Nongenomic effects of estrogen mediate the dose-related myocardial oxidative stress and dysfunction caused by acute ethanol in female rats

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El-Mas MM, Abdel-Rahman AA. Nongenomic effects of estrogen mediate the dose-related myocardial oxidative stress and dysfunction caused by acute ethanol in female rats. Am J Physiol Endocrinol Metab 306: E740–E747, 2014.—Acute ethanol lowers blood pressure (BP) and cardiac output in prooestrus and after chronic estrogen (E2) replacement in ovariectomized (OVX) female rats. However, whether rapid nongenomic effects of estrogen mediate these hemodynamic effects of ethanol remains unanswered. To test this hypothesis, we investigated the effect of ethanol (0.5 or 1.5 g/kg iv) on left ventricular (LV) function and oxidative markers in OVX rats pretreated 30 min earlier with 1 μg/kg E2 (OVXE2) or vehicle (OVX) and in proestrus sham-operated (SO) rats. In SO rats, ethanol caused significant and dose-related reductions in BP, rate of rise in LV pressure (LV dP/dtmax), and LV developed pressure (LVDp). These effects of ethanol disappeared in OVX rats and were restored in OVXE2 rats, suggesting rapid estrogen receptor signaling mediates the detrimental effects of ethanol on LV function. Ex vivo studies revealed that the estrogen-dependent myocardial dysfunction caused by ethanol was coupled with higher LV 1 generation of reactive oxygen species (ROS), 2 expression of malondialdehyde and 4-hydroxynonenal protein adducts, 3 phosphorylation of protein kinase B (Akt) and extracellular signal-regulated kinases (ERK1/2), and 4 catalase activity. ERK1/2 inhibition by PD-98059 (1 mg/kg iv) abrogated the myocardial dysfunction, hypotension, and the elevation in myocardial ROS generation caused by ethanol. We conclude that rapid estrogen receptor signaling is implicated in cellular events that lead to the generation of aldehyde protein adducts and Akt/ERK1/2 phosphorylation, which ultimately mediate the estrogen-dependent LV oxidative stress and dysfunction caused by ethanol in female rats.

Data from numerous studies demonstrate that ethanol administered acutely or chronically lowers blood pressure (BP) in female rats (16–18). Cardiac output and stroke volume are also reduced by ethanol, consistent with reduced myocardial contractility (17, 37) and ventricular dysfunction (42). The attenuation of these hemodynamic responses in ovariectomized (OVX) rats, and their full restoration, to levels in intact sham-operated (SO) rats after estrogen replacement suggests a primary role for estrogen in mediating the cardiovascular actions of ethanol (11, 14). Although the mechanism of the estrogen-dependent hemodynamic effects of ethanol is not fully understood, evidence suggests a role for the facilitation of myocardial phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/neuronal nitric oxide synthase (nNOS) in the reductions in BP and cardiac output produced by ethanol in female rats (18).

Oxidative stress has been implicated in cardiac abnormalities evoked by acute ethanol. In marine cardiac myocytes, ethanol reduced cell shortening and intracellular Ca2+ transients via increasing reactive oxygen species (ROS) generation (47). Because the ethanol effects were augmented and attenuated in myocytes with overexpressed aldehyde dehydrogenase and catalase, respectively, it is concluded that the metabolic product acetaldehyde might contribute to myocyte dysfunction and oxidative damage evoked by ethanol (47). Acute alcohol also increases the susceptibility of donor hearts to ischemia-reperfusion-induced myocardial contractile dysfunction in a rat heart transplant model by inducing oxidative stress, apoptosis, and mitochondrial dysfunction (28). Moreover, increased oxidative damage to mitochondrial DNA, proteins, and phospholipids is believed to contribute to cardiac dysfunction, possibly via myocyte apoptosis, cytosolic Ca2+ malfunction, and negative inotropy (31, 48). Furthermore, acute alcohol administration causes myocardial injury in mice due to rapid reduction in mitochondrial glutathione content and subsequent mitochondrial damage (26).

Because long-term (days or weeks) estrogen regimens were employed in our previous studies on estrogen-ethanol hemodynamic interaction (11, 14), our findings cannot ascertain whether nongenomic mechanisms contribute to the estrogen exacerbation of the myocardial depressant action of ethanol. Both genomic and nongenomic mechanisms are believed to facilitate the PI3K/Akt pathway (22, 23), which mediates the ethanol-evoked reductions in BP and cardiac output (18) and exhibits pro-oxidant activity (27). Therefore, integrative, cellular, and molecular studies were employed in the current study to test the hypothesis that rapid/nongenomic mechanisms triggered by estrogen mediate ethanol-evoked myocardial oxidative stress and hypotension in female rats. To test our hypothesis, we investigated the dose-related effects of ethanol (0.5 or 1.5 g/kg) in the presence (SO) of estrogen genomic and nongenomic effects as well as following acute (1 μg/kg: 30 min earlier) administration of estrogen (OVXE2) on LV performance [rate of rise in LV pressure (LV dP/dtmax), LV developed pressure (LVDp)], BP, and heart rate (HR). Ex vivo studies on tissues collected from ethanol- or vehicle-treated rats included measurements of LV ROS level, protein expressions of phosphorylated (p)-Akt and p-ERK1/2 and malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) protein adducts, and catalase activity as biomarkers/mediators of oxidative stress. Finally, we investigated the effect of pharmacological inhibition of ERK1/2 phosphorylation (PD-98059) to ascertain its causal role in the hemodynamic and oxidative effects of ethanol.

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A biochemical assay (20, 33) was used. The ventricles were found sufficient for achieving adequate estrogen. Ovariectomy (OVX) was performed as described in our previous studies. This was maintained at 22°C. All experiments were approved by the Institutional Animal Care and Use Committee and were carried out in accordance with the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Intravascular and intraventricular cannulation. Catheterization of femoral vessels was conducted as described in our previous studies (12, 15). Rats were anesthetized with intraperitoneal injection of a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). Catheters, each consisting of 5-cm polyethylene-10 tubing bonded to a 15-cm polyethylene-50 tubing, were placed into the abdominal aorta and vena cava via the left femoral vessels for measurement of arterial pressure and intravenous infusions, respectively. Intraventricular catheterization was performed by inserting polyethylene-50 tubing into the left ventricle through the carotid artery. Each rat received intramuscular injections of the analesic buprenorphine hydrochloride (Buprenex; 30 μg/kg) and penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen; 100,000 U/kg).

Ovariectomy. Two weeks before intravascular cannulation, bilateral OVX was performed as described in our previous studies. This 2-wk period was found sufficient for achieving adequate estrogen depletion (15, 16).

Quantification of cardiac ROS. The 2′,7′-dichlorofluorescein (DCF) biochemical assay (20, 33) was used. The ventricles were homogenized using Radnoti tissue grinders (Radnoti Glass Technology, Monrovia, CA) to increase protein yield, and kinetic readings (250 μg protein) were taken at 5-min intervals for 60 min at 37°C. ROS levels were calculated as relative DCF fluorescence per microgram of μg protein. Briefly, the assay buffer contained 130 mM KCl, 5 mM MgCl₂, 20 mM Na₂HPO₄, 20 mM Tris-HCl, and 30 mM glucose (pH 7.4), with a total volume of 3 ml. The assay was initiated with the addition of 5 μM dichlorohydrofluorescein diacetate (DCFH-DA) dissolved in 1.25 mM methanol (5 μM final concentration) and 50 μl (~1 mg protein) of tissue homogenate, and the mixture was incubated at 37°C for 15 min. This allowed DCFH-DA to be cleaved by intracellular esterase to derive free DCFH. The rate of oxidation from DCFH to DCF, which is indicative of oxidant production, was monitored at an excitation wavelength of 488 nm and emission wavelength of 525 nm for 30 min using a Hitachi F-2000 fluorescence spectrometer. The method was linear for least 60 min at various concentrations of protein present, corrected for the auto-oxidation rate of DCFH. The unit of oxidant production was expressed as micromoles of DCF formed per milligram of protein. The individual ROS generation curve were assessed by regression analysis, and slopes of the regression lines (regression coefficients) were taken as indices of ROS generation.

Western blots. For the measurement of total and phosphorylated Akt and ERK1/2 and expression of MDA protein adducts in ventricular tissues, the rat ventricle was homogenized on ice in a homogenization buffer [50 mM Tris (pH 7.5), 0.1 mM EGTA, 0.1 mM EDTA, 2 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 0.1% (vol/vol) Nonidet P-40, 0.1% SDS, and 0.1% deoxycholate]. After centrifugation (12,000 g for 10 min), protein in the supernatant was quantified (Bio-Rad protein assay system; Bio-Rad, Hercules, CA). Protein extracts (50 μg per lane) were run on a 4% to 12% SDS-polyacrylamide gel electrophoresis gel (Innovitrogen, Carlsbad, CA) and electro-blotted to nitrocellulose membranes. Blots were blocked for 2 h at room temperature in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) and then incubated overnight at 4°C in Odyssey blocking buffer with mouse antibodies to p-ERK1/2 (Thr202/Tyr204, 1:200; Cell Signaling Technology, Danvers, MA) or p-Akt (Ser473, 1:200; Cell Signaling Technology), rabbit antibodies to ERK1/2 or Akt (1:200; Cell Signaling Technology), or rabbit antibodies to MDA protein adducts (1:150; Academy Biomedical, Houston, TX). After three washes with PBS buffer containing Triton X-100 (PBS-T), the blots were incubated for 50 min at room temperature with the goat anti-mouse secondary antibody IRDye 800CW or the goat anti-rabbit secondary antibody IRDye 680CW (LI-COR Biosciences). After three washes with PBS-T buffer, the blots were detected using the Odyssey Infrared Scanning System. Equivalent sample loading was confirmed by stripping membranes with blot restore membrane rejuvination solution (SignaGen Laboratories, Gaithersburg, MD) and reprobing

### Table 1. Baseline values of MAP, HR, LV dP/dt\(_{max}\), and LVDP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SO</th>
<th>OVX</th>
<th>OVXE₂</th>
</tr>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>93 ± 8</td>
<td>96 ± 10</td>
<td>87 ± 7</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>348 ± 27</td>
<td>394 ± 19</td>
<td>372 ± 28</td>
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<tr>
<td>LV dP/dt(_{max}), mmHg/s</td>
<td>7,744 ± 370</td>
<td>8,935 ± 798</td>
<td>7,941 ± 830</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>164 ± 8</td>
<td>174 ± 12</td>
<td>157 ± 12</td>
</tr>
</tbody>
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Values are means ± SE of 7–8 observations in sham-operated (SO), ovariectomized (OVX), and estrogen-pretreated ovariectomized (OVXE₂) rats. MAP, mean arterial pressure; HR, heart rate; LV dP/dt\(_{max}\), rate of rise in left ventricular pressure; LVDP, left ventricular developed pressure.

Infrared Scanning System. Equivalent sample loading was confirmed by stripping membranes with blot restore membrane rejuvination solution (SignaGen Laboratories, Gaithersburg, MD) and reprobing.
with mouse β-actin antibody (1:5,000; ABCAM, Cambridge, MA). Protein bands were quantified by integrated intensities using Odyssey Infrared Imaging Software. Data were normalized to the corresponding total protein in the same sample, and results were then expressed as percentages of control values (18, 19).

**Dot blots.** The expression of 4-HNE protein adducts (29) in myocardial tissues was measured by dot blots. In brief, 100 μl of ventricular homogenate (10 μg protein) was transferred to a nitrocellulose membrane using the Minifold I vacuum dot-blot system (BioRad). Each slot was washed with 200 μl of PBS, and the membrane was dried for 15 min at 60°C. After blocking, the protein was detected by rabbit polyclonal HNE adducts antibody (1:1,000; Calbiochem, Billerica, MA) and mouse monoclonał β-actin antibody (1:5,000; ABCAM). All incubation and washing steps were performed according to the manufacturer’s instructions. Densitometric quantification of the dots was performed as described in Western blots.

**Catalase activity.** Ventricular homogenates were used for the determination of catalase activity as described by the manufacturer (Sigma Chemical, St. Louis, MO) and described previously (9).

**Blood ethanol concentration.** Blood ethanol concentration was measured using the enzymatic method described by Berni and Gutmann (5) and as used in our previous studies (16).

**Plasma estrogen.** Plasma estrogen was measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) as described in our previous studies (16).

### Experimental groups and protocols.
A total of 9 groups of female rats (3 SO, 3 OVX, and 3 OVXE2; n = 7–8 each) pre-instrumented 48 h earlier with intravascular and intraventricular catheters were used to investigate the hemodynamic and LV effects of ethanol (0.5 or 1.5 g/kg iv infusion over 30 min) or equal volume of saline. Each rat in a particular group received one treatment only. On the experiment day, the arterial and LV catheters were connected to pressure transducers. The data collected by fluid-filled LV catheters are comparable to those produced by the Millar catheter (44). The hemodynamic data were monitored and stored using LabChart 7 Pro software (PowerLab, ADInstruments, CO). SO rats were used during the proestrus phase, which exhibits the highest plasma estrogen levels (32), to avoid the impact of fluctuations in hormonal levels on the measured parameters. The proestrus phase of the cycle was identified through microscopic examination of vaginal smears (18).

A stabilization period of at least 30 min was allowed at the beginning of the experiment. Afterwards, baseline mean arterial pressure (MAP), HR, LV dp/dt\textsubscript{max}, and LVDP measurements were recorded. This was followed by the intravenous infusion of equal volumes (2 ml/kg) of ethanol (0.5 or 1.5 g/kg) or saline over 30 min in accordance with reported clinical studies (8). Ethanol was diluted in saline to final concentrations of 6.5% or 19.5%, respectively. Hemodynamic responses were monitored for 90 min following ethanol or saline administration. OVXE2 rats received a single bolus dose of estrogen (E2; 1 μg/kg iv) 10 min before ethanol or saline administration. After 30 min of ethanol or saline infusion, a volume of 0.5 ml of blood was drawn through the arterial cannula, collected into a heparinized tube, and centrifuged at 1,200 g for 5 min. The plasma was aspirated and stored at −80°C until analyzed for the ethanol and estrogen contents. At the conclusion of the experiment, euthanasia was induced with overdose of ketamine and xylazine mixture. The heart was excised immediately, and atria and great vessels were removed. The ventricles were blotted free of blood, homogenized, and used for biochemical and protein expression studies as detailed above. Another two groups of OVXE2 rats (n = 6–7 each) were used to investigate the effect of pharmacological inhibition of ERK1/2 phosphorylation by PD-98059 on the estrogen-dependent myocardial depression and oxidative stress caused by ethanol. PD-98059 (1 mg/kg iv) was administered 15 min before E2 (1 μg/kg iv) in OVX rats. Thirty minutes after E2 administration, the rats received ethanol (1.5 g/kg iv) or saline, and the hemodynamic changes were monitored for 90 min. At the conclusion of the experiment, the hearts were excised, following euthanasia, and used for the quantification of cardiac ROS as detailed above.

### Drugs.
Ketaject (ketamine), Xyla-ject (xylazine) (Phoenix Pharmaceuticals, St Joseph, MI), buprenorphine (Rickitt & Colman, Richmond, VA), Durapan (Vedco, Overland Park, KS), 17β-estradiol sulfate (Sigma Chemical), and ethanol (Midwest Grain Products, Weston, MO) were purchased from commercial vendors.

**Data analysis and statistics.** Values are means ± SE. The one-way or repeated-measures analysis of variance (ANOVA) followed by a Newman-Keuls post hoc test was used as appropriate for multiple comparisons. Statistical significance was defined as *P < 0.05.*

### RESULTS

**Baseline data.** The baseline values of hemodynamic (MAP and HR) and LV (LV dp/dt\textsubscript{max} and LVDP) parameters in conscious SO rats as well as in OVX rats treated with intravenous saline or E2 were not statistically different (Table 1). Blood ethanol concentrations measured 30 min after intravenous administration of ethanol (0.5 or 1.5 g/kg) correlated well with doses of ethanol employed (Table 1). Plasma alcohol levels, measured in SO rats, 30 min after the administration of.

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**Fig. 2.** Changes in rate of rise in left ventricular pressure (LV dp/dt\textsubscript{max}; A) and LV developed pressure (LVDP; B) caused by Etoh (0.5 or 1.5 g/kg iv), compared with an equal volume of saline, in sham-operated and OVXE2 or OVX rats. Values are means ± SE of 7–8 observations. *P < 0.05 vs. corresponding saline values. **P < 0.05 vs. OVX, Etoh 1.5 g/kg. #P < 0.05 vs. OVX, Etoh 0.5 g/kg.
the 0.5 and 1.5 g/kg doses of ethanol (38.8 ± 0.7 and 134.4 ± 0.9 mg%, respectively) were significantly higher than those seen in OVX (31.3 ± 1.5 and 108.6 ± 1.8 mg%, respectively) or OVXE2 rats (20.7 ± 0.7 and 110.0 ± 1.2 mg%, respectively). Ethanol (1.5 g/kg), compared with saline, had no effect on plasma E2 of SO rats (39 ± 3 vs. 46 ± 5 pg/ml). E2 levels were significantly lower in OVX rats (ethanol, 5 ± 1 pg/ml; saline, 5 ± 1 pg/ml) and increased to above SO values in OVXE2 rats (ethanol, 81 ± 4 pg/ml; saline, 85 ± 5 pg/ml).

**Estrogen-dependent BP and LV effects of ethanol.** Compared with an equal volume of saline, intravenous infusion of the 0.5 g/kg dose of ethanol had no effect on MAP, but the higher dose of ethanol (1.5 g/kg) caused gradual and significant (P < 0.05) decreases in MAP in SO rats (Fig. 1). The hypotensive effect of ethanol started at 10 min, reached its nadir (12–15 mmHg) at 40–50 min, and continued throughout the observation period (Fig. 1A). Ethanol (1.5 g/kg) had no effects on MAP in OVX rats (Fig. 1) but significantly lowered MAP in OVX rats pretreated with E2 (1 μg/kg iv) in a way that mimicked its effect in SO rats (Fig. 1).

Similar to its hypotensive effect, ethanol (0.5 and 1.5 g/kg) caused dose-related reductions in LV dP/dt max (Fig. 2A) and LVDp (Fig. 2B) in SO and OVXE2 rats in contrast to no effect in OVX rats. On the other hand, HR was not influenced by any of the ethanol doses in all rat preparations regardless of the estrogen state (data not shown).

**Acute estrogen uncovers ethanol-evoked LV oxidative stress.** Figures 3–6 illustrate the effect of ethanol on biomarkers/mediators of oxidative stress in ventricular tissues of female rats. The DCF kinetics assay revealed significantly (P < 0.05) greater ROS in myocardial tissue of saline-treated OVX rats compared with SO or OVXE2 rats (Fig. 3). Ethanol (0.5 and 1.5 g/kg) caused significantly greater increases in ROS generation in ventricular tissues obtained from SO or OVXE2, but not OVX, rats (Fig. 3). Regression analysis of the ROS generation curves also showed that ethanol increased the slopes of the regression lines (regression coefficients) in estrogen-replete but not depleted preparations (Fig. 3D). Similarly, ethanol caused significant (P < 0.05) and dose-related increases in the phosphorylation of ERK1/2 (Fig. 4A) and Akt (Fig. 4B),
expression of protein adducts of MDA (Fig. 5A) and 4-HNE (Fig. 5B), and catalase activity (Fig. 6) in ventricular tissues of SO and OVXE2, but not OVX, rats. Moreover, compared with SO rats, OVX rats exhibited reduced ventricular catalase activity, and this deficit was mitigated by acute estrogen administration in OVXE2 rats (Fig. 6).

**DISCUSSION**

In this study, we report the first evidence that rapid/non-genomic signaling contributes to the estrogen exacerbation of deleterious cellular and cardiovascular effects of ethanol in female rats. This conclusion is supported by several key findings of the present study. First, acute estrogen replacement restored ethanol-induced reductions in BP and myocardial contractility in OVX rats. Second, the findings clearly implicate rapid nongenomic effects of estrogen in the ethanol induction of mediators of oxidative stress in the myocardium.

**ERK1/2 inhibition blunts the E2-dependent hemodynamic and oxidative effect of ethanol.** In E2-treated OVX rats, the ERK1/2 phosphorylation inhibitor PD-98059 (1 mg/kg iv) virtually abolished the decreases in MAP (Fig. 7A), LV dP/dt max (Fig. 7B), and LVDP (Fig. 7C) caused by subsequently administered ethanol (1.5 g/kg iv). The hemodynamic profiles of PD-98059-treated OVXE2 rats, which subsequently received ethanol or saline, were similar (Fig. 7). Furthermore, the increased ROS generation caused by ethanol in cardiac tissues disappeared in OVXE2 rats pretreated with PD-98059 (Fig. 7D).
and support a causal role for these molecular events in the estrogen-dependent myocardial depressant effect of ethanol. Third, acute estrogen mitigated the significant reduction in catalase activity and higher oxidative stress (ROS) in the myocardium of OVX rats; however, the estrogen-dependent increase in this antioxidant enzyme activity might have contributed to the deleterious effects of ethanol because catalase catalyzes ethanol metabolism to the cardiotoxic aldehyde, acetaldehyde.

LV dysfunction is caused by acute ethanol administration in both experimental (28, 31, 48) and clinical settings (6). Although the precise mechanism of ethanol-evoked myocardial dysfunction remains unclear, recent findings from our laboratory implicated myocardial PI3K/Akt/endothelial NOS and inducible NOS in this deleterious effect (19). However, the latter studies were conducted in proestrus rats and were based on indirect measurements of LV function. In the current study, we provided the first evidence that links acutely administered estrogen to ethanol-evoked impairment of directly measured LV function. Both LV dP/dt max and LVDP, measures of LV performance, were dose-dependently reduced by ethanol in rats that exhibited physiological or supraphysiological estrogen levels (SO and OVXE2 rats) in contrast to no effect in estrogen-depleted rats (OVX). These findings highlight a critical role for estrogen in the compromised LV function elicited by ethanol in female rats (Fig. 2) and the estrogen-dependent reductions in cardiac output and stroke volume caused by ethanol in previous studies (11, 14). Notably, unlike previous studies (11, 14), an acute regimen of estrogen was used in the current study, which would suggest the involvement of estrogen nongenomic mechanisms in ethanol-evoked myocardial responses. Furthermore, the doses of ethanol used in the present study produced blood ethanol concentrations comparable to those attained in humans following consumption of mild to moderate amounts of ethanol (1, 24).

Oxidative damage caused by NADPH oxidase and subsequent generation of ROS and lipid peroxides have been correlated with a number of disease states including cardiac contractile dysfunction (28, 47). MDA and 4-HNE are among the most abundant and reactive aldehyde by-products of lipid peroxidation. The interaction of ROS with lipid cell membranes acts to disrupt protein function via interacting with amino acids such as histidine (2, 38). Therefore, one primary goal of the present study was to test the postulate that ROS generation and lipid peroxidation contribute to the estrogen dependence of ethanol-evoked and LV dysfunction and hypo-
tension in female rats. Our data showed that ethanol caused dose-dependent increases in ventricular ROS levels in SO rats that were coupled with enhanced protein expression of 4-HNE and MDA adducts. These oxidative effects of ethanol were estrogen specific, because they were not evident in ventricular tissues of OVX rats and were restored to SO levels when OVX rats were pretreated with intravenous E2. Thus the current data favor the novel hypothesis that ventricular oxidative insult caused by ROS and lipid aldehydes underlies the estrogen-dependent hypotensive and myocardial dysfunction caused by ethanol in female rats.

The nature of downstream cellular targets for ROS and lipid aldehydes in myocardial dysfunction, especially in the female population, remains poorly defined. One potential candidate is the redox-sensitive mitogen-activated protein kinases, including ERK1/2 (34). Studies have shown that the upregulation of kinases causes the formation of lipid aldehydes such as MDA (7) and 4-HNE (25). Furthermore, increased Akt/ERK activity following ROS generation correlates with cardiac dysfunction associated with ischemia-reperfusion injury (4). Consistent with these studies, we presently report the interesting observation that oxidative stress caused by ethanol was paralleled with enhanced Akt/ERK1/2 phosphorylation that was 1) proportional to the dose of ethanol and 2) estrogen specific, because it was evident in ventricular tissues obtained from rats with intact ovaries (SO) and OVX rats pretreated with acute E2 (OVX E2) but not in estrogen-depleted rats (OVX). These findings are bolstered by the pharmacological findings that ERK1/2 inhibition (PD-98059) abrogated the deleterious ethanol effects on the BP, LV, and ROS generation (Fig. 7). Collectively, our data support the postulate that the facilitation by ethanol of the oxidative cascade of the ROS/lipid peroxidation/Akt/ERK1/2 signaling contributes, at least in part, to the estrogen dependence of the myocardial depressant effect of ethanol. It is imperative to note that ethanol activation of the PI3K/Akt pathway, within minutes of E2 administration, most likely involved rapid/nongenomic mechanisms. This notion is supported by recent evidence that E2, administered within a similar time frame, lowered BP by triggering nontranscriptional activation of PI3K/Akt signaling (45).

Our findings on the individual and combined effects of ethanol and E2 on cardiac catalase activity deserve two comments. First, we have shown that acute administration of E2 alone reduced the LV ROS level (Fig. 3D) and increased catalase activity (Fig. 6) in OVX rats. These findings are consistent with a role for nongenomic mechanisms in the cardiovascular protective effect of acute E2, because acute E2 1) produced similar beneficial effects on the redox state in female aortas and cardiac tissues (3, 7) and 2) reversed the elevated cytokine production in cardiac myocytes of aged OVX (43). Furthermore, the increases in cardiac catalase activity in ethanol-treated SO or OVX E2 rats could plausibly be a feedback response to the deteriorated oxidative state observed in these rats. Such compensatory enhancement of catalase activity was apparently insufficient to offset the ethanol-evoked increases in cardiac oxidative damage. Second, it is also likely that the increased myocardial catalase activity might have exacerbated the oxidative stress rather than mitigated it. This counterintuitive contribution of the antioxidant enzyme could be mediated via catalase catalysis of ethanol metabolism to acetaldehyde (39). Accumulating evidence suggests that at least some of the neurobiological effects of ethanol are mediated via its oxidative product acetaldehyde. For example, acetaldehyde contributes to functional and oxidative disturbances evoked by acute ethanol in cardiac myocytes (29). We have also reported that local production of acetaldehyde, due to enhanced catalase activity, in the brain stem rostral ventrolateral medullary area mediates the pressor response elicited by ethanol (13). Although the heart exhibits lower catalase activity than other organs (25), local generation of the pro-oxidant metabolite acetaldehyde might have contributed to the LV oxidative stress and dysfunction caused by ethanol in rats with physiological E2 levels. Importantly, our findings lend credence to this conclusion, because acute ethanol treatment substantially enhanced myocardial catalase activity (Fig. 6) and restored LV oxidative stress and dysfunction in OVX rats to levels comparable to those observed in SO rats (Figs. 2–5).

Our data provide important insights into the chain of cellular oxidative processes that mediate the estrogen- and dose-dependent hypotension and LV dysfunction caused by acute ethanol in conscious female rats. The pro-oxidant effect of ethanol was evidenced by the enhanced generation of ROS along with increased protein expression of MDA and 4-HNE adducts and Akt/ERK1/2 phosphorylation in ventricular tissues. The presence of estrogen was essential for all the effects of ethanol to manifest. Clinically, this detrimental interaction between ethanol and nongenomic mechanisms triggered by estrogen could explain, at least in part, a worrying trend of increasing alcohol-related deaths in young women reported in a very recent study (41). Equally important, knowledge gained from this study might help, as suggested by others (34), in the development of pharmacological interventions to mitigate the ethanol-evoked myocardial oxidative stress and dysfunction in the presence of estrogen.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.M.E.-M. analyzed data; M.M.E.-M. and A.A.A.-R. interpreted results of experiments; M.M.E.-M. prepared figures; M.M.E.-M. drafted manuscript; A.A.A.-R. conception and design of research; A.A.A.-R. edited and revised manuscript; M.M.E.-M. and A.A.A.-R. approved final version of manuscript.

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