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Regular alcohol consumption mimics cardiac preconditioning by protecting against ischemia–reperfusion injury

(ethanol/adenosine/heart/guinea pig/myocardial infarction)

MASAMI MIYAMAE*†, IVAN DIAMOND†‡§¶, MICHAEL W. WEINER†‖, S. ALBERT CAMACHO*†, AND VINCENT M. FIGUEREDO*†¶**

Departments of *Medicine (Cardiology), ‡Neurology, and §Cellular and Molecular Pharmacology, and ¶Ernest Gallo Clinic and Research Center, San Francisco General Hospital, and ‖Magnetic Resonance Unit, Department Veterans Affairs Medical Center, †University of California, San Francisco, CA 94110

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ABSTRACT **Epidemiologic studies indicate that long-term alcohol consumption decreases the incidence of coronary disease and may improve outcome after myocardial infarction. Attenuation of ischemia–reperfusion injury after myocardial infarction improves survival. This study investigates the possibility that alcohol consumption can improve survival after myocardial infarction by reducing ischemia–reperfusion injury. Hearts were isolated from guinea pigs after drinking ethanol for 3–12 weeks and subjected to global ischemia and reperfusion. Hearts from animals drinking ethanol showed improved functional recovery and decreased myocyte damage when compared with controls. Adenosine A_1 receptor blockade abolished the protection provided by ethanol consumption. These findings indicate that long-term alcohol consumption reduces myocardial ischemia–reperfusion injury and that adenosine A_1 receptors are required for this protective effect of ethanol. This cardioprotective effect of long-term alcohol consumption mimics preconditioning and may, in part, account for the beneficial effect of moderate drinking on cardiac health.**

Epidemiologic studies indicate that long-term alcohol consumption is associated with a reduced incidence of coronary artery disease (1–3) and is correlated with beneficial effects on lipids and platelet aggregation (4–7). In addition, recent studies suggest that long-term alcohol consumption may improve survival in patients after myocardial infarction (8–10), but the mechanisms underlying this possible cardioprotective effect of alcohol are not understood.

Reperfusion injury, a paradoxical worsening of myocardial damage when circulation is restored to coronary arteries after prolonged ischemia, can increase infarct size and worsen outcome after myocardial infarction (11, 12). Recent evidence in experimental animals indicates that ischemia–reperfusion injury can be reduced by preconditioning the heart with brief episodes of ischemia and reperfusion prior to prolonged ischemia (13–16). However, no therapy is presently available that mimics ischemic preconditioning in patients to improve recovery after myocardial infarction.

Adenosine is a well known cardioprotective agent that appears to play a role in ischemic preconditioning (12–16). There is also substantial evidence that adenosine mediates many of the responses to ethanol in the brain and other organs (17, 18). Ethanol increases the extracellular concentration of adenosine (19) thereby increasing the activation of adenosine receptors (17). Also, brief exposure to ethanol protects against reperfusion injury in rats (20). In this study we examine the possibility that long-term consumption of ethanol can improve cardiac recovery after myocardial infarction by an adenosine-mediated response that mimics ischemic preconditioning.

METHODS

Male Hartley guinea pigs weighing 300 g were fed Lab Diet guinea pig food (PMI Feeds, St. Louis) and water *ad libitum*. Animals received ethanol in their drinking water for 3–12 weeks, and control animals were untreated (see Table 2). All animals accepted 2.5% or 5.0% ethanol in their drinking water throughout the course of the experiments. Animals consuming 10% or 20% ethanol were initially given 5% ethanol for 1 week to initiate drinking. Blood samples were obtained randomly throughout the day and night in a group of animals treated with 10% ethanol ($n = 11$) to assess serum ethanol concentration over a 24-h period. Peak serum ethanol concentration was assessed in a group of animals treated with 2.5% ethanol by obtaining blood samples at the end of the 12-h dark cycle.

Isolated Heart Perfusion and Measurement of Function. Guinea pigs were heparinized with 1,000 units i.p. and anesthetized with pentobarbital 60 mg/kg i.p. Hearts were excised and immediately arrested in cold isosmotic saline containing 20 mmol/liter KCl. Isolated hearts were then cannulated via the aorta and perfused at a constant pressure of 70 mmHg on a nonrecirculating isovolumic perfused heart apparatus, using a Krebs–Henseleit perfusate containing 123 mmol/liter NaCl, 4.7 mmol/liter KCl, 2.5 mmol/liter $CaCl_2$, 20 mmol/liter $NaHCO_3$, 1.7 mmol/liter $MgSO_4$, 1.2 mmol/liter KH_2PO_4, 11 mmol/liter glucose, and 20 units/liter insulin. The perfusate was bubbled continuously with a 95%O_2/5%CO_2 gas mixture and maintained at 37°C. Hearts were paced at 240 beats/min using two platinum-tipped electrodes connected to a Grass Instruments (Quincy, MA) SD-5 stimulus generator.

Left ventricular (LV) pressure was measured using a 2 French, high-fidelity micromanometer (Millar Instruments, Houston). A compliant latex balloon was attached to a 2-cm segment of rigid polyethylene tubing that was connected to a Y-adapter. One end of the Y-adapter was used to advance the micromanometer to the latex balloon. The other end of the Y-adapter was used to fill the LV balloon with bubble-free water 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). Coronary flow was con-

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Abbreviations: LV, left ventricular; CK, creatine kinase; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMPX, 3,7-dimethyl-1-propargylxanthine.

**To whom reprint requests should be addressed at: Division of Cardiology, Room 5G1, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, CA 94110. e-mail: margaux@itsa.ucsf.edu.

tinuously monitored by an in-line flow meter (Gilmont Instruments, Barrington, IL).

Creatine Kinase (CK). Coronary effluent samples were collected every 3 min beginning with reperfusion. CK released during the initial 18 min of reperfusion was measured with a commercially available kit (47–10; Sigma). Values were corrected for both dry heart weight and coronary flow rates and expressed in units/ml per gram-dry-weight. Ninety-three percent of the CK released occurred in the initial 18 min of reperfusion.

Experimental Protocol. Alcohol was withdrawn from the drinking water 12–16 h before sacrifice. Hearts were isolated and perfused as described above. After a 20-min equilibration period, baseline measurements were made of LV developed pressure and coronary flow. Hearts were then subjected to 45 min of no-flow ischemia, followed by reperfusion. During ischemia, hearts were maintained at 37°C by enclosure in a water-jacketed air chamber. Warmed perfusate kept in the lower part of the chamber saturated the air with humidity and prevented cooling by evaporation. Hemodynamic measurements were repeated every 6 min for a total of 48 min. After removing the atria and great vessels, hearts were then dried for 24 h at 80°C before being weighed.

Similar experiments were carried out as described above except that the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (200 nM) was added to the perfusate 10 min before ischemia. The K_i of DPCPX for adenosine A_1 receptors is 0.69 nM (21). In contrast, the K_i for A_2 and A_3 receptors is 502 and 49,300 nM, respectively. Based on these values, other investigators have used 60–200 nM to selectively antagonize adenosine A_1 receptors in perfused heart studies (14, 15, 22). This concentration of DPCPX has been shown to inhibit the negative chronotropic and inotropic effects, as well as 6-keto-prostaglandin $F_{1\alpha}$ production, caused by adenosine (10 μM) infusion (14, 22). Baseline LV developed pressure and coronary flow were measured before and after adding DPCPX.

An additional group of hearts from ethanol-treated animals ($n = 6$) was subjected to the same ischemia–reperfusion protocol in the presence of the adenosine A_2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX; 10 μM). This concentration of DMPX in the perfusate effectively blocked myocardial adenosine A_2 receptors as indicated by abolishing the increased coronary flow induced by rapid infusion of 10 μM adenosine (data not shown).

Ischemia–reperfusion experiments were also carried out on hearts from guinea pigs consuming 10% ethanol until sacrifice to exclude the possibility that protection was due to withdrawal ($n = 6$).

A preconditioning experiment without ethanol consumption was carried out as a positive control. Perfused hearts, isolated from age-matched controls ($n = 6$), were subjected to 2 min of global ischemia and 5 min of reperfusion immediately before prolonged ischemia and reperfusion, as described above.

Statistical Analyses. All data are expressed as mean ± SEM. Comparisons between groups were made using repeated measures ANOVA with multiple grouping factors. If significant differences were observed, a Tukey post-hoc test was used to confirm the significance of differences between the groups.

RESULTS

LV-developed pressure, coronary flow, and perfusion pressure did not differ between hearts from guinea pigs drinking 10% ethanol for 6 weeks and age-matched controls (Table 1). In control and ethanol-treated guinea pigs, dry heart weight to body weight ratios (3.96 ± 0.09 versus 3.94 ± 0.08) and body weights (637 ± 24 versus 595 ± 15) were not statistically different. After 6 weeks, *ad libitum* drinking of 10% ethanol resulted in a serum ethanol of 60 ± 32 mg/dl. This is comparable to values previously reported in guinea pigs drinking 10% ethanol (23, 24). Peak serum ethanol was 22 ± 7 mg/dl in guinea pigs drinking 2.5% ethanol for 6 weeks (samples drawn at end of dark cycle).

Table 1. Protection against reperfusion injury following chronic ethanol exposure: Effect of adenosine A_1 receptor antagonism

	Preischemia		Reperfusion	
	Control	Ethanol	Control	Ethanol
Developed pressure, mmHg	112 ± 4	116 ± 3	35 ± 3*	60 ± 2*†
Diastolic pressure, mmHg	10 ± 0	10 ± 0	46 ± 4*	22 ± 2*†
Perfusion pressure, mmHg	70 ± 0	70 ± 0	75 ± 1*	75 ± 1*
Coronary flow, ml/min	36 ± 1	35 ± 1	25 ± 1*	24 ± 2*
(+)DPCPX				
Developed pressure, mmHg	118 ± 4	113 ± 3	35 ± 6*	31 ± 4*‡
Diastolic pressure, mmHg	10 ± 0	10 ± 0	48 ± 6*	47 ± 5*‡
Perfusion pressure, mmHg	70 ± 0	70 ± 0	77 ± 1*	77 ± 1*
Coronary flow, ml/min	36 ± 1	34 ± 1	25 ± 1*	24 ± 2*

Isolated guinea pig hearts were subjected to 45 min of global ischemia and 48 min of reperfusion. Hearts from guinea pigs exposed to 10% ethanol in their drinking water for 6 weeks were compared to hearts from age-matched controls. Experiments were carried out in the presence and absence of the adenosine A_1 receptor antagonist, (+)DPCPX, ($n = 10$ for all groups). Data are presented as mean ± SEM.

*$P < 0.05$ versus preischemia value; †$P < 0.05$ ethanol versus control value; ‡$P < 0.05$ (+)DPCPX versus (−)DPCPX value.

Hemodynamics During Reperfusion. The hearts from animals treated with ethanol showed improved recovery of LV-developed pressure during reperfusion when compared with control hearts (Fig. 1; 52% versus 31% of preischemic values at 48 min; $P < 0.05$). The increase in diastolic pressure

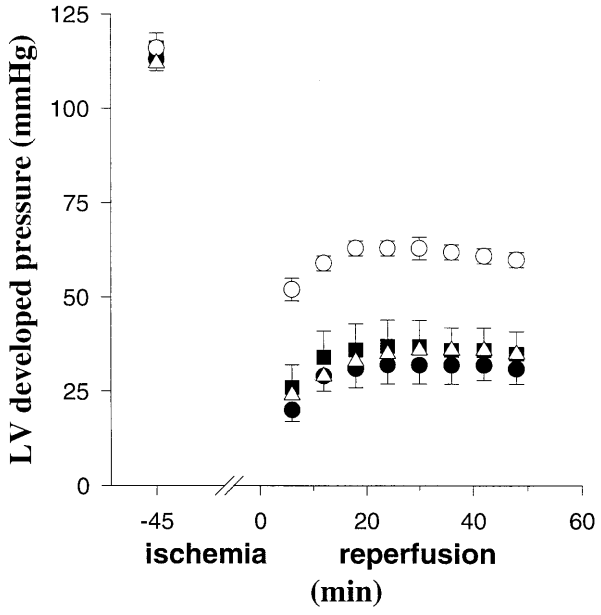

FIG. 1. Chronic exposure to ethanol improves recovery of LV-developed pressure during postischemic reperfusion via adenosine A_1 receptors. LV-developed pressure was measured during reperfusion after global ischemia in four groups of isolated guinea pig hearts ($n = 10$ for each group) following 6 weeks of 10% ethanol consumption (○), in age-matched controls (△), following 6 weeks of ethanol consumption in the presence of the adenosine A_1 receptor antagonist (DPCPX) (●), and in age-matched controls in the presence of DPCPX (■). Recovery of LV-developed pressure is significantly greater in hearts from ethanol-treated guinea pigs at each time point measured ($P < 0.05$). Adenosine A_1 receptor blockade completely abolished ethanol-induced cardioprotection. Data are mean ± SEM. Error bars are not included for open triangles but are less then SEM of closed symbols.

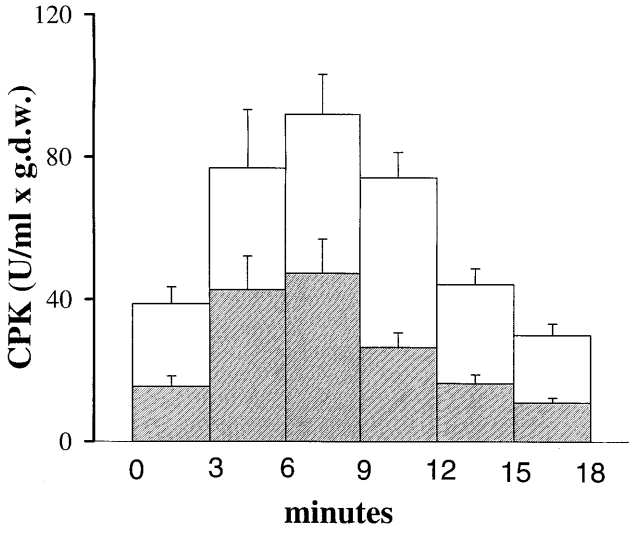

FIG. 2. Chronic exposure to ethanol reduces CK release during postischemic reperfusion. CK (units/ml per heart gram-dry-weight) was measured in the coronary effluent during 3-min intervals of postischemic reperfusion from hearts isolated from ethanol-treated guinea pigs as described in Fig. 1 (shaded bars) and controls (open bars). Release of creatine was significantly less from hearts of ethanol-treated animals compared with controls during all 3-min collection periods ($P < 0.05$). Adenosine A_1 receptor blockade by DPCPX completely abolished the protective effect of ethanol consumption on myocyte injury. Data are presented as mean ± SEM.

throughout reperfusion was lower in hearts from ethanol-treated animals than in control hearts (Table 1; 220% versus 460% of preischemic values at 48 min; $P < 0.05$). These data suggest that chronic exposure to ethanol improved functional recovery and decreased myocyte contracture or irreversible myocyte injury during reperfusion. There were no differences in coronary flow or coronary perfusion pressure during reperfusion (Table 1), suggesting that the protective effect of long-term ethanol consumption did not involve vasodilatation.

Release of CK. CK release was measured to assess the degree of myocyte injury following global ischemia and reperfusion (Fig. 2). The hearts from animals drinking 10% ethanol showed substantially less release of CK when compared with hearts from control animals (159 ± 25 versus 356 ± 26 units/ml per gram-dry-weight; $P < 0.05$). Twenty-eight percent of total CK released occurred between 6 and 9 min of reperfusion and decreased to ≈7% between 15 and 18 min (Fig. 2). These data suggest that chronic exposure to ethanol protects against myocyte injury following ischemia and reperfusion.

Ethanol Concentration and Duration of Consumption. The effect of varying concentrations of ethanol and time of exposure is shown in Table 2. The protective effect of ethanol consumption is independent of dose by 6 weeks of drinking (2.5–20%) and persists as long as ethanol is offered in the drinking water (12 weeks). A dose-response curve for ethanol-induced cardioprotection was evident by 3 weeks of consumption, with full protection observed in hearts isolated from animals drinking 10% ethanol. As little as 2.5% ethanol in the drinking water for 3 weeks protected against a rise in LV diastolic pressure during reperfusion (Table 2).

Role of Alcohol Withdrawal. The cardioprotective effect of long-term alcohol consumption was not related to withdrawal. Identical protection following ischemia–reperfusion was observed in hearts from animals consuming 10% ethanol until sacrifice. LV developed pressure recovered to 51% of preischemic levels at 48 min of reperfusion (116 ± 4 mmHg preischemia; 59 ± 4 mmHg at 48 min of reperfusion), LV diastolic pressure increased to 25 ± 4 mmHg at 48 min of reperfusion, and CK release was 219 ± 31 units/ml per gram-dry-weight. These data suggest that ethanol need not be present at the time of ischemia to confer cardioprotection.

Ischemic Preconditioning as a Control. Hearts from control animals were subjected to ischemia–reperfusion following a preconditioning protocol as a positive control in the absence of ethanol (Table 2). As expected, classic preconditioning produced striking cardioprotection that was identical to ethanol-induced cardioprotection. Thus, chronic alcohol's protective effect mimics cardioprotection by ischemic preconditioning.

Role of Adenosine A_1 Receptors. Adenosine mediates many cellular responses to ethanol (18). Experimental preconditioning requires adenosine A_1 receptor activation (14, 16, 25–27). Therefore, we searched for evidence that adenosine receptors are required for the cardioprotective effect of ethanol. Experiments described above were carried out in a second group of hearts in the presence of a selective adenosine A_1 receptor antagonist, DPCPX. Table 1 shows that DPCPX did not change preischemic LV-developed pressure and coronary flow. During reperfusion, however, DPCPX abolished the protective effect of ethanol. When exposed to DPCPX, hearts from animals consuming ethanol were no longer different than hearts from control animals. They exhibited similar recoveries of LV-developed pressure (Fig. 1) and increases of LV diastolic pressure (Table 1). Similar results were obtained when CK release was measured as a marker of myocardial damage during reperfusion. In the presence of DPCPX, CK release

Table 2. Effect of ethanol dose and duration of treatment on reperfusion injury

			Developed pressure, mmHg		Diastolic pressure, mmHg		CK, units/ml per heart gram-dry-weight
Time	Ethanol, %	*n*	Preischemia	Reperfusion	Preischemia	Reperfusion	
3 weeks	10	6	113 ± 4	62 ± 4*	10	16 ± 1*	156 ± 22*
	5	6	111 ± 3	39 ± 6	10	34 ± 3*	328 ± 76
	2.5	6	112 ± 4	38 ± 7	10	38 ± 2*	348 ± 67
	Control	6	115 ± 3	31 ± 7	10	57 ± 7	400 ± 60
6 weeks	20	10	118 ± 5	55 ± 4*	10	26 ± 6*	204 ± 42*
	10	10	116 ± 3	60 ± 2*	10	22 ± 2*	159 ± 25*
	5	6	113 ± 7	55 ± 4*	10	17 ± 2*	187 ± 33*
	2.5	6	105 ± 7	66 ± 7*	10	18 ± 4*	252 ± 20*
	Control	10	112 ± 4	35 ± 3	10	46 ± 4	356 ± 26
12 weeks	20	8	116 ± 6	56 ± 5*	10	26 ± 6*	181 ± 29*
PC	0	6	116 ± 4	62 ± 6*	10	26 ± 4*	167 ± 2*

LV-developed and diastolic pressures and release of CK were measured during postischemic reperfusion. Experiments were performed as described in Table 1 in hearts isolated from guinea pigs consuming varying concentrations of ethanol in their drinking water for 3, 6, or 12 weeks. A group of hearts from animals not exposed to ethanol were subjected to ischemic preconditioning (PC). Data are presented as mean(SEM).
*$P < 0.05$ versus control.

during reperfusion was identical in hearts from animals exposed to ethanol (374 ± 46 units/ml per gram-dry-weight) and controls (398 ± 35 units/ml per gram-dry-weight). These data were similar to CK measurements when control hearts were studied in the absence of DPCPX. Taken together, these data suggest that adenosine A_1 receptors are required for the improved functional recovery and protection against myocyte injury produced by chronic ethanol consumption.

To determine if the protective effect of ethanol was adenosine receptor-specific, a group of hearts from ethanol-treated animals was subjected to ischemia–reperfusion in the presence of a selective adenosine A_2 receptor antagonist, DMPX. In contrast to results with the adenosine A_1 antagonist, adenosine A_2 receptor blockade had no effect on ethanol-induced cardioprotection. In the presence of DMPX, LV-developed pressure recovered to 48% of preischemic levels and CK release was 179 ± 35 units/ml per gram-dry-weight. These data suggest that the protective effect of chronic exposure to ethanol is mediated selectively through adenosine A_1 receptors on myocytes.

DISCUSSION

There are two major findings in this study. First, long-term alcohol consumption protects against myocardial ischemia–reperfusion injury in guinea pig hearts, and second, this cardioprotective effect of ethanol requires adenosine A_1 receptor activation. Ethanol-induced cardioprotection was documented by improved recovery of contractile function and reduced release of CK, an indicator of myocyte damage. Therefore, the cardioprotective effect of ethanol appears to mimic ischemic preconditioning against ischemia–reperfusion injury. Protection was demonstrable whether or not ethanol was omitted from the drinking water 12–16 h before study, suggesting that prolonged alcohol consumption probably caused long-lasting changes in cellular function (17, 28) and/or in gene expression (29, 30). Moreover, because protection was abolished by blocking adenosine A_1 receptors, these long-term changes appear to involve adenosine receptor-dependent signaling pathways.

Ethanol and Ischemia–Reperfusion Injury. Clinical and epidemiologic studies show that moderate drinkers have less cardiovascular disease when compared with nondrinkers or heavy drinkers (1–3). This is primarily due to a decreased incidence of ischemic heart disease, which is present whether drinking moderate or excessive amounts of alcohol (2, 31). However, heavy drinkers succumb to cardiomyopathy and other alcohol-related diseases (1–3). There is also recent evidence that long-term alcohol consumption may improve survival after myocardial infarction (8–10). We find that prolonged consumption of ethanol protects against ischemia–reperfusion injury in guinea pig hearts. Specifically, hearts isolated from animals fed ethanol for many weeks show greater recovery and less myocyte damage following prolonged ischemia and reperfusion when compared with controls. Similar pathophysiologic changes in human beings would be expected to reduce infarct size and improve survival after myocardial infarction (32). Interestingly, this ethanol-induced cardioprotection was present whether moderate or heavy doses of ethanol were used. This correlates with epidemiological findings that the incidence of ischemic heart disease is reduced and outcome after an ischemic event is improved whether drinking moderate of excessive amounts of alcohol (2, 9, 10, 31).

Wannamethee *et al.* (8) found that moderate drinking or high levels of physical activity were associated with increased survival after myocardial infarction in an 11.5-year study of 7,735 middle-aged British males. In the Multicenter Study of Myocardial Ischemia, those patients who consumed alcohol regularly had improved survival and less reinfarction and unstable angina during a 26-month follow-up period (9). In a study of 14,407 subjects followed over 20 years using the National Institute of Alcoholism and Alcohol Abuse and National Institutes of Health Alcohol Epidemiologic Data System, Dufour *et al.* (10) reported that, "those dying of acute myocardial infarction were less likely to be continuous drinkers and more likely to be continuous nondrinkers." Thus, not only does moderate alcohol consumption appear to decrease the incidence of myocardial infarction, but there is emerging evidence that suggest that regular drinking may also improve survival after a myocardial infarct. The results in our study raise the possibility that long-term ethanol consumption improves survival because of an ethanol-induced cardioprotective effect that mimics ischemic preconditioning.

Measurement of CK was used in this study as an assay of myocyte damage but does not replace histological evidence of myocardial necrosis. Unfortunately, histological methods do not accurately quantitate relative degrees of myocardial injury in a perfused heart model of global ischemia. Nevertheless, despite this recognized methodological limitation, our results show that it is possible to determine the direct effects of chronic exposure to ethanol on the myocardium since CK release is a measure of myocyte injury, and probably necrosis.

Kobayashi *et al.* (20) demonstrated that ethanol added to the buffer of perfused rat hearts prior to anoxia followed by reoxygenation (a model of ischemia–reperfusion injury) decreased myocardial injury. However, this study did not determine whether chronic ethanol consumption produced protection against reperfusion injury in the absence of ethanol. McDonough and Causey (33) fed rats ethanol for 8–10 weeks and studied contractile recovery after brief ischemia in isolated hearts. However, this study is more consistent with myocardial stunning than infarction because the episode of ischemia was brief and resulted in ≈80% recovery of LV-developed pressure (82% in ethanol treated rats; 78% in controls; P = not significant). Furthermore, there was no measurement of myocardial damage. To our knowledge, the current study is the first to demonstrate that chronic exposure to ethanol protects against irreversible myocardial injury caused by prolonged ischemia and reperfusion.

In general, the concentration of ethanol required to produce an adaptive biological response is inversely related to the time of exposure (18). In this study, 2.5% and 5% ethanol produced partial cardioprotection after 3 weeks of exposure, but protection was complete by 6 weeks. As expected, higher concentrations of ethanol produced maximal protection at 3 weeks; this was sustained as long as ethanol was consumed (up to 12 weeks). These data suggest that duration of consumption may be a more important variable than the dose of ethanol consumed. This is consistent with clinical experience that regular alcohol consumption, whether by moderate drinkers or alcoholics, is associated with decreased incidence of coronary events (2, 31) and improved survival following myocardial infarction (8–10). However, alcoholics have a substantial risk for developing nonischemic cardiomyopathy, cirrhosis of the liver, and other life-threatening medical complications. As a result, alcoholics have a much higher rate of morbidity and mortality that overcomes any potential benefit against coronary artery disease.

Potential Mechanisms for the Cardioprotective Effect of Long-Term Alcohol Consumption. Adenosine mediates many of the acute and long-term effects of ethanol on cellular and organ function (17, 18). Ethanol inhibits adenosine uptake which results in increased extracellular levels of adenosine (19) and activation of adenosine receptors (34). After chronic exposure to ethanol, however, there is an adaptive change in adenosine receptor signaling that appears to depend on the expression of adenosine receptor subtypes on particular cells. For example, chronic exposure to ethanol of cells with adenosine A_1 receptors causes hypersensitization of cAMP production (35), in contrast to cells expressing adenosine A_2 receptors where ethanol causes heterologous desensitization of

cAMP signal transduction (30, 36). In this study, the cardioprotective effect of chronic ethanol consumption required adenosine A_1 receptors, just like ischemic preconditioning (14, 16, 25–27). Adenosine A_1 receptor blockade abolished the protective effect of ethanol but adenosine A_2 receptor blockade was without effect. Ischemic preconditioning appears to be mediated through activation of adenosine A_1 and/or A_3 receptors on myocytes (13–15). The results in this study suggest that the protective effect of chronic ethanol use is mediated through selective activation of adenosine A_1 receptors on myocytes, just as in preconditioning.

Experiments in pigs (25), rabbits (14), dogs (26, 27), and studies with human tissue (16) indicate that adenosine A_1 receptors mediate ischemic preconditioning. Adenosine has been shown to protect guinea pig myocytes from ischemia–reperfusion injury through adenosine A_1 receptors (37). In this study, a preconditioning experiment in the absence of alcohol consumption also provided cardioprotection in guinea pig hearts, just like that produced by long-term ethanol treatment. Recent studies in rabbit myocardium suggest involvement of A_3 receptors in preconditioning, although those reports also suggested that A_1 receptors may still be important because adenosine A_1 antagonists partially blocked protection (13, 15). Cardiac adenosine A_3 receptors have been found in rats (38), sheep (21), and humans (39), and may be present in rabbits (13, 15), but no data are available for guinea pigs. On the other hand, A_1 receptors have a higher density in guinea pig myocardium when compared with other species (40), and selective adenosine A_1 blockade completely abolished cardioprotection in our study. While this does not rule out a contributing role for A_3 receptors, it is clear that adenosine A_1 receptors are required for the cardioprotective response to ethanol. Studies are underway to determine whether specific adenosinergic agents can potentiate the protective effect of prolonged alcohol consumption against ischemia–reperfusion injury.

Conclusions. Long-term alcohol consumption is associated with a reduced incidence of coronary artery disease. Moreover, long-term drinking may be associated with improved survival after a myocardial infarction. Long-term alcohol consumption protects against myocardial ischemia–reperfusion injury. Moreover, this alcohol-induced cardioprotection appears to be mediated by adenosine A_1 receptors, just as in ischemic preconditioning. There is no therapy available today to reproduce the benefits of preconditioning in patients at risk for myocardial infarction. Our study, demonstrating that long-term alcohol consumption mimics ischemic preconditioning, opens a new avenue for developing novel therapies to improve outcome in patients at risk for myocardial infarction.

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1. Klatsky, A. L., Armstrong, M. A. & Friedman, G. D. (1990) *Am. J. Cardiol.* **66,** 1237–1242.
2. Rimm, E. B., Giovannucci, E. L., Willett, W. C., Colditz, G. A., Ascherio, A., Rosner, B. & Stampfer, M. J. (1991) *Lancet* **338,** 464–468.
3. Fuchs, C. S., Stampfer, M. J., Colditz, G. A., Giovannucci, E. L., Manson, J. E., Kawachi, I., Hunter, D. J., Hankinson, S. E., Hennekens, C. H., Rosner, B., Speizer, F. E. & Willett, W. C. (1995) *N. Engl. J. Med.* **332,** 1245–1250.
4. Gaziano, J. M., Buring, J. E., Breslow, J. L., Goldhaber, S. Z., Rosner, B., VanDenburgh, M., Willett, W. & Hennekens, C. H. (1993) *N. Engl. J. Med.* **329,** 1829–1834.
5. Fumeron, F., Betoulle, D., Luc, G., Behague, I., Ricard, S., Poirier, O., Jemaa, R., Evans, A., Arveiler, D., Marques-Vidal, P., Bard, J., Fruchart, J., Ducimetiere, P., Apfelbaum, M. & Cambien, F. (1995) *J. Clin. Invest.* **96,** 1664–1671.
6. Pikaar, N. A., Wedel, M., van der Beek, E. J., van Dokkum, W., Kempen, H. J., Kluft, C., Ockhuizen, T. & Hermus, R. J. (1987) *Metabolism* **36,** 538–543.
7. Renaud, S. C., Beswick, A. D., Fehily, A. M., Sharp, D. S. & Elwood, P. C. (1992) *Am. J. Clin. Nutr.* **55,** 1012–1017.
8. Wannamethee, G., Whincup, P. H., Shaper, A. G., Walker, M. & MacFarlane, P. W. (1995) *Br. Heart J.* **74,** 324–331.
9. Nakamura, Y., Kawai, C., Kinoshito, M. & Moss, A. J. (1995) *Circulation* **92,** I-708 (abstr.).
10. Dufour, M. C., Caces, M. F., Whitmore, C. C. & Hanna, E. Z. (1996) *Alchol. Clin. Exp. Res.* **20,** 97A (abstr.).
11. Figueredo, V. M., Dresdner, K. P., Jr., Wolney, A. C. & Keller, A. M. (1991) *Cardiovasc. Res.* **25,** 337–342.
12. Forman, M. B., Velasco, C. E. & Jackson, E. K. (1993) *Cardiovasc. Res.* **27,** 9–17.
13. Armstrong, S. & Ganote, C. E. (1994) *Cardiovasc. Res.* **28,** 1049–1056.
14. Liu, G. S., Thornton, J., Van Winkle, D. M., Stanley, A. W., Olsson, R. A. & Downey, J. M. (1991) *Circulation* **84,** 350–356.
15. Liu, G. S., Richards, S. C., Olsson, R. A., Mullane, K., Walsh, R. S. & Downey, J. M. (1994) *Cardiovasc. Res.* **28,** 1057–1061.
16. Walker, D. M., Walker, J. M., Pugsley, W. B., Pattison, C. W. & Yellon, D. M. (1995) *J. Mol. Cell. Cardiol.* **27,** 1349–1357.
17. Diamond, I. & Gordon, A. S. (1997) *Physiol. Rev.* **77**, 1–20.
18. Diamond, I. & Gordon, A. S. (1994) in *The Role of Adenosine in Mediating Cellular and Molecular Responses to Ethanol*, eds. Jansson, B., Jornvall, H., Rydberg, U., Terenius, L. & Vallee, B. L. (Birkhauser, Basel), pp. 175–183.
19. Nagy, L. E., Diamond, I., Casso, D. J., Franklin, C. & Gordon, A. S. (1990) *J. Biol. Chem.* **265,** 1946–1951.
20. Kobayashi, H., Ashraf, M., Rahamathulla, P. M. & Minami, M. (1987) *Pathol. Res. Pract.* **182,** 810–816.
21. Linden, J. (1994) *Trends Pharmacol. Sci.* **15,** 298–306.
22. Cano, C. & Malik, K. U. (1992) *J. Pharmacol. Exp. Ther.* **261,** 660–668.
23. Schreiber, S. S., Reff, F., Evans, C. D., Rothschild, M. A. & Oratz, M. (1986) *Alcohol. Clin. Exp. Res.* **10,** 531–534.
24. Schreiber, S. S., Evans, C. D., Reff, F., Oratz, M. & Rothschild, M. A. (1984) *Alcohol. Clin. Exp. Res.* **8,** 54–61.
25. Van Winkle, D. M., Chien, G. L., Wolff, R. A., Soifer, B. E., Kuzume, K. & Davis, R. F. (1994) *Am. J. Physiol.* **266,** H829–H839.
26. Kitakaze, M., Hori, M., Takashima, S., Sato, H., Inoue, M. & Kamada, T. (1993) *Circulation* **87,** 208–215.
27. Auchampach, J. A. & Gross, G. J. (1993) *Am. J. Physiol.* **264,** H1327–H1336.
28. Dohrman, D. P., Diamond, I. & Gordon, A. S. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 10217–10221.
29. Miles, M. F., Wilke, N., Elliot, M., Tanner, W. & Shah, S. (1994) *Mol. Pharmacol.* **46,** 873–879.
30. Mochly-Rosen, D., Chang, F. H., Cheever, L., Kim, M., Diamond, I. & Gordon, A. S. (1988) *Nature (London)* **333,** 848–850.
31. Stampfer, M. J., Colditz, G. A., Willett, W. C., Speizer, F. E. & Hennekens, C. H. (1988) *N. Engl. J. Med.* **319,** 267–273.
32. The Multicenter Postinfarction Research Group (1983) *N. Eng. J. Med.* **309,** 331–336.
33. McDonough, K. H. & Causey, K. M. (1994) *Alcohol. Clin. Exp. Res.* **18,** 1423–1429.
34. Sapru, M. K., Diamond, I. & Gordon, A. S. (1994) *J. Pharmacol. Exp. Ther.* **271,** 542–548.
35. Nagy, L. E. & DeSilva, S. E. F. (1994) *Biochem. J.* **304,** 205–210.
36. Gordon, A. S., Collier, K. & Diamond, I. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2105–2108.
37. Cordeiro, J. M., Ferrier, G. R. & Howlett, S. E. (1995) *Am. J. Physiol.* **269,** H121–H129.
38. Zhou, Q. Y., Li, C., Olah, M. E., Johnson, R. A., Stiles, G. L. & Civelli, O. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7432–7436.
39. Salvatore, C. A., Jacobson, M. A., Taylor, H. E., Linden, J. & Johnson, R. G. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10365–10369.
40. Musser, B., Morgan, M. E., Leid, M., Murray, T. F., Linden, J. & Vestal, R. E. (1993) *Eur. J. Pharmacol.* **246,** 105–111.