve. The addition of 3% ethanol at the peak of the curve resulted in a significant decrease in force and $[Ca^{2+}]_i$. However, with increased transsarcolemmal calcium flux there was little change in contraction despite an increase in $[Ca^{2+}]_i$ to near maximum values, suggesting a more potent effect on the ability of the myofilaments to produce force in response to calcium. This shift in the relationship between $[Ca^{2+}]_i$ and stress is consistent with the hypothesis that ethanol alters the relationship between calcium and the myofilaments.

Discussion

The goal of this study was to ascertain at what level of electromechanical coupling ethanol affects myocardial contractile function. The primary finding in this study is that ethanol, in concentrations found in man during acute intoxication, causes an alteration in electromechanical coupling, which may be at the level of the myofilaments. The effect is reversible by increasing the amount of calcium presented to the myofilaments or by washing out the ethanol.

Table III. Action Potential at High ETOH

	Control	3%
Vm, *mV*	−82±5	−80±4
APD50, *ms*	182±10	160±8*
APD90, *ms*	212±8	190±9*
dV/dT_{max}, *V/s*	148±10	109±15*

Results are mean±SD; $n = 4$.
* $P < 0.05$.

Table IV. Stress/Light at High Concentrations of ETOH

	Control	1%	2%	3%
Stress, g/mM^2	4.1±0.9	2.0±0.9*	1.1±0.4*	0.5±0.2*
Time to peak stress, *ms*	100±10	92±9	88±8	86±10*
Peak luminescence (L/L_{max}), $\times 10^{-5}$	5.4±0.2	4.1±0.8	2.2±0.4*	1.8±0.6*
Time to peak light, *ms*	52±4	45±6	41±9*	36±8*
Time to 75% light dissipation, *ms*	78±8	71±8	65±10*	56±6*

Results are mean±SD; $n = 6$; 50–100 beats averaged.
* $P < 0.05$.

Ethanol at threshold concentration did not appear to alter the action potential, suggesting that electromechanical coupling was not altered at the level of the sarcolemma. It is, of course, possible that ethanol had multiple effects on ion currents, which would lead to no net change in inward or outward currents and thus leave the action potential unaltered. Indeed, at higher concentrations ethanol appears to be a potent antagonist of both the sodium and calcium channel (see below).

With the advent of the photoluminescent calcium indicators, it is now possible to study the relationship between free intracellular calcium ($[Ca^{2+}]_i$) and the force generated by the cardiac twitch (15). In these experiments the peak and the time course (shape) of the aequorin transient were unchanged at a time when ethanol had decreased the force of contraction by ~ 10%. These data in turn suggest that the amplitude and time course of the cytosolic calcium transient were not changed at the threshold concentration of ethanol. That we could also demonstrate that the cardiac action potential was not altered is consistent with the hypothesis that the effect was at the level of the myofilaments, and not at the sarcolemma or sarcoplasmic reticulum (16).

It should be noted that all methods, including aequorin, for determining the relationship between $[Ca^{2+}]_i$ and force production (hence, myofilament sensitivity) have problems (17, 18). In part this is due to the nonlinear features of the calcium detector (aequorin) and the indirect manner in which myofilament sensitivity is inferred (15, 16). Other methodologies that might further corroborate our data would be an examination of myofilament sensitivity by skinned preparations or by defining the relationship between maximal force activation and light (19, 20).

It is possible that ethanol may have other nonspecific effects, which might explain our results. For example, ethanol may cause selective damage to the endocardium, which in turn may exert a unique modulating effect on the contraction of isolated cardiac muscle (21). Certainly, at the higher concentration of ethanol the multiple and perhaps nonspecific effects of ethanol must be considered.

The effects of ethanol were easily reversible. Notably, at the threshold range the force of contraction could be restored by small additions of superfusate calcium. This observation supports the clinical data which show that the acute negative inotropic effects of alcohol are readily reversed by catecholamines

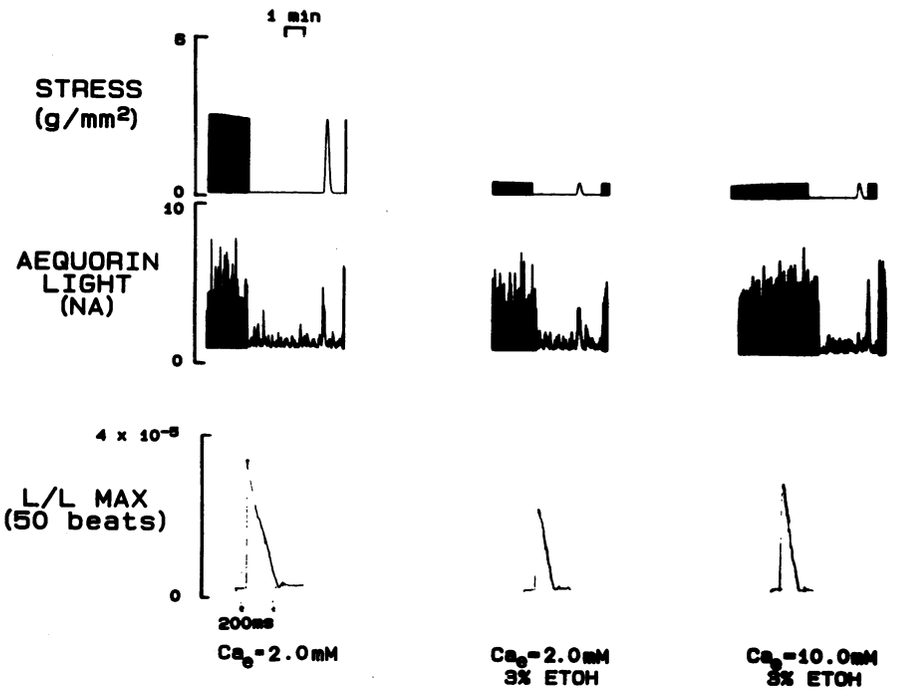

Figure 7. Stress and light after exposure to 3% ETOH. The twitch is depressed by 80% with a concomitant decrease in light. With increased perfusate Ca^{2+} there is an increase in $[Ca^{2+}]_i$, but little change in contraction.

or, conversely, unmasked by autonomic blockade (3–5). Both interventions are well-known modulators of intracellular calcium transport.

In higher concentrations, ethanol appears to affect electromechanical coupling at several levels. The aequorin transient was diminished in size and duration, suggesting that sarcoplasmic reticulum calcium cycling was significantly inhibited, or that less sarcoplasmic reticulum loading occurred. Though the cardiac action potential did not change (see above), the latter explanation cannot be excluded. This result is consistent with a recent preliminary communication by Danzinger et al. (22). Additionally, the concentrations of ethanol abbreviated the action potential, particularly the plateau (23). In fact, there is clear evidence for calcium channel blockade by ethanol in tetrodotoxin-treated single bullfrog cells (17). The mechanism of the action at the sarcolemma appears to be diffuse, as both the calcium and sodium channel seem to be involved.

The mechanism of the acute decrease in inotropy caused by alcohol intoxication has never been defined, though the phenomenon has been repeatedly demonstrated (1–6). In a series of experiments, Puszkin and Rubin advanced the notion that alcohol (180 mM or 0.69 vol %) interfered with actomyosin function in human skeletal muscle (24, 25). These data suggested that in the contractile apparatus of skeletal muscle ethanol inhibited the calcium myofilament interaction. Our data in cardiac muscle do not specify at what point in the troponin actomysin reaction alcohol acts. Ethanol has also been demonstrated to interfere with Na^+/K^+ pump activity and with sarcolemmal permeability, both perturbations leading to an increase in $[Na^+]_i$ (26–29). It is difficult to ascribe a negative inotropic effect of ethanol at the sarcolemma since the resultant increase in $[Na^+]_i$ should lead to an increase in $[Ca^{2+}]_i$ through Na^+/Ca^{2+} exchange. The results of our study are not to be confused with the voluminous data concerning the effects of chronic alcohol intake on myocardial structure and function (13, 14). Rather, our data provide, for the first time, a mechanism for the acute decrease in contractile function seen with alcohol consumption.

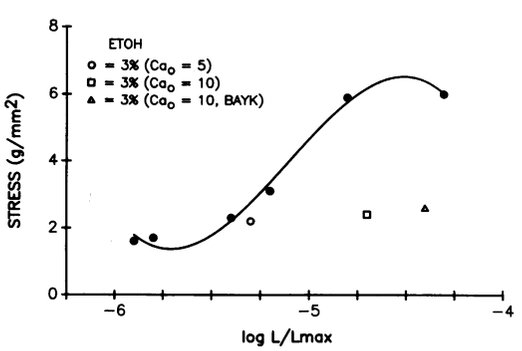

Figure 8. Aequorin luminescence versus stress generated by increasing the perfusate Ca^{2+} concentration from 0.5 to 5.0 mM (*solid circles*). At 5 mM Ca^{2+}, 3% ethanol decreases stress and $[Ca^{2+}]_i$. Increasing perfusate Ca^{2+} or adding Bay-K 8644 increases $[Ca^{2+}]_i$ to near maximum levels, but leaves stress relatively unchanged.

In summary, these data suggest a mechanism for the acute decrease in ventricular function that is seen in alcohol intoxication. Low concentrations of ethanol may inhibit the calcium myofilament interaction, and any manuever that either increases the amount of calcium presented to the myofilaments, or decreases the ethanol will reverse the negative inotropic effect.

Acknowledgments

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References

1. Delgado, C. E., N. J. Fortuin, and R. S. Ross. 1985. Acute effects of low doses of alcohol on left ventricular function by echocardiography. *Circulation.* 51:535–540.

2. Timmis, G. C., R. C. Ramos, S. Gordon, and V. Gangadharan. 1975. The basis for differences in ethanol-induced myocardial depression in normal subjects. *Circulation.* 51:1144–1148.

3. Child, J. S., R. B. Kovick, J. A. Levisman, and M. L. Pearce. 1979. Cardiac effects of acute ethanol ingestion unmasked by autonomic blockade. *Circulation.* 59:120–125.

4. Ahmed, S. S., G. E. Levinson, and T. J. Regan. 1973. Depression of myocardial contractility with low doses of ethanol in normal man. *Circulation.* 48:378–385.

5. Kelbaek, H., T. Gjorup, I. Brynjolf, N. J. Christensen, and J. Godtfredsen. 1985. Acute effects of alcohol on left ventricular function in healthy subjects at rest and during upright exercise. *Am. J. Cardiol.* 55:164–167.

6. Regan, T. J., G. Koroxenidis, C. B. Moschos, H. A. Oldewurthel, P. H. Lethan, and H. K. Hellems. 1966. The acute metabolic and hemodynamic responses of the left ventricle to ethanol. *J. Clin. Invest.* 45:270–280.

7. Symbas, P. N., D. H. Tyras, R. E. Ware, and B. J. Baldwin. 1972. Alteration of cardiac function by hemodialysis during experimental alcoholic intoxication. *Circulation.* 45:46. (Abstr.)

8. Sarma, J. S. M., S. Ikeda, R. Fischer, Y. Maruyama, R. Weishaar, and R. J. Bing. 1976. Biochemical and contractile properties of heart muscle after prolonged alcohol administration. *J. Mol. Cell. Cardiol.* 8:951–972.

9. Bing, R. J., H. Tillmanns, J. Fauvel, K. Seeler, and J. C. Mao. 1974. Effect of prolonged alcohol administration on calcium transport in heart muscle of the dog. *Circ. Res.* 35:33–38.

10. Kino, M., K. A. Thorp, O. H. L. Bing, and W. H. Abelmann. 1981. Impaired myocardial performance and response to calcium in experimental alcoholic cardiomyopathy. *J. Mol. Cell. Cardiol.* 13:981–989.

11. Rubin, E. 1979. Alcoholic myopathy in heart and skeletal muscle. *N. Engl. J. Med.* 301:28–33.

12. Knochel, J. P. 1983. Cardiovascular effects of alcohol. *Ann. Intern. Med.* 98:849–854.

13. Tepper, D., J. M. Capasso, and E. H. Sonnenblick. 1986. Excitation–contraction coupling in rat myocardium: alterations with long-term ethanol consumption. *Cardiovasc. Res.* 20:369–374.

14. Noren, G. R., N. A. Staley, S. Einzig, F. L. Mikell, and R. W. Asinger. 1983. Alcohol-induced congestive cardiomyopathy: an animal model. *Cardiovasc. Res.* 17:81–87.

15. Blinks, J. R., W. G. Wier, P. Hess, and F. Prendergast. 1982. Measurement of Ca^{2+} concentration in living cells. *Prog. Biophys. Mol. Biol.* 40:1–114.

16. Endott, M., and J. R. Blinks. 1962. Actions of sympathomimetic amines on the Ca^{2+} transient and contractions of rabbit myocardium: reciprocal changes in myofibrillar responsiveness to Ca^{2+} mediated through α and β-adrenoreceptors. *Circ. Res.* 62:247–251.

17. Yue, D. T., and W. G. Weir. 1985. Estimation of intracellular $[Ca^{2+}]$ by nonlinear indicators. *Biophys. J.* 48:533–537.

18. Stern, M. D. 1985. Bounds on the estimation of calcium by aequorin luminescence in the presence of inhomogeneity. *Biophys. J.* 48:853–857.

19. Fabiato, A., and F. Fabiato. 1976. Techniques of skinned cardiac cells and of isolated cardiac fibers with disrupted sarcolemmas with reference to the effects of catecholamine and caffeine. *In* The Sarcolemma: Recent Advances in Cardiac Structure and Metabolism. P. E. Ray and N. S. Dhalla, editors. Vol. IX. University Park Press, Baltimore. 71–78.

20. Yue, D. T., E. Marban, and W. G. Weir. 1986. Relationship between force and intracellular $[Ca^{2+}]$ in tetanized mammalian heart muscle. *J. Gen. Physiol.* 87:223–242.

21. Brutsaert, D. L., K. R. Meulemans, K. R. Sipido, and S. U. Sys. 1988. Effects of damaging the endocardial surface on the mechanical performance of isolated cardiac muscle. *Circ. Res.* 62:358–366.

22. Danziger, R. S., M. Sakai, R. G. Hansford, and E. G. Lakatta. 1988. Reversible abolition of cardiac contraction by ethanol is associated with depletion of sarcoplasmic reticulum calcium content. *J. Am. Coll. Cardiol.* 11(Suppl. A):203A. (Abstr.)

23. Gimeno, A. L., M. F. Gimeno, and J. L. Webb. 1962. Effects of ethanol on cellular membrane potentials and contractility of isolated rat atrium. *Am. J. Physiol.* 203:194–196.

24. Puszkin, S., and E. Rubin. 1976. Effects of ADP, ethanol and acetaldehyde on the relaxing complex of human muscle and its adsorption by polystyrene particles. *Arch. Biochem. Biophys.* 177:574–576.

25. Puszkin, S., and E. Rubin. 1975. Adenosine diphosphate effect on contractility of human muscle actomyosin: inhibition by ethanol and acetaldehyde. *Science (Wash. DC).* 188:1319–1320.

26. Israel, Y., H. Kalant, and I. Laufer. 1965. Effects of ethanol on Na, K, Mg-stimulated microsomal ATPase activity. *Biochem. Pharmacol.* 14:1803–1814.

27. Kischuk, R. P., M. D. Otten, and P. I. Polimeni. 1986. Effect of acute alcoholic intoxication on myocardial electrolyte and water distributions. *J. Mol. Cell. Cardiol.* 18:197–205.

28. Williams, J. W., M. Tada, A. M. Katz, and E. Rubin. 1975. Effect of ethanol and acetaldehyde on the $(Na^+ + K^+)$-activated adenosine triphosphatase activity of cardiac plasma membranes. *Biochem. Pharmacol.* 24:27–29.

29. Retig, J. N., M. A. Kirchberger, E. Rubin, and A. M. Katz. 1977. Effects of ethanol on calcium transport by microsomes phosphorylated by cyclic AMP-dependent protein kinase. *Biochem. Pharmacol.* 26:393–396.