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Oxidative stress and autonomic dysregulation contribute to the acute time-dependent myocardial depressant effect of ethanol in conscious female rats

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Abstract

BACKGROUND—The molecular mechanisms of the acute hypotensive and indirectly assessed cardiac depressant effect of ethanol-evoked myocardial depression and hypotension in female rats are not known. We tested the hypothesis that a time-dependent myocardial depression caused by ethanol is initiated by its direct and indirect (cardiac vagal dominance) effects, and is exacerbated by gradual development of oxidative stress.

METHODS—In conscious female rats, we directly measured left ventricular developed pressure (LVDP), the maximal rise of ventricular pressure/time ($dP/dt_{\max}$), blood pressure (BP), heart rate (HR) and sympathovagal activity following intragastric (i.g.) ethanol (1 g/kg) or water over 90 min. Catalytic activity of acetaldehyde (ACA)-generating (alcohol dehydrogenase, ADH, and catalase) and eliminating (ALDH2) enzymes along with mediators of oxidative stress were measured in myocardial tissues collected at 30, 60 or 90 min after ethanol or water.

RESULTS—Ethanol reduced myocardial function (LVDP and $dP/dt_{\max}$) within 5–10 min before the steady fall in BP in conscious proestrus rats. Further, ethanol shifted the sympathovagal balance, analyzed by spectral analysis of high-frequency (HF α) and low-frequency (LF α) of interbeat intervals, toward vagal dominance. Prior vagal blockade (atropine) or antioxidant (tempol) treatment attenuated ethanol-evoked myocardial depression and hypotension. Ex vivo studies revealed time-dependent: (i) enhancement of ADH but not ALDH2 activity (indicative of elevated ACA levels), (ii) increases in phosphorylated Akt and ERK1/2, NADPH-oxidase activity, reactive oxygen species (ROS), malondialdehyde and 4-hydroxy-2-nonenal-modified proteins. These molecular responses along with reduced myocardial catalase activity were most evident at 90 min post-ethanol when the reductions in cardiac function and BP reached their nadir.

CONCLUSIONS—Vagal dominance and time-dependent myocardial oxidative stress along with the accumulation of cardiotoxic aldehydes mediate ethanol-evoked myocardial dysfunction and hypotension in conscious proestrus female rats.

Keywords

Ethanol; ROS; Hypotension; Female Rats; Myocardial Dysfunction

Introduction

We have previously reported that ethanol causes acute reductions in cardiac output and blood pressure (BP) in proestrus female but not in male or ovariectomized rats (El-Mas and Abdel-Rahman, 1999b, El-Mas and Abdel-Rahman, 1999a). These findings are clinically relevant because ethanol lowers BP in young but not in older women (Klatsky, 1990). However, the reported ethanol-evoked myocardial dysfunction in female rats was based on indirect measures of cardiac function (cardiac output/stroke volume). Notably, recent advances in cardiovascular research have facilitated measurements of LVDP and $dP/dt_{\max}$ as direct indices of cardiac contractility (Zhao et al., 2012, Lieber et al., 2008, Wang et al., 2004) in conscious animals along with assessment of sympathovagal control of the heart. Data based on the latter implicated cardiac vagal dominance in the E_2 -dependent chronic hypotensive effect of ethanol in female rats (El-Mas and Abdel-Rahman, 2012b, El-Mas and Abdel-Rahman, 2009). However, it is not known if acute ethanol similarly influences the sympathovagal control of heart rate in proestrus female rats, and the mechanisms of the myocardial depressant effect of alcohol are not fully understood. Our findings implicated myocardial phosphatidylinositol 3-kinase (PI3K)/Akt-neuronal nitric oxide synthase/nitric oxide (nNOS/NO) signaling in these effects (El-Mas et al., 2009). However, it is not known if ethanol activation of PI3K/Akt signaling pathway, including its downstream kinases e.g. ERK1/2, is temporally related to the hemodynamic responses. This is important because pharmacologic inhibition of the PI3K/Akt or NOS-NO signaling does not fully abrogate ethanol-evoked reduction in cardiac output (El-Mas et al., 2009), which inferred the involvement of additional molecular mechanism(s) in ethanol-evoked myocardial effects.

Ethanol is metabolized via ADH and catalase to ACA, which is eliminated by ALDH2 (El-Mas and Abdel-Rahman, 2012a, Quertemont et al., 2005, Zimatkin et al., 2006). ACA plays crucial role in ethanol evoked cellular responses and in myocardial dysfunction in male animals (Duan et al., 2003, Ren et al., 1997). Realizing that: (i) ACA causes oxidative stress (Duan et al., 2003, Haorah et al., 2008, Oei et al., 1986), and (ii) E_2 enhances ethanol metabolism to ACA (Jeavons and Zeiner, 1984), it is likely that greater ACA accumulation in the female myocardium accounts for the exacerbated ethanol-evoked myocardial dysfunction in proestrus rats (highest endogenous E_2 level). At the molecular level, ACA increases ROS production via multiple signaling pathways that lead to NADPH-oxidase (Nox) activation, at least partly, via the PI3K/Akt-ERK1/2 signaling cascade (Baumer et al., 2008, Sheu et al., 2004, Du et al., 2011, Zhou et al., 2011, Zhang et al., 2006, Li et al., 2006).

In this study, we hypothesized that time-dependent oxidative stress, due to imbalance between ACA production and elimination within the myocardium, accounts for the acute ethanol-evoked myocardial dysfunction in female rats. We investigated the effects of intragastric (i.g.) ethanol (1 g/kg), compared with water, on directly measured cardiac function along with BP, heart rate (HR), and sympathovagal activity (spectral analysis) for 90 min. Thereafter, the hearts were collected from these rats and at two other time points (30 and 60 min) after ethanol or water for ex vivo measurements of myocardial ADH, catalase, ALDH2 along with the oxidative stress mediators Akt and ERK1/2. Finally, we measured

myocardial levels of the cardiotoxic molecule malondialdehyde (MDA) (Del Rio et al., 2005) and 4-hydroxy-2-nonenal (4-HNE)-modified proteins (Haorah et al., 2008, Musicki et al., 2010) known to be generated following ethanol administration. Finally, we investigated the impact of pharmacologic vagal blockade (atropine) or antioxidant therapy (tempol) on ethanol-evoked myocardial depression and myocardial oxidative stress. The integrative studies were conducted in conscious proestrus rats, an established model for ethanol-evoked hypotension (El-Mas and Abdel-Rahman, 1999a), to avoid the confounding effects of anesthesia on cardiac contractility (Wang et al., 2004).

Material and Methods

Animals

Female Sprague-Dawley rats (200–250 g, Charles River, Raleigh, NC) were housed in a room with controlled environment at a constant temperature of $23 \pm 1^\circ\text{C}$, humidity of $50\% \pm 10\%$ and a 12:12-h light/dark cycle. Food (Prolab Rodent Chow, Granville Milling, Creedmoor, NC) and water were provided ad libitum. All surgical, experimental, and animal care procedures were performed in accordance with, and approved by, the Institutional Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition (2011), The National Academies Press, Washington, D.C.

Arterial, Left Ventricular and Nasogastric Cannulations

These surgeries were performed under sterile conditions and induction of anesthesia with ketamine (9 mg/100 g) and xylazine (1 mg/100 g, i.p.). For measurement of arterial pressure, a catheter was placed into the abdominal aorta via the left femoral artery, and nasogastric catheterization was performed by inserting 20 cm PE50 tubing into the stomach through a nostril as detailed in our previous study (El-Mas et al., 2009). For cardiac function measurements, the tip of a fluid-filled 8 cm PE50 catheter connected to Gould-Statham pressure transducers (Oxnard, CA) was inserted into the left ventricle (LV) via the right carotid artery (Zhao et al., 2012, Wang et al., 2004), then secured in place by suturing to adjacent muscle. Optimal placement of the LV catheter was verified by obtaining typical ventricular pressure waveforms (see Fig. 1). Finally, the catheters were tunneled subcutaneously and exteriorized at the back of the neck between the scapulae. Vascular and cardiac catheters were flushed with heparin (100 U/ml), and plugged with stainless steel pins. Incisions were closed by surgical clips and swabbed with povidone-iodine solution. Each rat received intramuscular injections of the analgesic buprenorphine hydrochloride (Buprenex; 30 $\mu\text{g/kg}$, Rickitt & Colman, Richmond, VA) and penicillin G benzathine and penicillin G procaine in an aqueous suspension (100,000 U/ kg, Durapen; Vedco, Inc., Overland Park, KS) and was housed in a separate cage. The experiments were performed 2 days later in the conscious freely moving animals.

Hemodynamic measurements

On the day of the experiment, the arterial and cardiac catheters were flushed with heparinized saline (100 IU/ml) and connected to Gould-Statham pressure transducers (Oxnard, CA). Measurements of BP and HR were conducted in conscious unrestrained rats

as in our previous studies (Ibrahim and Abdel-Rahman, 2012) and LV function as described by others (Zhao et al., 2012, Wang et al., 2004). All cardiovascular variables were recorded by ML870 (PowerLab 8/30), and analyzed using LabChart 7 software (ADInstruments, Colorado Spring, CO).

Frequency Domain Analysis

Frequency domain analysis was conducted as reported (Parati et al., 1995) using software designed for rats (Nevrokard SA-BRS; Izola, Slovenia). RRI power spectral densities oscillations were computed by 512-point fast Fourier transform and integrated over the specified frequency range (LF, 0.25–0.75 Hz; HF, 0.75–5.0 Hz) as detailed in our previous studies (Shaltout and Abdel-Rahman, 2005) and by others (Parati et al., 1995). The changes in the powers of RRI spectra in the LF and HF range (LF_{RRI} and HF_{RRI}) before (baseline) and after treatments were calculated in normalized units. The LF_{RRI}/HF_{RRI} ratio, a measure of sympathovagal balance, was also determined (Laitinen et al., 1999).

Alcohol dehydrogenase (ADH), catalase and ALDH2 activities assay

Commercially available colorimetric assay kits from biomedical Research Services center (University of the State of New York at Buffalo, Buffalo, NY) and Sigma-Aldrich (Saint Louis, MO) were used for these measurements. Catalytic enzyme activity was determined in equal amounts of protein extracted from ventricular tissues according to the manufacturer protocol and our recent study (El-Mas and Abdel-Rahman, 2012a). The ADH activity in our samples was derived from a calibration curve constructed using pure ADH (Sigma-Aldrich; Saint Louis, MO). ALDH2 (mitochondrial ALDH) activity was measured by a modified protocol reported in previous studies (Yoon et al., 2006) where sub-cellular fractions were prepared from ventricular tissues to isolate the mitochondrial portion.

NADPH oxidase activity assay

NADPH oxidase activity was determined by fluorescence assay using Amplex Red (Molecular Probes, OR). Briefly, 50 µg protein/sample, extracted from ventricular tissues of ethanol or water treated animals, were incubated with Amplex Red (10 µM), horseradish peroxidase (300 U/ml), superoxide dismutase (7500 U/ml) and NADPH (10 mM) in Krebs's HEPES buffer, pH 7.4. The fluorescence intensities were measured with Fluoromax-3 spectrofluorometer (Horiba Jobin Yovin, Edison, NY) at excitation 530 nm and emission 590 nm in a 96-well fluoroplate. Measurements were made every 2 min for 10 min and compared to a calibration curve constructed by varying concentrations of hydrogen peroxide.

Measurement of Reactive Oxygen Species (ROS)

Myocardial ROS levels were measured by a fluorometric assay using 2',7'-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Inc., Eugene, OR). Briefly, DCFH-DA (25 µM) was added to 250 µg protein/sample extracted from ventricular tissues of ethanol-treated and control animals in PBS. The fluorescence intensities were measured with Fluoromax-3 spectrofluorometer at 485 nm and emission 530 nm at 37°C in a 96-well fluoroplate every 5 min for 30 min as reported (McGee and Abdel-Rahman, 2012).

Western blotting

Modified protocols from our recent studies (Ibrahim and Abdel-Rahman, 2012) were followed for measurements of phosphorylated Akt (pAkt), ERK1/2 (pERK1/2) and the levels of MDA, and 4-HNE-modified proteins in the myocardium. Protein extracts from rat ventricle (80 µg per lane) were run on a 4–12% SDS-PAGE gel (Invitrogen, CA) and electroblotted to nitrocellulose membranes. Blots were blocked for 120 min at room temperature in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) and incubated overnight at 4°C with a mixture of monoclonal anti-phospho-Akt antibody (pSer473) (1:1000) and rabbit polyclonal anti-Akt antibody (1:1000) or mouse monoclonal antiphospho-ERK1/2 (T202/Y204) antibody or antibodies against malondialdehyde MDA (1:150), 4-HNE-modified proteins (1:1000), and beta actin (1:5000) in blocking buffer. Antibodies for Akt, ERK1/2 and pERK1/2 were from Cell Signaling Technology, Inc. (Danvers, MA) and anti-pAkt was from BD Transduction Laboratories. MDA antibody was from Academy Bio-Medical Company, Inc. (Houston, TX), anti-4-HNE was from Calbiochem (Darmstadt, Germany) and anti-beta actin was from Abcam Inc. (Cambridge, MA). It is important to note that the anti-4-HNE antibody recognizes all proteins that form complex adduct with 4-HNE (Tanito et al., 2006, Anderson et al., 2009). Analysis of the amount of 4-HNE-modified proteins included the averaged densitometric ratio of all proteins in each lane normalized to beta actin. After 3 washes with phosphate-buffered saline containing 0.1% Tween 20 (PBS-T), the blots were incubated for 50 min at room temperature with goat anti-mouse secondary antibody IRDye® 800CW or goat anti-rabbit secondary antibody IRDye® 680, respectively (LI-COR Biosciences, Lincoln, NE). After 3 washes with PBS-T buffer, the blots were detected by Odyssey Infrared Scanning System. Protein bands were quantified by integrated intensities using Odyssey Infrared Imaging Software.

Statistical analysis

Changes in MAP, heart rate, LVDP, $dP/dt_{\max}$ and Tau from their respective baseline, following ethanol or water, are expressed as mean $\pm$ S.E.M. Data were analyzed by repeated measures ANOVA using SPSS 16.0 statistical package for Windows®, for differences in time and treatment trends followed by a one way ANOVA to assess individual differences at different time points among the treatment and control groups. Tukey's (equal variance) and Games Howell (unequal variance) tests were used for post hoc analysis. Western blot and enzymatic activity data for each protein were expressed as percent of control (water) value, and analyzed by unpaired t-test using GraphPad Prism V5 for Windows®. $P < 0.05$ was considered significant.

Results

Ethanol depresses myocardial function and enhances cardiac vagal dominance

We investigated the acute myocardial effects of ethanol in freely moving female rats by directly measuring the contractility indices LVDP, $dP/dt_{\max}$ and the cardiac isovolumic relaxation constant Tau along with BP and HR. A total of 48 proestrus rats were divided into 3 ethanol and 3 water groups ($n = 6-8$). At least 30 min was allowed at the beginning of the experiment for hemodynamic stabilization. Ethanol (1g/kg; 10 ml/kg of 13% ethanol diluted

in water) or equal volume of water was administered i.g. as in our previous studies (El-Mas et al., 2009). In one of the ethanol groups, and its control, hemodynamic measurements were continued for 90 min after which the animals were euthanized by pentobarbital overdose (100 mg/kg). The two other ethanol and their corresponding control groups were sacrificed at 30 and 60 min after i.g. ethanol or water. The hearts were collected for conducting the ex vivo studies (see below). To investigate the effect of ethanol on cardiac autonomic control, offline spectral analyses (sympathovagal balance) were calculated by the frequency domain analysis.

The BP, HR, and the cardiac contractility indices (LVDP, $dP/dt_{\max}$ and Tau) obtained, in freely moving proestrus female rats, before ethanol or water administration were not significantly different (Tables 1). These values are consistent with reported values in conscious rats (Lieber et al., 2008, Zhao et al., 2012). In proestrus rats, acute ethanol (1 g/kg, i.g.), compared to water, caused slowly developing reductions in MAP (Figs. 1A) without noticeable effects on HR (Fig. 1B). The hypotensive response was significant ($P < 0.05$) at 40 min following ethanol, reached its nadir at 70 min, and lasted till the experiment ended at 90 min. However, significant ($P < 0.05$) reductions in myocardial contractility indices (LVDP and $dP/dt_{\max}$) started 10 min after ethanol, which preceded the hypotensive response, and continued thereafter till the end of the experiment (Figs. 1C, D and F). Finally, the exponential time constant of cardiac isovolumic relaxation (Tau), which reflects the lusitropic state of the heart, was significantly ($P < 0.05$) higher in ethanol, compared to water, treated rats (Fig. 1E); basal values in the two groups were similar (31 ± 7 vs. 24 ± 3 ms). The high-frequency (HF α) component of spectral analysis of RRI, which reflects the cardiac vagal control, was significantly ($P < 0.05$) higher in ethanol-treated rats starting at 20 min and continued throughout the experiment (Fig. 2A). However, the spectral measure of sympathetic activity, low-frequency (LF α) component of RRI, was not affected by ethanol (Fig. 2B). This resulted in net decreases in the sympathovagal (LF $_{\text{RRI}}$ /HF $_{\text{RRI}}$) ratio (Fig. 2C), which reflects vagal dominance.

Biochemical effects of acute ethanol in proestrus rats

To elucidate the role of pro-oxidant and antioxidant mechanisms in the ethanol-evoked myocardial dysfunction, we measured the catalytic activities of ACA generating (ADH and catalase) and eliminating (ALDH2) enzymes in cardiac tissues collected at 30, 60 or 90 min. Further, we measured, in the same cardiac tissues, Nox activity (Chen et al., 2008, Roe et al., 2011, Schulz et al., 2008), phosphorylated Akt and ERK1/2 (Sheu et al., 2004, Turdi et al., 2007, El-Mas et al., 2009, Zhou et al., 2011), and myocardial levels of the cardiotoxic aldehyde MDA and 4-HNE protein adducts (Haorah et al., 2008, Del Rio et al., 2005). Myocardial ADH activity was enhanced ($P < 0.05$) in ethanol treated rats at 60 and 90 min (Fig. 3A) while catalase activity was significantly ($P < 0.05$) reduced at 90 min (Fig. 3B). Myocardial ALDH2 activity was not significantly altered at 30 or 60 min, but increased at 90 min in ethanol-treated rats (Fig. 3C); the increase in ALDH2 at 90 approached, but did not achieve, statistical significance (Fig. 3C).

Acute ethanol enhances Akt and ERK1/2 phosphorylation and increases levels of cytotoxic aldehydes in the myocardium of proestrus rats

As shown in Fig. 4, levels of phosphorylated Akt (pAkt) (Western blotting) were significantly ($P < 0.05$) higher in myocardial tissues of ethanol treated animals at 30, 60 and 90 min. Phosphorylated ERK1/2 (pERK1/2) levels were significantly ($P < 0.05$) higher at 60 and 90 min following ethanol treatment (Fig. 4). Additionally, the levels of the cardiotoxic MDA and 4-HNE-modified proteins were significantly ($P < 0.05$) higher at 60 and 90 min in cardiac tissues of ethanol-treated female rats (Fig. 5).

Ethanol causes time-dependent enhancement of myocardial oxidative stress

There were no statistical differences between the rates of ROS production or Nox activity at 30 (data not shown) or at 60 min between ethanol and water treated groups (Fig. 6).

However, the rate of myocardial ROS production and Nox activity were significantly ($P < 0.05$) higher in ethanol-treated rats, than the corresponding control (water), values at 90 min (Fig. 6).

Vagal blockade or antioxidant treatment abrogates ethanol-evoked myocardial dysfunction

Four groups of rats were used to investigate the role of increased vagal tone or myocardial ROS in ethanol-evoked cardiac depression. Two groups of rats received atropine (5 mg/kg) 20 min before ethanol (1 g/kg) or equal volume of water, and the other two groups of rats received tempol (20 mg/kg) 10 min before ethanol or water. The doses and pretreatment time period are based on reported studies (Da Silva et al., 2012, Rashid et al., 2012). Hemodynamic measurements continued for 90 min after which the animals were euthanized.

Pretreatment with atropine significantly ($P < 0.05$) increased myocardial ROS level (Fig. 8) in the water treated group. Further, pretreatment with atropine or tempol significantly ($P < 0.05$) attenuated the reductions in MAP and myocardial function indices (Fig. 7), and abrogated the elevation of myocardial ROS production (Fig. 8) caused by ethanol.

Discussion

The present study is the first to elucidate the temporal relationship between directly measured myocardial function and the molecular events caused by acute ethanol in proestrus rats. The following are the most important findings of the present study: (i) vagal dominance contributed to the early ethanol-evoked reduction in LVDP, $dP/dt_{\max}$ and BP; (ii) enhancement of myocardial ADH, but not ALDH2 activity, at 60 and 90 min, reflected myocardial ACA accumulation; (iii) ethanol/ACA enhanced phosphorylation of myocardial Akt and ERK1/2 and subsequently Nox activation (high ROS level); (iv) myocardial oxidative stress coupled with reduced catalase activity, and elevated levels of cytotoxic aldehydes (MDA and 4-HNE-modified proteins) were evident during ethanol-evoked myocardial depression; (v) atropine (vagal blockade) or tempol (anti-oxidant) pretreatment abrogated ethanol-evoked myocardial dysfunction and hypotension and myocardial oxidative stress. Together, the integrative and ex vivo biochemical findings identified time-dependent molecular mechanisms for the acute myocardial depression caused by ethanol in proestrus rats.

We directly investigated, for the first time, the acute effects of a moderate dose of ethanol on myocardial function in conscious proestrus rats. We chose this animal model because it exhibits the highest levels of circulating E_2 and manifests the E_2 -dependent reductions in cardiac output and BP caused by the same dose of ethanol (El-Mas et al., 2009, El-Mas and Abdel-Rahman, 1999a). It is important to note that the technique used in our present study for the direct LVDP and $dP/dt_{\max}$ measurements has been verified in recent studies in conscious freely moving rats and mice (Zhao et al., 2012, Wang et al., 2004). The basal values of these two cardiac indices are very similar to the corresponding values in the reported studies. Further verification of the technique using graded doses of isoproterenol produced the expected dose-related increases in $dP/dt_{\max}$ (data not shown).

Our experimental approach permitted the unequivocal conclusion that the reductions in myocardial contractility indices, LVDP and $dP/dt_{\max}$ were temporally (causally) linked to the ethanol-evoked hypotension in conscious proestrus rats (Fig. 1). Additionally, ethanol caused short-lived prolongation of Tau (Fig. 1), a measure of the left ventricular isovolumic relaxation constant (Weiss et al., 1976, Frederiksen et al., 1978). Notably, the time-course and the magnitude of the hypotensive response caused by i.g. ethanol (1 g/kg) in this study, fully agree with similar findings in proestrus rats (El-Mas et al., 2009) as well as in ovariectomized rats pretreated with E_2 (El-Mas and Abdel-Rahman, 1999a). The power spectral analysis of hemodynamic variability, which revealed ethanol-evoked increase in the high (HF α), but not the low (LF α) frequency component of RRI, is consistent with a shift in the sympathovagal balance towards vagal dominance (Laitinen et al., 1999). These findings suggest that enhanced vagal dominance is implicated in the chronic cardiodepressant effect of ethanol in female rats (El-Mas et al., 2009, El-Mas and Abdel-Rahman, 2012b), and occurs within minutes following acute ethanol administration (Fig. 2). Equally important, the abrogation of ethanol-evoked myocardial depression and hypotension by atropine (Fig. 7) supports a causal role for the enhanced vagal dominance in ethanol-evoked hemodynamic effects. Another important finding was the atropine abrogation of ethanol-evoked elevation in myocardial ROS (Fig. 8). Importantly, atropine caused significant elevation in myocardial ROS, which might have dampened the impact of ethanol on myocardial ROS (Fig. 8). Whether the higher atropine dose (5 mg/kg), which was based on reported studies (Da Silva et al., 2012) to ensure vagal blockade, contributed to ROS elevation in our model system remains to be investigated. On the other hand, our new finding on atropine-evoked elevation in myocardial ROS is consistent with antioxidant effect of vagal stimulation and its abrogation by atropine in ischemia-reperfusion injury model (Kong et al., 2012). Importantly, differences in the methods of induction of vagal activation, and the resultant sympathovagal balance in the two model systems, and the use of anesthesia in the reported study must be taken into consideration when interpreting our findings. More studies are warranted to elucidate the mechanistic role of the vagal tone in the cellular and cardiodepressant effect of ethanol. Nonetheless, the present findings provide direct evidence for the involvement of vagal dominance in the myocardial depressant effect of a moderate dose of ethanol in proestrus female rats. Another major objective of the study was to investigate the molecular mechanisms of the myocardial dysfunction caused by ethanol in our model system.

We hypothesized that the acute ethanol-evoked myocardial dysfunction in female rats is mediated, at least partly, by the accumulation of locally formed ACA within the myocardium due to a mismatch between its production and elimination. Notably, measurement of myocardial ACA level, which has been reported (Duan et al., 2002) was rather challenging. An alternative approach to address this limitation was the measurement of the myocardial catalytic activity of ACA generating (ADH and catalase) and eliminating (ALDH2) enzymes. This approach was employed in our recent studies in neuronal tissues (El-Mas and Abdel-Rahman, 2012a). The myocardium of ethanol-treated rats exhibited higher ADH, but not ALDH, activity (Fig. 3) reflecting higher myocardial ACA levels at 60 and 90 min after ethanol administration. These biochemical findings coincided with the significant reductions in LVP, $dP/dt_{\max}$, and BP (Fig. 1), and occurred at times when blood ethanol concentrations were declining in the same model system (El-Mas et al., 2009). Further, a delayed (90 min) reduction in catalase activity, which serves antioxidant role (Ren et al., 2007, Li et al., 2007, Turdi et al., 2007), is expected to exacerbate the myocardial oxidative stress. Equally important, the lack of increase in myocardial ALDH2 activity at 30 and 60 min might underlie the accumulation of cardiotoxic MDA and 4-HNE-modified proteins (Fig 5). ALDH2 serves a detoxifying role against ACA as well as these cardiotoxic aldehydes (Budass et al., 2010, Del Rio et al., 2005, Li et al., 2006).

Our conclusion that ethanol-evoked myocardial depression in proestrus rats is mediated, at least partly, via ACA is supported by experimental and clinical findings. First, ACA plays a crucial role in the higher female propensity to alcohol induced myocardial dysfunction (Ren et al., 1997, Urbano-Marquez et al., 1995, Duan et al., 2003, Duan et al., 2002). Second, compared to men, higher ACA levels are achieved in premenopausal women and in women taking oral contraceptives following the consumption of similar amounts of ethanol (Eriksson et al., 1996). Third, ADH overexpression (overproduction of ACA) accelerated cardiomyopathy more in female, than in male, cardiac myocytes (Liang et al., 1999, Duan et al., 2002, Duan et al., 2003). Notably, none of these reported studies linked the molecular events in the myocardium to the ethanol-evoked myocardial dysfunction at the integrative level. We reasoned that myocardial ACA accumulation triggers molecular events conducive to the generation of oxidative stress. In support of this notion, myocardial Nox-mediated oxidative stress was evident at 90 min in ethanol-treated rats (Fig. 6). Further, the antioxidant tempol abrogated myocardial oxidative stress (Fig. 8) and preserved myocardial function (Fig. 7) in ethanol-treated rats. These results are consistent with ACA mediation of ethanol-evoked oxidative stress and myocardial dysfunction in vitro (Oei et al., 1986, Duan et al., 2003). Our conclusion gains further credence from the time-dependent increases in Akt and ERK1/2 phosphorylation and Nox activation in ethanol-treated rats (Figs. 4 and 6). These biochemical findings are consistent with (i) ethanol-derived ACA activation of Nox (Oei et al., 1986, Duan et al., 2003, Haorah et al., 2008) and ERK1/2 (Li et al., 2006) in different tissues, and (ii) the PI3K/Akt mediation of Nox activation (ROS production) in macrophages (Wang et al., 2007). Together, these findings support and extend our earlier findings, which implicated PI3K/Akt activation in the acute ethanol-evoked reductions in cardiac output and BP in proestrus female rats (El-Mas et al., 2011, El-Mas et al., 2009). Importantly, in the latter study, the molecular responses were only measured at 90 min, and cardiac function was indirectly measured. Equally important, p-Akt mediates ROS

generation via eNOS uncoupling (Roe et al., 2011, Karuppiyah et al., 2011, Chen et al., 2008), which could be exacerbated by the accumulation of the cardiotoxic aldehyde MDA and 4-HNE adducts (Fig. 5). Finally, the higher MDA level, observed in ethanol-treated animals (Fig. 5), is generated via peroxidation of polyunsaturated fatty acids (Del Rio et al., 2005), and reflects eNOS uncoupling (Xia et al., 2010). Further studies are needed to investigate this possibility in our model system.

In conclusion, this study is the first to demonstrate the direct myocardial depressant effect of acute ethanol, and to elucidate the time-dependent molecular mechanisms for this effect in proestrus female rats. The findings are clinically relevant because the deleterious cardiac effects were produced by a dose of ethanol that leads to blood ethanol concentrations compatible with those achieved following social drinking. The observed biochemical and molecular responses, which encompassed ACA accumulation and inhibition of myocardial catalase activity, paralleled the drastic ethanol-evoked reductions in cardiac function and BP in ethanol treated rats. The findings also yield new insight into the role of cardiac vagal activation and oxidative stress in the acute ethanol-evoked myocardial dysfunction in female rats. Finally, the present findings might help identify pharmacologic interventions to ameliorate the ethanol-evoked cardiovascular derangements in general and in females in particular.

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References

- Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neuffer PD. Substrate-Specific Derangements in Mitochondrial Metabolism and Redox Balance in the Atrium of the Type 2 Diabetic Human Heart. *Journal of the American College of Cardiology*. 2009; 54:1891–1898. [PubMed: 19892241]
- Baumer AT, Ten Freyhaus H, Sauer H, Wartenberg M, Kappert K, Schnabel P, Konkol C, Hescheler J, Vantler M, Rosenkranz S. Phosphatidylinositol 3-kinase-dependent membrane recruitment of Rac-1 and p47phox is critical for alpha-platelet-derived growth factor receptor-induced production of reactive oxygen species. *Journal of Biological Chemistry*. 2008; 283:7864–7876. [PubMed: 18070887]
- Budas GR, Disatnik MH, Chen CH, Mochly-Rosen D. Activation of aldehyde dehydrogenase 2 (ALDH2) confers cardioprotection in protein kinase C epsilon (PKC ϵ) knockout mice. *J Mol Cell Cardiol*. 2010; 48:757–764. [PubMed: 19913552]
- Chen CA, Druhan LJ, Varadharaj S, Chen YR, Zweier JL. Phosphorylation of endothelial nitric-oxide synthase regulates superoxide generation from the enzyme. *Journal of Biological Chemistry*. 2008; 283:27038–27047. [PubMed: 18622039]
- Da Silva VJ, Gneccchi-Ruscione T, Bellina V, Oliveira M, Maciel L, de Carvalho AC, Salgado HC, Bergamaschi CM, Tobaldini E, Porta A, Montano N. Acute adenosine increases cardiac vagal and reduces sympathetic efferent nerve activities in rats. *Exp Physiol*. 2012; 97:719–729. [PubMed: 22366563]
- Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis*. 2005; 15:316–328. [PubMed: 16054557]

- Du J, Xu R, Hu Z, Tian Y, Zhu Y, Gu L, Zhou L. PI3K and ERK-Induced Rac1 Activation Mediates Hypoxia-Induced HIF-1 α Expression in MCF-7 Breast Cancer Cells. *PLoS ONE*. 2011; 6:e25213. [PubMed: 21980400]
- Duan J, Esberg LB, Ye G, Borgerding AJ, Ren BH, Aberle NS, Epstein PN, Ren J. Influence of gender on ethanol-induced ventricular myocyte contractile depression in transgenic mice with cardiac overexpression of alcohol dehydrogenase. *Comp Biochem Physiol A Mol Integr Physiol*. 2003; 134:607–614. [PubMed: 12600669]
- Duan J, McFadden GE, Borgerding AJ, Norby FL, Ren BH, Ye G, Epstein PN, Ren J. Overexpression of alcohol dehydrogenase exacerbates ethanol-induced contractile defect in cardiac myocytes. *Am J Physiol Heart Circ Physiol*. 2002; 282:H1216–H1222. [PubMed: 11893554]
- El-Mas MM, Abdel-Rahman AA. Estrogen-dependent hypotensive effects of ethanol in conscious female rats. *Alcoholism-Clinical and Experimental Research*. 1999a; 23:624–632.
- El-Mas MM, Abdel-Rahman AA. Sexually dimorphic hemodynamic effects of intragastric ethanol in conscious rats. *Clinical and Experimental Hypertension*. 1999b; 21:1429–1445. [PubMed: 10574422]
- El-Mas MM, Abdel-Rahman AA. Longitudinal assessment of the effects of oestrogen on blood pressure and cardiovascular autonomic activity in female rats. *Clin Exp Pharmacol Physiol*. 2009; 36:1002–1009. [PubMed: 19413598]
- El-Mas MM, Abdel-Rahman AA. Enhanced catabolism to acetaldehyde in rostral ventrolateral medullary neurons accounts for the pressor effect of ethanol in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2012a; 302:H837–H844. [PubMed: 22159996]
- El-Mas MM, Abdel-Rahman AA. Exacerbation of myocardial dysfunction and autonomic imbalance contributes to the estrogen-dependent chronic hypotensive effect of ethanol in female rats. *Eur J Pharmacol*. 2012b; 679:95–100. [PubMed: 22305881]
- El-Mas MM, Fan M, Abdel-Rahman AA. Facilitation of Myocardial PI3K/Akt/nNOS Signaling Contributes to Ethanol-Evoked Hypotension in Female Rats. *Alcoholism: Clinical and Experimental Research*. 2009; 33:1158–1168.
- El-Mas MM, Fan M, Abdel-Rahman AA. Upregulation of cardiac NOS due to endotoxemia and vagal overactivity contributes to the hypotensive effect of chronic ethanol in female rats. *Eur J Pharmacol*. 2011; 650:317–323. [PubMed: 20970417]
- Eriksson CJ, Fukunaga T, Sarkola T, Lindholm H, Ahola L. Estrogen-related acetaldehyde elevation in women during alcohol intoxication. *Alcoholism-Clinical and Experimental Research*. 1996; 20:1192–1195.
- Frederiksen JW, Weiss JL, Weisfeldt ML. Time constant of isovolumic pressure fall: determinants in the working left ventricle. *American Journal of Physiology*. 1978; 235:H701–H706. [PubMed: 736158]
- Haorah J, Ramirez SH, Floreani N, Gorantla S, Morsey B, Persidsky Y. Mechanism of alcohol-induced oxidative stress and neuronal injury. *Free Radic Biol Med*. 2008; 45:1542–1550. [PubMed: 18845238]
- Ibrahim BM, Abdel-Rahman AA. Differential Modulation of Brainstem Phosphatidylinositol 3-Kinase/Akt and Extracellular Signal-Regulated Kinase 1/2 Signaling Underlies WIN55,212-2 Centrally Mediated Pressor Response in Conscious Rats. *Journal of Pharmacology and Experimental Therapeutics*. 2012; 340:11–18. [PubMed: 21946192]
- Jeavons CM, Zeiner AR. Effects of elevated female sex steroids on ethanol and acetaldehyde metabolism in humans. *Alcoholism-Clinical and Experimental Research*. 1984; 8:352–358.
- Karuppiiah K, Druhan LJ, Chen CA, Smith T, Zweier JL, Sessa WC, Cardounel AJ. Suppression of eNOS-derived superoxide by caveolin-1: a biopterin-dependent mechanism. *American Journal of Physiology - Heart & Circulatory Physiology*. 2011; 301:H903–H911. [PubMed: 21724868]
- Klatsky, AL. Blood pressure and alcohol intake, in *Hypertension: Pathophysiology, Diagnosis, and Management*. In: Laragh, JH.; Brenner, BM., editors. *Hypertension: Pathophysiology, Diagnosis, and Management*. Raven Press Ltd: 1990. p. 277-294.
- Kong SS, Liu JJ, Yu XJ, Lu Y, Zang WJ. Protection against Ischemia-Induced Oxidative Stress Conferred by Vagal Stimulation in the Rat Heart: Involvement of the AMPK-PKC Pathway. *Int J Mol Sci*. 2012; 13:14311–14325. [PubMed: 23203066]

- Laitinen T, Hartikainen J, Niskanen L, Geelen G, Lansimies E. Sympathovagal balance is major determinant of short-term blood pressure variability in healthy subjects. *American Journal of Physiology*. 1999; 276:H1245–H1252. [PubMed: 10199849]
- Li Q, Wu S, Li SY, Lopez FL, Du M, Kajstura J, Anversa P, Ren J. Cardiac-specific overexpression of insulin-like growth factor 1 attenuates aging-associated cardiac diastolic contractile dysfunction and protein damage. *Am J Physiol Heart Circ Physiol*. 2007; 292:H1398–H1403. [PubMed: 17085535]
- Li SY, Li Q, Shen JJ, Dong F, Sigmon VK, Liu Y, Ren J. Attenuation of acetaldehyde-induced cell injury by overexpression of aldehyde dehydrogenase-2 (ALDH2) transgene in human cardiac myocytes: role of MAP kinase signaling. *J Mol Cell Cardiol*. 2006; 40:283–294. [PubMed: 16403513]
- Liang Q, Carlson EC, Borgerding AJ, Epstein PN. A transgenic model of acetaldehyde overproduction accelerates alcohol cardiomyopathy. *J Pharmacol Exp Ther*. 1999; 291:766–772. [PubMed: 10525098]
- Lieber SC, Qiu H, Chen L, Shen Y-T, Hong C, Hunter WC, Aubry N, Vatner SF, Vatner DE. Cardiac dysfunction in aging conscious rats: altered cardiac cytoskeletal proteins as a potential mechanism. *American Journal of Physiology - Heart and Circulatory Physiology*. 2008; 295:H860–H866. [PubMed: 18567712]
- McGee M, Abdel-Rahman AA. Enhanced Vascular nNOS-derived NO Production Underlies the Pressor Response Caused by Peripheral NMDA Receptor Activation in Conscious Rats. *J Pharmacol Exp Ther*. 2012
- Musicki B, Liu T, Lagoda GA, Strong TD, Sezen SF, Johnson JM, Burnett AL. Hypercholesterolemia-induced erectile dysfunction: endothelial nitric oxide synthase (eNOS) uncoupling in the mouse penis by NAD(P)H oxidase. *Journal of Sexual Medicine*. 2010; 7:3023–3032. [PubMed: 20626609]
- Oei HH, Zoganas HC, McCord JM, Schaffer SW. Role of acetaldehyde and xanthine oxidase in ethanol-induced oxidative stress. *Res Commun Chem Pathol Pharmacol*. 1986; 51:195–203. [PubMed: 3754347]
- Parati G, Frattola A, Dirienzo M, Castiglioni P, Pedotti A, Mancia G. Effects of Aging on 24-H Dynamic Baroreceptor Control of Heart-Rate in Ambulant Subjects. *American Journal of Physiology-Heart and Circulatory Physiology*. 1995; 268:H1606–H1612.
- Quertemont E, Grant KA, Correa M, Arizzi MN, Salamone JD, Tambour S, Aragon CM, McBride WJ, Rodd ZA, Goldstein A, Zaffaroni A, Li TK, Pisano M, Diana M. The role of acetaldehyde in the central effects of ethanol. *Alcoholism: Clinical & Experimental Research*. 2005; 29:221–234.
- Rashid M, Kotwani A, Fahim M. Long-acting phosphodiesterase 5 inhibitor, tadalafil, and superoxide dismutase mimetic, tempol, protect against acute hypoxia-induced pulmonary hypertension in rats. *Hum Exp Toxicol*. 2012; 31:626–636. [PubMed: 22144729]
- Ren J, Davidoff AJ, Brown RA. Acetaldehyde depresses shortening and intracellular Ca²⁺ transients in adult rat ventricular myocytes. *Cell Mol Biol (Noisy-le-grand)*. 1997; 43:825–834. [PubMed: 9359629]
- Ren J, Li Q, Wu S, Li SY, Babcock SA. Cardiac overexpression of antioxidant catalase attenuates aging-induced cardiomyocyte relaxation dysfunction. *Mech Ageing Dev*. 2007; 128:276–285. [PubMed: 17250874]
- Roe ND, Thomas DP, Ren J. Inhibition of NADPH oxidase alleviates experimental diabetes-induced myocardial contractile dysfunction. *Diabetes, Obesity & Metabolism*. 2011; 13:465–473.
- Schulz E, Jansen T, Wenzel P, Daiber A, Munzel T. Nitric oxide, tetrahydrobiopterin, oxidative stress, and endothelial dysfunction in hypertension. *Antioxidants & Redox Signaling*. 2008; 10:1115–1126. [PubMed: 18321209]
- Shaltout HA, Abdel-Rahman AA. Mechanism of fatty acids induced suppression of cardiovascular reflexes in rats. *J Pharmacol Exp Ther*. 2005; 314:1328–1337. [PubMed: 15937146]
- Sheu ML, Ho FM, Chao KF, Kuo ML, Liu SH. Activation of phosphoinositide 3-kinase in response to high glucose leads to regulation of reactive oxygen species-related nuclear factor-kappaB activation and cyclooxygenase-2 expression in mesangial cells. *Mol Pharmacol*. 2004; 66:187–196. [PubMed: 15213311]

- Tanito M, Haniu H, Elliott MH, Singh AK, Matsumoto H, Anderson RE. Identification of 4-hydroxynonenal-modified retinal proteins induced by photooxidative stress prior to retinal degeneration. *Free Radical Biology and Medicine*. 2006; 41:1847–1859. [PubMed: 17157187]
- Turdi S, Li Q, Lopez FL, Ren J. Catalase alleviates cardiomyocyte dysfunction in diabetes: role of Akt Forkhead transcriptional factor and silent information regulator 2. *Life Sci*. 2007; 81:895–905. [PubMed: 17765928]
- Urbano-Marquez A, Estruch R, Fernandez-Sola J, Nicolas JM, Pare JC, Rubin E. The greater risk of alcoholic cardiomyopathy and myopathy in women compared with men. *JAMA*. 1995; 274:149–154. [PubMed: 7596003]
- Wang Q, Brunner HR, Burnier M. Determination of cardiac contractility in awake unsedated mice with a fluid-filled catheter. *American Journal of Physiology - Heart and Circulatory Physiology*. 2004; 286:H806–H814. [PubMed: 14512276]
- Wang Y, Zeigler MM, Lam GK, Hunter MG, Eubank TD, Khramtsov VV, Tridandapani S, Sen CK, Marsh CB. The role of the NADPH oxidase complex, p38 MAPK, and Akt in regulating human monocyte/macrophage survival. *American Journal of Respiratory Cell & Molecular Biology*. 2007; 36:68–77. [PubMed: 16931806]
- Weiss JL, Frederiksen JW, Weisfeldt ML. Hemodynamic determinants of the time-course of fall in canine left ventricular pressure. *Journal of Clinical Investigation*. 1976; 58:751–760. [PubMed: 956400]
- Xia N, Daiber A, Habermeier A, Closs EI, Thum T, Spanier G, Lu Q, Oelze M, Torzewski M, Lackner KJ, Munzel T, Forstermann U, Li H. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. *J Pharmacol Exp Ther*. 2010; 335:149–154. [PubMed: 20610621]
- Yoon M, Madden MC, Barton HA. Developmental expression of aldehyde dehydrogenase in rat: a comparison of liver and lung development. *Toxicol Sci*. 2006; 89:386–398. [PubMed: 16291827]
- Zhang Y, Wang H, Li J, Dong L, Xu P, Chen W, Neve RL, Volpe JJ, Rosenberg PA. Intracellular zinc release and ERK phosphorylation are required upstream of 12-lipoxygenase activation in peroxynitrite toxicity to mature rat oligodendrocytes. *Journal of Biological Chemistry*. 2006; 281:9460–9470. [PubMed: 16431921]
- Zhao X, Park J, Ho D, Gao S, Yan L, Ge H, Iismaa S, Lin L, Tian B, Vatner DE, Graham RM, Vatner SF. Cardiomyocyte overexpression of the α 1a-adrenergic receptor in the rat phenocopies second but not first window preconditioning. *American Journal of Physiology - Heart and Circulatory Physiology*. 2012; 302:H1614–H1624. [PubMed: 22307672]
- Zhou F, Qu L, Lv K, Chen H, Liu J, Liu X, Li Y, Sun X. Luteolin protects against reactive oxygen species-mediated cell death induced by zinc toxicity via the PI3K–Akt–NF- κ B–ERK-dependent pathway. *Journal of Neuroscience Research*. 2011; 89:1859–1868. [PubMed: 21800350]
- Zimatkin SM, Pronko SP, Vasiliou V, Gonzalez FJ, Deitrich RA. Enzymatic mechanisms of ethanol oxidation in the brain. *Alcoholism: Clinical & Experimental Research*. 2006; 30:1500–1505.

Figure 1.

Time-dependent changes in mean arterial pressure (MAP; A), heart rate (HR; B), and the myocardial contractility indices, maximal rise of ventricular pressure over time ($dP/dt_{\max}$; C), left ventricular developed pressure (LVDP; D) and the exponential time constant of cardiac isovolumic relaxation (Tau; E) following intragastric ethanol (ETOH; 1 g/kg), compared with equal volume of water (H₂O), in conscious proestrous female Sprague-Dawley rats. Values are mean $\pm$ S.E.M. * $P < 0.05$ versus corresponding control (water) values. (F) Representative tracings depicting the changes in $dP/dt_{\max}$ and arterial pressure (AP) at baseline (BL) and 90 min after intragastric (i.g.) water (H₂O; top) or ethanol (ETOH; 1 g/kg; bottom) in conscious proestrous Sprague-Dawley rats.

Figure 2.

Time-dependent changes in: (A) the high-frequency component of spectral analysis of RRI (RRI-HF α ; 0.75–3 Hz) index of cardiac vagal control, (B) low-frequency component of spectral analysis of RRI (RRI-LF α ; 0.25–0.75 Hz), index of cardiac sympathetic control (C) and LF_{RRI}/HF_{RRI} ratio as a measure of sympathovagal balance in intragastric ethanol (ETOH; 1 g/kg), compared with water (H₂O), treated conscious proestrus Sprague-Dawley rats. Values are mean $\pm$ S.E.M. * $P < 0.05$ versus corresponding control (water) values.

Figure 3.

Activity of alcohol dehydrogenase (ADH; A), catalase (B), and aldehyde dehydrogenase (ALDH2; C) in myocardial tissues collected at 30, 60 or 90 min after intragastric ethanol (ETOH; 1 g/kg) or water (H₂O) in conscious proestrus female Sprague-Dawley rats. Values are means + S.E.M of 5–7 observations. *P < 0.05 versus corresponding control (water) values.

Figure 4.

Levels of myocardial pAkt (A), pERK1/2 (B) at 30, 60 or 90 min after intragastric administration of ethanol (ETOH; 1 g/kg) or water (H₂O). Data are presented as integrated density ratio of pAkt (pSer473) and pERK1/2 to the corresponding total protein tAkt and tERK1/2, respectively, and expressed as percent of control (water) value for each time point. Values are mean $\pm$ S.E.M. of 5 to 7 observations. *P < 0.05 versus corresponding control (water) values.

Figure 5.

Levels of malondialdehyde (MDA; A) and 4-hydroxy-2-nonenal multiple proteins adducts (4-HNE; B) in myocardial tissues isolated from proestrus female Sprague-Dawley rats at 30, 60 or 90 min after intragastric ethanol (ETOH; 1 g/kg) or water (H₂O). The amount of 4-HNE-modified proteins represents the averaged densitometric ratios of all proteins in each lane normalized to beta actin. MDA and 4-HNE integrated density values were compared to those of beta actin and expressed as percent of control (water) value for each time point. Values are mean ± S.E.M. of 5 to 7 observations. *P < 0.05 versus corresponding control (water) values.

Figure 6.

Rate of reactive oxygen species (ROS) production (A and B) and NADPH-oxidase activity (C and D) in myocardial tissues isolated from proestrus female Sprague-Dawley rats 60 min (A and C) or 90 min (B and D) following intragastric ethanol (ETOH; 1 g/kg) or an equal volume of water (H₂O) as detailed under methods. Values are means + S.E.M of 5–7 observations and expressed as μM/mg protein. Inset in each panel depicts the average change in the rate of ROS production (A and B) or NADPH-Oxidase activity (C and D) and expressed as μM/mg protein/min. *P < 0.05 versus corresponding control (water) values.

Figure 7.

Changes in mean arterial pressure (MAP), heart rate (HR), and the myocardial contractility indices, maximal rise of ventricular pressure over time (dp/dt_{max} ; C), and left ventricular developed pressure (LVDP; D), following intragastric ethanol (EtOH; 1 g/kg), compared with equal volume of water (H₂O), in conscious proestrus female Sprague-Dawley pretreated with tempol (20 mg/kg) or atropine (5 mg/kg). Values are mean ± S.E.M. *P < 0.05 versus corresponding control (saline + water) values.

Figure 8.

Changes in the rate of reactive oxygen species (ROS) production in myocardial tissues isolated from proestrus female Sprague-Dawley rats 90 min following intragastric ethanol (ETOH; 1 g/kg) or equal volume of water (H₂O). The rats received tempol (20 mg/kg) or atropine (5 mg/kg) before ethanol or water (see methods for details). Values (expressed as μM/mg protein) are means + S.E.M of 5–7 observations. Inset in each panel depicts the average change in the rate of ROS production expressed as μM/mg protein/min. *P < 0.05 versus corresponding control (saline + water) values.

Table 1

Baseline mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), and the myocardial contractility indices: maximal rise of ventricular pressure over time ($dP/dt_{\max}$, mmHg/sec), and left ventricular developed pressure (LVDP, mmHg) before intragastric administration of ethanol (ETOH; 1 g/kg) or an equal volume of water (H_2O) in conscious proestrus female Sprague-Dawley rats. Values are mean $\pm$ S.E.M.

Treatment	n	MAP	HR	$dP/dt_{\max}$	LVDP
H_2O	7	96.0 $\pm$ 6.0	395 $\pm$ 34	8037 $\pm$ 884	154 $\pm$ 10
ETOH	8	102.0 $\pm$ 6.0	424 $\pm$ 27	9629 $\pm$ 449	182 $\pm$ 9
Atropine + H_2O	4	99.7 $\pm$ 5.6	445 $\pm$ 26	8490 $\pm$ 110	175 $\pm$ 8
Atropine + ETOH	4	93.5 $\pm$ 2.0	463 $\pm$ 24	9027 $\pm$ 100	182 $\pm$ 6
Tempol + H_2O	4	77.7 $\pm$ 2.6	358 $\pm$ 51	9170 $\pm$ 827	169 $\pm$ 9
Tempol + ETOH	5	84.8 $\pm$ 5.1	403 $\pm$ 13	8210 $\pm$ 606	172 $\pm$ 11