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Estrogen Receptor α and β Play Major Roles in Ethanol-Evoked Myocardial Oxidative Stress and Dysfunction in Conscious Ovariectomized Rats

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Abstract

Background—We documented the dependence of ethanol-evoked myocardial dysfunction on estrogen (E_2), and our recent estrogen receptor (ER) blockade study, in proestrus rats, implicated ERα signaling in this phenomenon. However, a limitation of selective pharmacological loss of function approach is the potential contribution of the other 2 ERs to the observed effects because crosstalk exists between the 3 ERs. Here, we adopted a "regain" of function approach (using selective ER subtype agonists) to identify the ER subtype(s) required for unraveling the E_2-dependent myocardial oxidative stress/dysfunction caused by ethanol in conscious ovariectomized (OVX) rats.

Method—OVX rats received a selective ERα (PPT), ERβ (DPN) or GPER (G1) agonist (10 μg/kg; i.v.) or vehicle 30 min before ethanol (1.0 g/kg; infused i.v. over 30 min) or saline, and the hemodynamic recording continued for additional 60 min. Thereafter, left ventricular tissue was collected for conducting ex vivo molecular/biochemical studies.

Results—Ethanol had no hemodynamic effects in OVX rats, but reduced the left ventricular contractility index, dP/dt_{max}, and MAP after acute ERα (PPT) or ERβ (DPN) activation. These responses were associated with increases in the phosphorylation of ERK1/2 and eNOS, and in ROS and MDA levels in the myocardium. GPER activation (G1) only unraveled a modest ethanol-evoked hypotension and elevation in myocardial ROS. PPT enhanced catalase, DPN reduced ALDH2, while G1 had no effect on the activity of either enzyme, and none of the agonists influenced ADH or CYP2E1 activities in the myocardium. Blood ethanol concentration (96.0 mg/dL) was significantly reduced following ERα (59.8 mg/dL) or ERβ (62.9 mg/dL), but not GPER (100.3 mg/dL), activation in ethanol-treated OVX rats.

Conclusions—ERα and ERβ play major roles in the E_2-dependent myocardial dysfunction caused by ethanol by promoting combined accumulation of cardiotoxic (ROS and MDA) and cardio-depressant (NOS-derived NO) molecules in female myocardium.

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Keywords

Ovariectomized Rats; Estrogen Receptors; Ethanol; Myocardial Function; Oxidative Stress; Blood Pressure

INTRODUCTION

We documented the estrogen (E_2)-dependence of ethanol-evoked myocardial dysfunction and hypotension because these effects are absent in ovariectomized (OVX) and male rats, and manifest in both preparations following E_2 administration (El-Mas and Abdel-Rahman, 2014, 2015). These findings, which are reminiscent of E_2-dependent cardiovascular deficits following chronic ethanol administration (El-Mas and Abdel-Rahman, 2000), might be clinically relevant because ethanol reduces blood pressure (BP) in young, but not in older women, or men (Klatsky et al., 1990, Bae et al., 2012). Our recent study identified a pivotal role for the estrogen receptor (ER) subtype ERα in the E_2-dependent myocardial dysfunction caused by ethanol (Yao and Abdel-Rahman, 2016). However, the use of selective ER blockade in proestrus rats (highest level of endogenous E_2) in the latter study or the use of genetic ER knockout models (Sharma and Prossnitz, 2016) might confound data interpretation because a functional crosstalk exists between the 3 ERs (Shi et al., 2013). This limitation could be circumvented by adopting a “regain” of function approach to identify the ER subtype(s) required for restoring ethanol-evoked myocardial oxidative stress/dysfunction in conscious OVX rats. Such studies have become feasible following the availability of highly selective ERα (PPT), ERβ (DPN) and G protein-coupled ER (GPER, G1) agonists (Mata et al., 2015).

The elucidation of the ER subtype(s) implicated in a paradoxical transformation of E_2 into a pro-inflammatory hormone in the presence of conditions that promote cellular oxidative stress (White et al., 2010) is important for understanding the molecular mechanisms of the sex (E_2)-dependent adverse hemodynamic effects of alcohol in females. All 3 ER subtypes, ERα, ERβ and GPER, are distributed throughout the cardiovascular system (Meyer et al., 2006, Meyer et al., 2011), and confer favorable redox and functional cardiac effects under physiopathological conditions via both genomic and rapid signaling mechanisms (Weil et al., 2010, Lee et al., 2014). In OVX rats, acute E_2 administration enhances the activity of two cardiac enzymes, catalase and mitochondrial aldehyde dehydrogenase 2 (mitALDH2) (El-Mas and Abdel-Rahman, 2014), which serve antioxidant roles (Ren et al., 2007, Ma et al., 2010, Kandadi et al., 2012). Notably, these two enzymes also catalyze ethanol oxidative metabolism (Soffia and Penna, 1987, Kinoshita et al., 2001), and determine the level of the pro-oxidant metabolite acetaldehyde (Comporti et al., 2010). Therefore, identifying the ER(s) that activate(s) these two enzymes is critical for our understanding of paradoxical myocardial oxidative stress caused by combining E_2 and ethanol.

Ethanol-evoked accumulation of cardiotoxic molecules such as malondialdehyde (MDA) and reactive oxygen species (ROS) (El-Mas and Abdel-Rahman, 2014) is exacerbated in the presence of E_2 (El-Mas and Abdel-Rahman, 2014, Ibrahim et al., 2014). Further, E_2 is required for mediating the protein kinase B (Akt)/nitric oxide synthase (NOS)-dependent

myocardial dysfunction caused by ethanol in female rats (El-Mas et al., 2009, 2012). Collectively, these cellular effects are expected to compromise the antioxidant/detoxifying effects of mitALDH2 (Ren et al., 2007, Ma et al., 2010, Kandadi et al., 2012), and ultimately result in ethanol-evoked myocardial oxidative stress/dysfunction.

The main aim of the present study was to identify the ER subtype(s) implicated in the E_2-dependent adverse biochemical and cardiac effects of ethanol. To achieve this goal, we conducted targeted ERα, ERβ or GPER restoration of function studies, using the respective highly selective agonist (PPT, DPN or G1), in the absence or presence of ethanol in conscious OVX rats. Notably, in OVX rats, the activation of one or more ER subtype function, by the endogenous non-selective ER agonist E_2, is required for the manifestation of ethanol-evoked myocardial oxidative stress and dysfunction (El-Mas and Abdel-Rahman, 2014). This experimental approach also generated important data on the role of the individual ER subtype(s) in the E_2-dependent activation of two myocardial enzymes, catalase and mitALDH2, which exert favorable redox function and, along with ADH and CYP2E1, catalyze oxidative metabolism of ethanol.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (female, 175–200 g) were purchased from Charles River (Raleigh, NC), and kept in the university animal care center keeping the temperature at 23 ± 1°C, humidity at 50 ± 10%, and a 12-h light/dark cycle. Rats were housed in pairs in plastic boxes and allowed food (Prolab Rodent Chow, Granville Milling, Creedmoor, NC) and water freely for at least one week. Two weeks after ovariectomy (OVX), vascular and left ventricular catheterization was conducted one day before the hemodynamic studies. All surgical procedures were conducted under sterile conditions. The animal was anesthetized (i.p.) by a mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg). Buprenorphine (0.03 mg/kg; s.c) was used for pre- and postoperative analgesia, and Durapen (10,000 unites/kg) to guard against infection. Only one rat was kept in a cage after surgery. All the surgical procedure, post-operative care, and experimental protocols were approved by the East Carolina University Institutional Animal Care and Use Committee and were consistent with the Guide for the Care and Use of Laboratory Animals (2011).

Ovariectomy

Ovariectomy, detailed in our previous studies (El-Mas and Abdel-Rahman, 2000), was conducted via a longitude skin incision (about 2 cm) on the lower part of the back after separating the muscles overlying the ovaries on both sides. The ovaries were isolated, tied off with sterile suture and removed. The muscles were closed by sterile suture (Roboz Surgical Instrument Co., Gaithersburg, MD) and the skin was closed by Suture Wound Clips (Mikron Precision Inc., Gardena, CA). Povidone-iodine solution was used for disinfection. The rats were kept for 2 weeks after ovariectomy to ensure depletion of endogenous E_2 in accordance with an established protocol in our lab (El-Mas and Abdel-Rahman, 2000).

Vascular and left ventricular catheterizations were performed as detailed in our recent study (Yao and Abdel-Rahman, 2016). Briefly, gas sterilized arterial and venous catheters (Konigsberg Instruments Inc., Pasadena, CA), filled with heparinized saline, were placed into the abdominal aorta and vena cava via the femoral artery/vein for monitoring BP, and for the administration of ethanol and/or the pharmacological intervention, respectively. A PE50 tubing was placed into left ventricle via the right carotid artery for the measurement of left ventricular function. All catheters were subcutaneously tunneled to the back of the neck, existed between the scapulae, plugged with sterile stainless steel pins, and attached to the skin with 3M VetbondTM tissue adhesive (Animal Care Products, St. Paul, MN).

Hemodynamic recording

Left ventricular function and BP measurements were conducted in conscious freely moving rats as detailed in our previous studies (Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016). The arterial and left ventricular catheters were connected to Gould-Statham pressure transducers (Oxnard-CA) and flushed with heparinized saline (100 IU/ml). The animal was allowed to adapt to the environment for at least 30 min. BP and left ventricular indices were simultaneously recorded by ML870 (PowerLab 8/30), and analyzed by LabChart (v.7) pro software (AD Instruments, Colorado Spring, CO).

Determination of plasma ethanol concentration

Measurement of ethanol concentration is detailed in our previous studies (Yao and Abdel-Rahman, 2016). Briefly, sample and standard reactions were conducted in 96-well plate. The absorbance was detected at 340 nm by an Infinite® 200 PRO multimode microplate readers (Tencan Group Ltd., Männedorf, Schweiz). The ethanol concentration was determined based on concurrently constructed ethanol standard calibration curve.

Measurement of myocardial catalase and mitALDH2 activity

Left ventricular tissue was homogenized in PBS on ice and centrifuged at 4°C, at 10,000 rpm for 10 min. The supernatant was collected for measurements. As descripted in the manufacturer's protocol and in our studies (El-Mas et al., 2012, Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016), 5 μg of sample protein was used to measure the catalytic activity by colorimetric catalase assay kit (Sigma-Aldrich, St. Louis, MO). The absorbance was read at 520 nm. A mitochondrial ALDH2 activity assay kit (Abcam, Ann Arbor, MI) was used for the measurement of mitALDH2 activity in 500 μg sample protein; the absorbance was read at 450 nm every 3 min for 1 hr.

Measurement of alcohol dehydrogenase (ADH)

We followed the protocol for Enzymatic Assay of Alcohol Dehydrogenase (EC 1.1.1.1) (Sigma-Aldrich, St. Louis, MO) to measure ADH activity. Heart tissue was homogenized in PBS (PH 7.5) and centrifuged at 10,000 rpm for 10 min. Baseline absorbance was read at 340 nm after adding 90 μl of sodium pyrophosphate buffer (50 mM, PH 8.8), 100 μl of β-nicotinamide adenine dinucleotide (β-NAD, 15 mM, freshly prepared) and 10 μl of 95% ethanol in each well of a 96-well plate. Next, we added 20 μl of the supernatant of each sample homogenate, and 20 μl of escalated concentrations of freshly prepared ADH (0–1.2

units/ml), in adjacent 6 wells, to obtain a standard curve. Absorbance was measured again at 340 nm, and the difference between baseline and final absorbance was used to calculate the ADH activity from the standard curve.

Measurement of cytochrome P450 2E1 (CYP2E1) activity

CYP2E1 activity was measured in heart tissue homogenate by the method of oxidation of p-Nitrophenol (PNP, 4-Nitrophenol) to p-nitrocatechol by CYP2E1 in the presence of NADPH as reported (Zhang et al., 2011) with modification. Briefly, 200 μL reaction medium containing 500 μg protein, 100 mM potassium-phosphate buffer (pH 7.4), 2 mM PNP and 1 mM NADPH was incubated at 37°C for 60 min, then terminated by 30 μL of 20% trichloroacetic acid. After centrifugation (10,000 rpm for 10 min), 200 μL of the supernatant was mixed with 10 μL of 10 M NaOH in a 96-well plate, and the optical density was read at 546 nm.

Measurement of myocardial nitrite/nitrate (NOx) level

Left ventricular tissue was homogenized in PBS on ice and centrifuged at 5000 rpm for 10 min, at 4 °C. The supernatant was collected and ultrafiltered by a 10 kDa molecular weight filter for measurement of NOx using Nitrite/Nitrate Colorimetric Assay Kit (Cayman Chemical Company, Ann Arbor, MI) as described in our previous studies (El-Mas et al., 2012, Yao and Abdel-Rahman, 2016). The filtrate (80 μL containing 30 μg of protein) was used for a reaction with nitrate reductase mixture and enzyme cofactor mixture. The O.D value was read at 550 nm using Infinite® 200 PRO multimode microplate readers (Tencan Group Ltd., Männedorf, Schweiz). NOx concentration was determined based on standard curve data.

Measurement of myocardial ROS level

Myocardial ROS levels were measured by a fluorometric assay using 2′,7′-dichlorofluorescin diacetate (DCFH-DA, Molecular Probes-Thermo Fisher Scientific Inc, Raleigh, NC). Briefly, myocardial tissue was homogenized in PBS on ice and centrifuged at 5000 rpm for 10 min. DCFH-DA (25 μM) was added to 250 μg of sample protein supernatant. The fluorescence intensities were measured in a 96-well plate every 5 min for 30 min with Infinite® 200 PRO multimode microplate readers (Tencan Group Ltd., Männedorf, Schweiz) at 485 nm/530 nm for excitation/emission wavelengths at 37 °C as in our previous reports (Yao and Abdel-Rahman, 2016).

Measurement of myocardial MDA level

TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure MDA level. Briefly, myocardial tissue was homogenized in RIPA buffer with protease inhibitors on ice, and centrifuged at 10,000 rpm for 15 min. The supernatant (50 μL) was used to react with thiobarbituric acid (TBA) under high temperature (90–100°C) and acidic conditions. The MDA-TBA adduct was measured colorimetrically at 530–540 nm. A standard curve was used to calculate the MDA concentration.

Western blot

We followed the protocols described in our studies (Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016). Left ventricular tissue was homogenized on ice in lysis buffer with protease inhibitor cocktail (Roche, IN), and centrifuged (12,000 rpm for 15 min). Briefly, the supernatant protein (80 μg) was separated in a 4–12% gel electrophoresis (Novex Tis-Glycine gel, Life Technologies, CA), and semidry transferred to nitrocellulose membrane. The membranes were blocked in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) for 2 hrs, and incubated in primary antibody overnight at 4°C with shaking. The primary antibodies used were: mouse anti-p-Akt (1:500) and rabbit anti-Akt (1:500, Cell Signaling, Danvers, MA); mouse anti-p-ERK1/2 (1:500) and ERK1/2 antibodies (1:500, Cell Signaling, Danvers, MA); mouse anti-p-p38 (1:500) and rabbit anti-p38 (1:500, Cell Signaling, Danvers, MA); rabbit anti-p-nNOS (Ser1417) (1:200) and mouse polyclonal anti-nNOS antibody (1:200, BD Biosciences, San Jose, CA); rabbit anti-p-eNOS (1:200, Thermo Fisher Scientific, Waltham, MA) and mouse polyclonal anti-eNOS antibody (1:200). Thereafter, the membranes were incubated with secondary antibody prepared by IRDye680-conjugated goat anti-mouse and IRDye800-conjugated goat anti-rabbit (1:15000, LI-COR Biosciences, Lincoln, NE) for 60 min after washing. Bands were detected by Odyssey Infrared Imager and quantified by integrated intensities with Odyssey application software version 3 (LI-COR Biosciences, Lincoln, NE).

Experimental groups and protocols

The experimental design/schedule is presented in Fig. 1.

Experiment 1. Effect of prior selective ERα, ERβ or GPER activation on myocardial function and BP in ethanol-treated OVX female rats—Based on our findings that acute E_2 administration unravels ethanol evoked myocardial dysfunction in OVX rats (El-Mas and Abdel-Rahman, 2014), we investigated the roles of ERα, ERβ or GPER in this adverse effect of ethanol in 56 OVX rats, divided into 8 groups (6–8 rats/group). All rats were used in the conscious state, 2 weeks after ovariectomy, and 1 day after left ventricular and vascular catheterization. At least 30 min was allowed for the hemodynamic variables to stabilize at baseline levels. Six groups were divided into 3 pairs and each pair received an i.v. (10 μg/kg) injection of an ERα (PPT), ERβ (DPN) or GPER (G1) agonist 30 min before ethanol (1.0 g/kg; agonist + ethanol) or equal volume of saline (agonist + saline). The remaining 2 groups received vehicle (DMSO:saline, 1:50; control for ER agonists) 30 min before saline (control, vehicle + saline) or ethanol (vehicle + ethanol). The doses of the ER agonists were based on reported findings (Shaolong Yang, 2007, Lu et al., 2009) and the dose of ethanol was based on our previous study (Ibrahim et al., 2014). Mean arterial pressure (MAP), left ventricular developed pressure (LVDP) and maximum rate of left ventricular pressure rise (dP/dt_{max}) were recorded for additional 60 min after 30 min infusion period of ethanol or saline, and analyzed by Lab chart. At the conclusion of the hemodynamic measurements, the rats were euthanized, tissue were collected, flash-frozen, and stored at −80°C for conducting the molecular studies as described below.

Experiment 2. Effect of selective ER subtype activation on modulators of myocardial redox state and ethanol metabolizing enzymes—The catalytic activity

of catalase and ALDH2, which serve antioxidant roles (Ren et al., 2007, Ma et al., 2010), and catalyze ethanol metabolism (Soffia and Penna, 1987, Kinoshita et al., 2001), is enhanced in presence of E_2 (Campos et al., 2014), but the ER subtype that mediate these effects in the absence or presence of ethanol is not known. Therefore, these ex vivo studies were conducted to elucidate the effect of selective activation of each of the 3 ER subtype on myocardial alcohol metabolizing enzymes (ADH, CYP2E1 and catalase), anti-oxidative enzymes (catalase and mitALDH2) and mediators of oxidative stress (MAKs) in the absence or presence of ethanol. Blood and left ventricular tissues were obtained from all rats employed in experiment 1 for the measurements of blood ethanol concentration and biochemical studies as detailed under methods and in our recent studies (Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016).

Drugs

Ethanol (Midwest Grain Products Co., Weston, MO) was diluted in saline. Propylpyrazole Triol (PPT), 2, 3-bis (4-Hydroxyphenyl) (DPN), and G1 were purchased from Cayman Chemical (Ann Arbor, MI, USA). The drugs were firstly dissolved in DMSO for stock solution. The working solution was the 1:50 diluted stock solution with saline.

Data analysis and statistics

Values are presented as mean ± SEM. The baseline data in table 1 were analyzed by one-way ANOVA. Differences in hemodynamic variables between before and after an ER subtype agonist administration (Table 1) were analyzed by paired t-test. Other data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. These statistical analyses were conducted by Prism version 5 (GraphPad Software, Inc. La Jolla, CA). $p < 0.05$ was considered significant.

RESULTS

Selective ERα or ERβ activation unraveled ethanol-evoked myocardial dysfunction and hypotension in OVX rats

The baseline hemodynamic variables measured in all conscious OVX rat groups were not statistically different (Table 1). When administered alone, none of the selective ER subtype agonists (10 μg/kg, i.v.) influenced the measured hemodynamic variables (Table 1). Similarly, ethanol (1.0 g/kg) administered alone had no effect on the measured variables (Figs. 2 and 3). Prior ERα (PPT) or ERβ (DPN) activation unraveled ($p < 0.05$) ethanol-evoked reductions in dP/dt_{max} (Figs. 2A and A1), LVDP (Figs. 2B and B1) and MAP (Fig. 3). However, prior GPER activation (G1) only unraveled significant ($p < 0.05$), but modest (approx. 5 mmHg) hypotensive effect of ethanol, which was much smaller than that unraveled by ERα or ERβ activation (Figs. 2 and 3).

Ethanol and PPT produced additive enhancement of myocardial catalase activity in OVX rats

Here, we examined, for the first time, the effect of individual ER subtype activation in the absence or presence of ethanol on myocardial catalase, mitALDH2, ADH and CYP2E1 activities in E_2-depeleted (OVX) rats. Notably, the two antioxidant enzymes, catalase and

mitALDH2, are involved in ethanol oxidative metabolism, and their activities are suppressed in OVX hearts and restored following acute E_2 (nonselective ER agonist) administration in OVX rats (El-Mas and Abdel-Rahman, 2014, 2015). ERα (PPT) significantly ($p < 0.05$) enhanced both myocardial catalase and mitALDH2 activities, and alcohol (1.0 g/kg) caused further increase ($p < 0.05$) in catalase, but not mitALDH2 activity, in the presence of PPT (Figs. 4A and B). While neither ERβ nor GPER influenced myocardial catalase (Fig. 4A), they significantly ($p < 0.05$) reduced mitALDH2 activity (Fig. 4B), and these effects were not altered by alcohol (Figs. 4A and B). None of the ER agonists influenced myocardial ADH (Fig. 5A) or CYP2E1 (Fig. 5B) activity in the absence or presence of ethanol. Finally, blood ethanol level was significantly ($p < 0.05$, Fig. 4C) lower in OVX rats pretreated with the ERα agonist PPT (59.8 ± 6.3 mg/dL) or ERβ agonist DPN (62.9 ± 7.7 mg/dL), but not with the GPER agonist G1 (100.3 ± 10 mg/dL), compared with saline pretreatment (96.0 ± 8.6 mg/dL).

Alcohol reversed the ERα, ERβ or GPER-mediated improvement of myocardial redox status in OVX rats

ERα (PPT), ERβ (DPN) or GPER (G1) activation significantly ($p < 0.05$) suppressed myocardial ROS level (Fig. 6A), but only PPT or DPN significantly ($p < 0.05$) suppressed MDA (Fig. 6B) levels in OVX rats. Consequent ethanol administration reversed ($p < 0.05$) ROS suppression caused by PPT, DPN or G1 (Fig. 6A) and MDA suppression caused by PPT or DPN (Fig. 6B) in the myocardium of OVX rats that received the individual ER agonist plus alcohol. GPER activation (G1) had no effect on Akt, ERK1/2 and p38 phosphorylation in the absence or presence of ethanol (Fig. 7). However, both PPT and DPN significantly ($p < 0.05$) increased Akt (Fig. 7A), but only PPT decreased ERK1/2 (Fig. 7B) phosphorylation, and these effects were reversed by ethanol (Figs. 7A and B). While PPT alone had no effect, it uncovered significant ($p < 0.05$) increase in p38 phosphorylation when combined with ethanol (Fig. 7C). On the other hand, DPN significantly ($p < 0.05$) and similarly increased p38 phosphorylation in the presence or absence of ethanol (Fig. 7C). Finally, ethanol produced significant ($p < 0.05$) increases in eNOS phosphorylation when administered following PPT or DPN (Fig. 8A), and significant ($p < 0.05$) increases in nNOS phosphorylation (Fig. 8B) and NOx (Fig. 8C) when administered following DPN. GPER activation (G1) alone or in combination with ethanol had no effect on these molecular responses (Fig. 8).

DISCUSSOIN

In the present study we generated important data on the role of the individual ER subtype ERα, ERβ or GPER in the sex (E_2)-dependent myocardial oxidative stress/dysfunction caused by alcohol. We show that a pre-existing ERα or ERβ activation is required for the manifestation of the adverse myocardial effects of ethanol in females. Our multidimensional approach helped us to elucidate the molecular mechanisms implicated in the counterintuitive transformation of the favorable E_2-mediated myocardial redox status into deleterious cardiac effects in the presence of alcohol. These findings are relevant to women's cardiovascular health given the steady rise in alcohol use by women in the US, and the potential use of selective ER subtype agonists, in lieu of E_2, as hormone replacement therapy.

Our recent study presented indirect evidence for the contribution of all 3 ER subtypes to ethanol-evoked myocardial oxidative stress/dysfunction, but emphasized a role for ERα in initiating these adverse effects (Yao and Abdel-Rahman, 2016). A limitation of the approach used in our previous study, which is shared with genetic ER subtype knockout models (Sharma and Prossnitz, 2016), is the concurrent signaling triggered by endogenous E_2 chronic activation of the other two ER subtypes when the targeted ER subtype is pharmacologically blocked or deleted. We resolved this limitation in the present study, and generated physiologically relevant data by adopting the selective gain of function approach in E_2-depeleted (OVX) rat model because the loss of E_2 in OVX rats: (i) abolishes ethanol evoked myocardial dysfunction (El-Mas and Abdel-Rahman, 2014), (ii) suppresses the activities of myocardial catalase and mitALDH2 (El-Mas and Abdel-Rahman, 2015), which confer favorable redox effects (Ren et al., 2007, Ma et al., 2010, Kandadi et al., 2012), and catalyze ethanol metabolism (Soffia and Penna, 1987, Kinoshita et al., 2001). Importantly, these biochemical and ethanol-evoked hemodynamic responses are fully restored in OVX rats treated with chronic or acute E_2 (El-Mas and Abdel-Rahman, 1999, 2012, 2014). Replacing the nonselective ER agonist E_2 with a highly selective ERα (PPT), ERβ (DPN) or GPER (G1) agonist in OVX rats was adopted in the present study to determine the required ER subtype(s) for restoring ethanol-evoked adverse cardiac effects and hypotension in OVX rats.

Our pharmacological approach is supported by the ability of PPT, DPN and G1, which exhibit several hundred fold selectivity to ERα, ERβ and GPER, respectively, to regain the targeted ER function (Stauffer et al., 2000, Meyers et al., 2001, Bologa et al., 2006). Consistent with our previous studies (El-Mas and Abdel-Rahman, 1999, 2014), ethanol had no effect on cardiac function (Fig. 2) or BP (Fig. 3) in OVX rats. We show that regaining ERα or ERβ function, following acute administration of its respective agonist, unraveled ethanol-evoked myocardial oxidative stress/dysfunction and hypotension in OVX rats (Figs. 2, 3 and 6). These findings are reminiscent of the ability of acutely administered E_2 (nonselective ER agonist) to unravel the adverse cardiovascular effects of ethanol in OVX (El-Mas and Abdel-Rahman, 1999, 2014) as well as in male (El-Mas and Abdel-Rahman, 2015) rats, and directly implicate ERα and ERβ in the studied phenomenon. On the other hand, regaining GPER function only unraveled modest ethanol-evoked hypotension in OVX rats (Fig. 3).

It is important to consider the underlying biochemical environment following the regaining of each individual ER subtype function in OVX rats to understand the basis for their relative contribution to ethanol-evoked biochemical and hemodynamic effects. While reported studies including ours (Campos et al., 2014, Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016) showed that E_2 enhances myocardial catalase and mitALDH2 activities in OVX rats, the present study is the first to show that these biochemical effects were only replicated following regaining ERα function (Fig. 4). This is in marked contrast to an inhibitory role for ERβ or GPER on mitALDH2 (Fig. 4B). In addition to the physiological ramifications of these ER subtype-dependent alterations in the catalytic activity of these 2 myocardial antioxidant enzymes (White et al., 2010), we must consider their impact on ethanol metabolism. This is important because catalase (Oshino et al., 1973) and mitALDH2 (Matsumoto et al., 1996, Doser et al., 2009) enhance ethanol oxidation to acetaldehyde and

acetate, respectively. The ability of ethanol to further enhance catalase, but not mitALDH2 in OVX rats with regained ERα function (Figs. 4A and B) is suggestive of acetaldehyde accumulation, which exerts greater cardiotoxic effects than ethanol (Sarc and Lipnik-Stangelj, 2009). On the other hand, acetaldehyde accumulation following ERβ or GPER regaining of function might result, at least partly, from the lower mitALDH2 activity (Fig. 4B) although this effect might be mitigated somewhat by ethanol-evoked increase in mitALDH2 in DPN-treated OVX rats (Fig. 4B). Nonetheless, the contribution of ADH to ethanol metabolism in our model system must also be considered because E_2 suppresses hepatic ADH activity (Chrostek et al., 2003).

Similar to our finding in the heart (Ibrahim et al., 2014), ethanol (1.0 g/kg) enhanced ADH activity in the liver (Supplemental Fig. 1A) of sham-operated rats in an E_2-dependent manner because ethanol had no effect on cardiac (Fig. 5A) or liver (Supplemental Fig. 1A) ADH activity in OVX rats. Further, the inability of ethanol to enhance cardiac (Fig. 5A) or liver (Supplemental Fig. 2A) ADH activity in OVX rats, pretreated with PPT, DPN or G1, inferred that concomitant activation of 2 or all 3 ER subtypes is required for this action. We addressed this question in a validated in vitro model system in which ethanol (100 mg/dL; attained after 1.0 g/kg dose in vivo), enhanced ADH activity in isolated liver tissue from sham-operated rats (Supplemental Fig. 3A). Next, we showed that adding 2 or the 3 ER agonists to the incubation medium in reported concentrations (Shen and Shi, 2016), compatible with those attained following their acute administration in the present study, did not reproduce the E_2-dependent enhancement of hepatic ADH activity caused by alcohol in sham-operated rats in vivo (Supplemental Fig. 1A) or in vitro (Supplemental Fig. 3A). It is likely that concomitant chronic activation of 2 or the 3 ER subtypes is required to unravel ethanol (1.0 g/kg)-evoked enhancement of ADH activity in the heart (Ibrahim et al., 2014) and liver (Supplemental Fig. 1A) of sham-operated rats. This conclusion gains credence from the findings that ethanol enhanced liver ADH activity in tissues isolated from sham-operated rats (Supplemental Fig. 3A), but not in tissues from OVX rats subjected to combined acute activation of the 3 ER subtypes (Supplemental Fig. 3B). Whether this phenomenon applies to E_2-dependent effects of ethanol on other enzymes or kinases remains to be elucidated in future studies.

We also investigated the influence of activating the individual ER subtype on cardiac CYP2E1 activity, another enzyme that catalyzes alcohol oxidative metabolism, because its expression is increased by E_2 (Konstandi et al., 2013). Consistent with the latter finding, hepatic CYP2E1 activity is significantly higher in sham-operated, compared with OVX, rats (Supplemental Fig. 1B). Further, acute (Haorah et al., 2005) or chronic (Takahashi et al., 1993) ethanol administration enhances CYP2E1 activity in different organs. Here, ethanol had no effect on cardiac CYP2E1 activity in the absence or the presence of PPT, DPN or G1 (Fig. 5B). It is likely that the reported effect of ethanol on CYP2E1 activity is organ dependent and influenced by the hormonal status. In support of this notion, ethanol enhanced CYP2E1 activity in the liver (Supplemental Fig. 2B), but not the heart (Fig. 5B) of OVX rats, and had no effect on hepatic CYP2E1 activity in sham-operated rats (Supplemental Fig. 1B). More studies are needed to understand the mechanism of the dampening effect of the three ER subtypes on ethanol-evoked activation of hepatic CYP2E1 in the liver of OVX rats.

In our previous studies, acute nonselective activation of all ERs by E_2 had no effect on blood alcohol concentration (BAC) in OVX (el-Mas and Abdel-Rahman, 2012) or male (El-Mas and Abdel-Rahman, 2015) rats. While averaging BAC in all OVX rats pretreated with the individual ER agonist agree with our previous findings, the present study contributes new knowledge by demonstrating reduced blood ethanol level in PPT- or DPN-treated OVX rats. As discussed above, this reduction in BAC due, at least partly, to the alterations in catalase and mitALDH2, is indicative of higher levels of acetaldehyde or acetate, which are more cardiotoxic than ethanol (Sarc and Lipnik-Stangelj, 2009). These findings may also explain the higher acetaldehyde level, derived from similar amounts of alcohol, in women on birth control pills (Jeavons and Zeiner, 1984).

The present study directly discerned the contribution of the individual ER subtype to the E_2-dependent myocardial dysfunction caused by alcohol, which was a challenging task when we adopted the selective ER subtype blockade in our previous study (Yao and Abdel-Rahman, 2016). While both studies suggest major contributions of ERα and ERβ to ethanol-evoked myocardial dysfunction, here we show that ERα mediated the reduction in dP/dt_{max} (Figs. 2A and A1) and ERβ mediated the reduction in LVDP (Figs. 2B and B1) caused by ethanol. These divergent responses might be explained, at least partly, by the differences in the autonomic and endocrine control of dP/dt_{max} and LVDP (Gordan et al., 2015). Notably, ERα and ERβ play prominent roles in the heart and CNS, respectively (Arnal et al., 2012), and play different roles in cardio-protection (Murphy, 2011). Our findings suggest a minimal role for GPER in these E_2-dependent adverse hemodynamic effects of ethanol (Figs. 2 and 3). Nonetheless, these effects are expected to be additive to those mediated by ERα and ERβ when the endogenous levels of E_2 are high in proestrus rats in our previous studies (Ibrahim et al., 2014) or in women receiving E_2 replacement therapy or birth control pills (Jeavons and Zeiner, 1984).

Our findings showed that both PPT and DPN increased Akt and p38 phosphorylation (Fig. 7), which agrees with favorable roles for these kinases in the salutary effects of E_2 on cardiac function (Hsu et al., 2007, Favre et al., 2010). Further, the increases in ERK1/2 and p38 phosphorylation caused by PPT or DPN, but not by G1 (Fig. 7), must be considered in the context of their roles in regulating cardiac function, particularly in the presence of ethanol. This is important because our earlier studies implicated enhanced AKt and ERK1/2 phosphorylation, and their mediation of NOS phosphorylation and ROS generation, in the E_2 dependent myocardial dysfunction caused by similar doses of ethanol (El-Mas et al., 2009, Ibrahim et al., 2014, Yao and Abdel-Rahman, 2016). Here, we extend these earlier findings by showing that ERα mediates ERK1/2 (Fig. 7B), p38 (Fig. 7C) and eNOS (Fig. 8A) phosphorylation while ERβ mediates ERK1/2 (Fig. 7B), eNOS (Fig. 8A) and nNOS (Fig. 8B) phosphorylation caused by ethanol in the female myocardium. GPER had no role in these molecular responses (Figs. 7 and 8). These molecular findings might also explain the relatively greater reductions in dP/dt_{max} (Fig. 2A) and LVDP (Fig. 2B) caused by ethanol following ERα and ERβ activation, respectively. Finally, simultaneous ERα and ERβ activation might be required to replicate the E_2-dependent activation of Akt caused by ethanol (El-Mas et al., 2009) because activating either ER subtype was not sufficient to reproduce this effect of ethanol (Fig. 7).

It is imperative to discuss the roles of the ER subtypes in mediating the E_2-dependent oxidative stress caused by ethanol because the OVX rat myocardium exhibits greater levels of two mediators of oxidative stress, ROS and MDA, and these elevations are reversed by E_2 to proestrus rat levels (Abbas and Elsamanoudy, 2011, El-Mas and Abdel-Rahman, 2014). All ER subtypes mediated ROS reduction (Fig. 6A), but only ERα or ERβ mediated MDA reduction (Fig. 6B) in OVX rats, which is consistent with more favorable redox roles for ERα and ERβ (Urata et al., 2006, Liu et al., 2009). Ethanol alone had no effect on the already elevated myocardial ROS and MDA in OVX rats (Fig. 6), but reversed the ERα- or ERβ-dependent reductions in ROS and MDA to OVX level (Fig. 6). However, myocardial dysfunction occurred only when ethanol was combined with ERα or ERβ agonist (Figs. 2 and 3). These findings raise the interesting possibility that a pre-existing higher oxidative stress in OVX (El-Mas and Abdel-Rahman, 2014) or male (El-Mas and Abdel-Rahman, 2015), compared to proestrus female rats, might protect the heart from the adverse effects of ethanol. In support of this premise, the resilience of the OVX or male rat heart to the adverse effect of ethanol is lost when myocardial oxidative stress was suppressed in both preparations by acute E_2 administration (El-Mas and Abdel-Rahman, 1999, 2014, 2015). The present findings implicate ERα and ERβ in this phenomenon.

The current study yields new insight into the role of ERα, ERβ or GPER in the E_2-dependent myocardial dysfunction caused by alcohol via accumulation of cardiotoxic (ROS and MDA) and cardiodepressant (NOS-derived NO) molecules. Pharmacological and biochemical evidence support pivotal roles for ERα and ERβ in this phenomenon although differences existed between their mediation of ethanol-evoked reductions in myocardial function indices. By contrast, GPER only contributed a modest role in ethanol-evoked hypotension. The findings highlight potential adverse hemodynamic interaction between selective ER agonists and ethanol in women with surgical menopause. If selective ER agonists are utilized as alternatives to E_2 replacement, the selective GPER agonist G1 represents a safer option for women with history of alcohol consumption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Abbas AM, Elsamanoudy AZ. Effects of 17beta-estradiol and antioxidant administration on oxidative stress and insulin resistance in ovariectomized rats. Can J Physiol Pharmacol. 2011; 89:497–504. [PubMed: 21812527]

Arnal JF, Valera MC, Payrastre B, Lenfant F, Gourdy P. Structure-function relationship of estrogen receptors in cardiovascular pathophysiological models. Thromb Res. 2012; 130(Suppl 1):S7–11.

Bae KY, Kim SW, Shin HY, Kim JM, Shin IS, Kim SJ, Kim JK, Yoon JS. The acute effects of ethanol and acetaldehyde on physiological responses after ethanol ingestion in young healthy men with different ALDH2 genotypes. Clin Toxicol (Phila). 2012; 50:242–249. [PubMed: 22455355]

Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuck NP, Sklar LA, Oprea TI, Prossnitz ER. Virtual and biomolecular screening converge on a selective agonist for GPR30. Nat Chem Biol. 2006; 2:207–212. [PubMed: 16520733]

Campos C, Casali KR, Baraldi D, Conzatti A, Araujo AS, Khaper N, Llesuy S, Rigatto K, Bello-Klein A. Efficacy of a low dose of estrogen on antioxidant defenses and heart rate variability. Oxid Med Cell Longev. 2014; 2014:218749. [PubMed: 24738017]

Chrostek L, Jelski W, Szmitkowski M, Puchalski Z. Gender-related differences in hepatic activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in humans. J Clin Lab Anal. 2003; 17:93–96. [PubMed: 12696080]

Comporti M, Signorini C, Leoncini S, Gardi C, Ciccoli L, Giardini A, Vecchio D, Arezzini B. Ethanol-induced oxidative stress: basic knowledge. Genes Nutr. 2010; 5:101–109. [PubMed: 20606811]

Doser TA, Turdi S, Thomas DP, Epstein PN, Li SY, Ren J. Transgenic overexpression of aldehyde dehydrogenase-2 rescues chronic alcohol intake-induced myocardial hypertrophy and contractile dysfunction. Circulation. 2009; 119:1941–1949. [PubMed: 19332462]

El-Mas MM, Abdel-Rahman AA. Estrogen-dependent hypotensive effects of ethanol in conscious female rats. Alcohol Clin Exp Res. 1999; 23:624–632. [PubMed: 10235298]

El-Mas MM, Abdel-Rahman AA. Ovariectomy alters the chronic hemodynamic and sympathetic effects of ethanol in radiotelemetered female rats. Clin Exp Hypertens. 2000; 22:109–126. [PubMed: 10685729]

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. 2012; 679:95–100. [PubMed: 22305881]

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. 2014; 306:E740–747. [PubMed: 24368668]

El-Mas MM, Abdel-Rahman AA. Estrogen modulation of the ethanol-evoked myocardial oxidative stress and dysfunction via DAPK3/Akt/ERK activation in male rats. Toxicol Appl Pharmacol. 2015; 287:284–292. [PubMed: 26111663]

El-Mas MM, Fan M, Abdel-Rahman AA. Facilitation of myocardial PI3K/Akt/nNOS signaling contributes to ethanol-evoked hypotension in female rats. Alcohol Clin Exp Res. 2009; 33:1158–1168. [PubMed: 19389198]

El-Mas MM, Fan M, Abdel-Rahman AA. Differential modulation by vascular nitric oxide synthases of the ethanol-evoked hypotension and autonomic dysfunction in female rats. Alcohol. 2012; 46:727–735. [PubMed: 23046587]

Favre J, Gao J, Henry JP, Remy-Jouet I, Fourquaux I, Billon-Gales A, Thuillez C, Arnal JF, Lenfant F, Richard V. Endothelial estrogen receptor {alpha} plays an essential role in the coronary and myocardial protective effects of estradiol in ischemia/reperfusion. Arterioscler Thromb Vasc Biol. 2010; 30:2562–2567. [PubMed: 20847304]

Gordan R, Gwathmey JK, Xie LH. Autonomic and endocrine control of cardiovascular function. World J Cardiol. 2015; 7:204–214. [PubMed: 25914789]

Haorah J, Knipe B, Leibhart J, Ghorpade A, Persidsky Y. Alcohol-induced oxidative stress in brain endothelial cells causes blood-brain barrier dysfunction. J Leukoc Biol. 2005; 78:1223–1232. [PubMed: 16204625]

Hsu JT, Hsieh YC, Kan WH, Chen JG, Choudhry MA, Schwacha MG, Bland KI, Chaudry IH. Role of p38 mitogen-activated protein kinase pathway in estrogen-mediated cardioprotection following trauma-hemorrhage. Am J Physiol Heart Circ Physiol. 2007; 292:H2982–2987. [PubMed: 17293487]

Ibrahim BM, Fan M, Abdel-Rahman AA. Oxidative stress and autonomic dysregulation contribute to the acute time-dependent myocardial depressant effect of ethanol in conscious female rats. Alcohol Clin Exp Res. 2014; 38:1205–1215. [PubMed: 24754626]

Jeavons CM, Zeiner AR. Effects of elevated female sex steroids on ethanol and acetaldehyde metabolism in humans. Alcohol Clin Exp Res. 1984; 8:352–358. [PubMed: 6091490]

Kandadi MR, Yu X, Frankel AE, Ren J. Cardiac-specific catalase overexpression rescues anthrax lethal toxin-induced cardiac contractile dysfunction: role of oxidative stress and autophagy. BMC Med. 2012; 10:134. [PubMed: 23134810]

Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K, Jiri I, Harbuz MS. Acetaldehyde, a metabolite of ethanol, activates the hypothalamic-pituitary-adrenal axis in the rat. Alcohol Alcohol. 2001; 36:59–64. [PubMed: 11139417]

Klatsky AL, Armstrong MA, Friedman GD. Risk of cardiovascular mortality in alcohol drinkers, ex-drinkers and nondrinkers. Am J Cardiol. 1990; 66:1237–1242. [PubMed: 2239729]

Konstandi M, Cheng J, Gonzalez FJ. Sex steroid hormones regulate constitutive expression of Cyp2e1 in female mouse liver. Am J Physiol Endocrinol Metab. 2013; 304:E1118–1128. [PubMed: 23548611]

Lee TM, Lin SZ, Chang NC. Both GPER and membrane oestrogen receptor-alpha activation protect ventricular remodelling in 17beta oestradiol-treated ovariectomized infarcted rats. J Cell Mol Med. 2014; 18:2454–2465. [PubMed: 25256868]

Liu S, Le May C, Wong WP, Ward RD, Clegg DJ, Marcelli M, Korach KS, Mauvais-Jarvis F. Importance of extranuclear estrogen receptor-alpha and membrane G protein-coupled estrogen receptor in pancreatic islet survival. Diabetes. 2009; 58:2292–2302. [PubMed: 19587358]

Lu CL, Hsieh JC, Dun NJ, Oprea TI, Wang PS, Luo JC, Lin HC, Chang FY, Lee SD. Estrogen rapidly modulates 5-hydroxytrytophan-induced visceral hypersensitivity via GPR30 in rats. Gastroenterology. 2009; 137:1040–1050. [PubMed: 19344720]

Ma H, Yu L, Byra EA, Hu N, Kitagawa K, Nakayama KI, Kawamoto T, Ren J. Aldehyde dehydrogenase 2 knockout accentuates ethanol-induced cardiac depression: role of protein phosphatases. Journal of molecular and cellular cardiology. 2010; 49:322–329. [PubMed: 20362583]

Mata KM, Li W, Reslan OM, Siddiqui WT, Opsasnick LA, Khalil RA. Adaptive increases in expression and vasodilator activity of estrogen receptor subtypes in a blood vessel-specific pattern during pregnancy. Am J Physiol Heart Circ Physiol. 2015; 309:H1679–1696. [PubMed: 26408543]

Matsumoto H, Matsubayashi K, Fukui Y. Mitochondrial ALDH polymorphism affects ethanol-derived acetate disposition in Wistar rats. Alcohol Clin Exp Res. 1996; 20:284A–288A. [PubMed: 8730219]

Meyer MR, Haas E, Barton M. Gender differences of cardiovascular disease: new perspectives for estrogen receptor signaling. Hypertension. 2006; 47:1019–1026. [PubMed: 16651458]

Meyer MR, Prossnitz ER, Barton M. The G protein-coupled estrogen receptor GPER/GPR30 as a regulator of cardiovascular function. Vascul Pharmacol. 2011; 55:17–25. [PubMed: 21742056]

Meyers MJ, Sun J, Carlson KE, Marriner GA, Katzenellenbogen BS, Katzenellenbogen JA. Estrogen receptor-beta potency-selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. J Med Chem. 2001; 44:4230–4251. [PubMed: 11708925]

Murphy E. Estrogen signaling and cardiovascular disease. Circ Res. 2011; 109:687–696. [PubMed: 21885836]

Oshino N, Oshino R, Chance B. The characteristics of the “peroxidatic” reaction of catalase in ethanol oxidation. Biochem J. 1973; 131:555–563. [PubMed: 4720713]

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]

Sarc L, Lipnik-Stangelj M. Comparison of ethanol and acetaldehyde toxicity in rat astrocytes in primary culture. Arh Hig Rada Toksikol. 2009; 60:297–305. [PubMed: 19789159]

Shaolong Yang LZ, Shunhua Hu, Chatham John C, Bland Kirby I, Machase Richard B, Chaudry Irshad H. The Protective Effect of Estrogen Receptor (ER)-beta Agonist on Cardiac Function Following Trauma-Hemorrhage: Downregulation of Cardiomyocyte L-Type Calcium Current (ICa-L). The FASEB Journal. 2007; 21:33.

Sharma G, Prossnitz ER. GPER/GPR30 Knockout Mice: Effects of GPER on Metabolism. Methods Mol Biol. 2016; 1366:489–502. [PubMed: 26585159]

Shen M, Shi H. Estradiol and Estrogen Receptor Agonists Oppose Oncogenic Actions of Leptin in HepG2 Cells. PLoS One. 2016; 11:e0151455. [PubMed: 26982332]

Shi H, Kumar SP, Liu X. G protein-coupled estrogen receptor in energy homeostasis and obesity pathogenesis. Prog Mol Biol Transl Sci. 2013; 114:193–250. [PubMed: 23317786]

Soffia F, Penna M. Ethanol metabolism by rat heart homogenates. Alcohol. 1987; 4:45–48. [PubMed: 3828063]

Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS, Katzenellenbogen JA. Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-alpha-selective agonists. J Med Chem. 2000; 43:4934–4947. [PubMed: 11150164]

Takahashi T, Lasker JM, Rosman AS, Lieber CS. Induction of cytochrome P-4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. Hepatology. 1993; 17:236–245. [PubMed: 8428720]

Urata Y, Ihara Y, Murata H, Goto S, Koji T, Yodoi J, Inoue S, Kondo T. 17Beta-estradiol protects against oxidative stress-induced cell death through the glutathione/glutaredoxin-dependent redox regulation of Akt in myocardiac H9c2 cells. J Biol Chem. 2006; 281:13092–13102. [PubMed: 16549430]

Weil BR, Manukyan MC, Herrmann JL, Wang Y, Abarbanell AM, Poynter JA, Meldrum DR. Signaling via GPR30 protects the myocardium from ischemia/reperfusion injury. Surgery. 2010; 148:436–443. [PubMed: 20434187]

White RE, Gerrity R, Barman SA, Han G. Estrogen and oxidative stress: A novel mechanism that may increase the risk for cardiovascular disease in women. Steroids. 2010; 75:788–793. [PubMed: 20060403]

Yao F, Abdel-Rahman AA. Estrogen receptor ERalpha plays a major role in ethanol-evoked myocardial oxidative stress and dysfunction in conscious female rats. Alcohol. 2016; 50:27–35. [PubMed: 26695589]

Zhang W, Lu D, Dong W, Zhang L, Zhang X, Quan X, Ma C, Lian H, Zhang L. Expression of CYP2E1 increases oxidative stress and induces apoptosis of cardiomyocytes in transgenic mice. FEBS J. 2011; 278:1484–1492. [PubMed: 21352494]

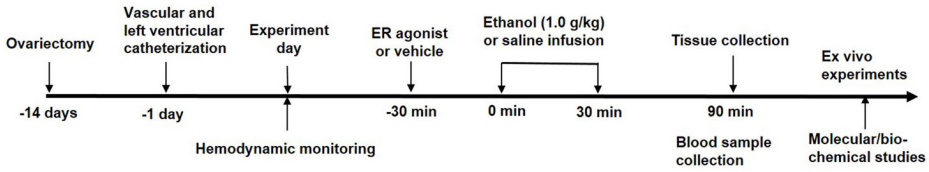

Fig. 1.

A schematic timeline of the experimental design including surgery, hemodynamic recording, pharmacological interventions (ERα, ERβ or GPER agonist in the absence or presence of ethanol) in conscious OVX rats and tissue collection for ex vivo biochemical/molecular studies.

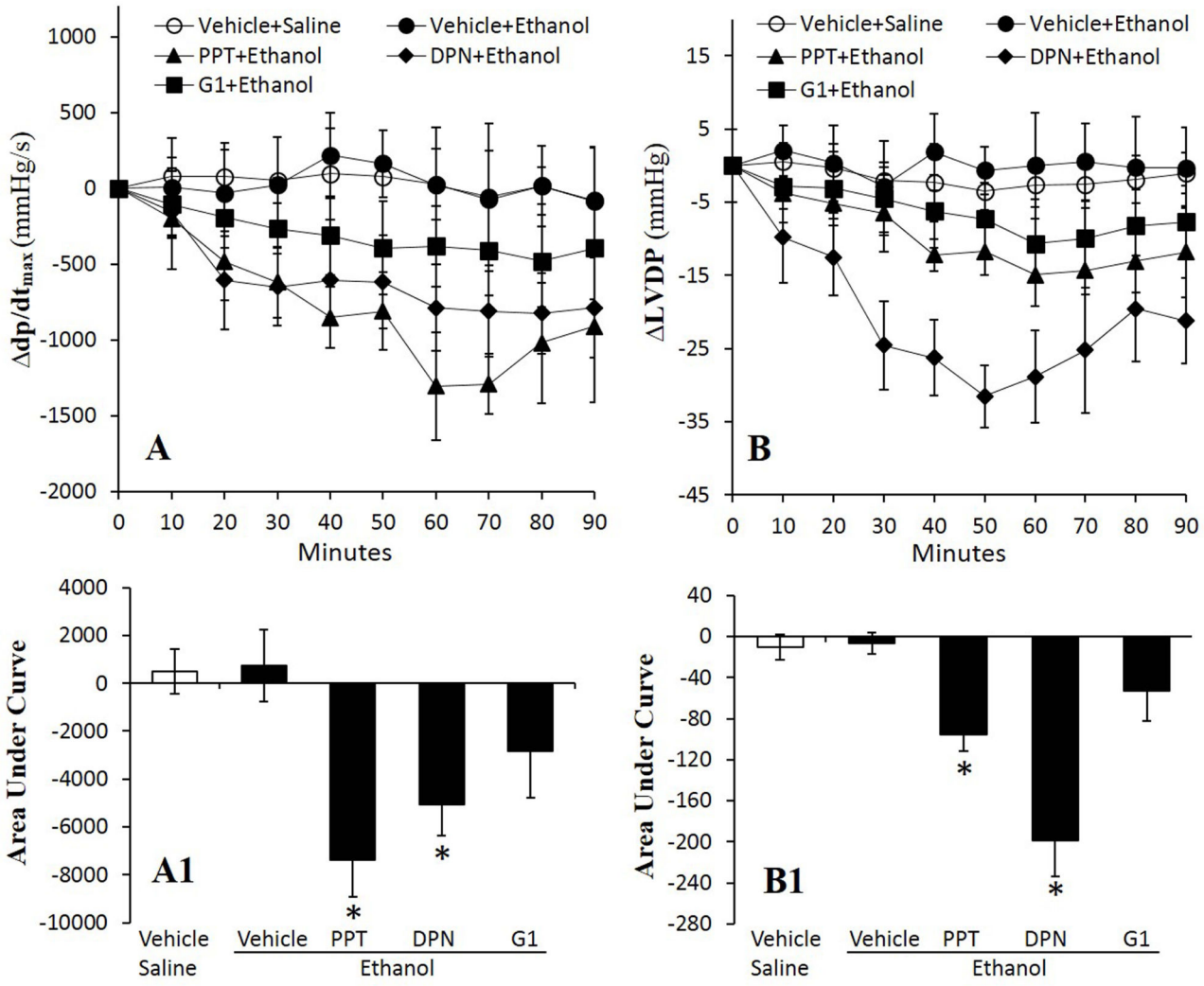

Fig. 2.

Time course of the changes in maximum rate of left ventricular pressure rise (dP/dt_{max}, **A**) and left ventricular developed pressure (LVDP, **B**) caused by ethanol (1.0 g/kg; i.v) in the absence or presence of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1, in conscious OVX rats. Bar graphs (**A1** and **B1**) show the area under curve for each treatment. Values are mean ± SEM. The data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus vehicle + ethanol.

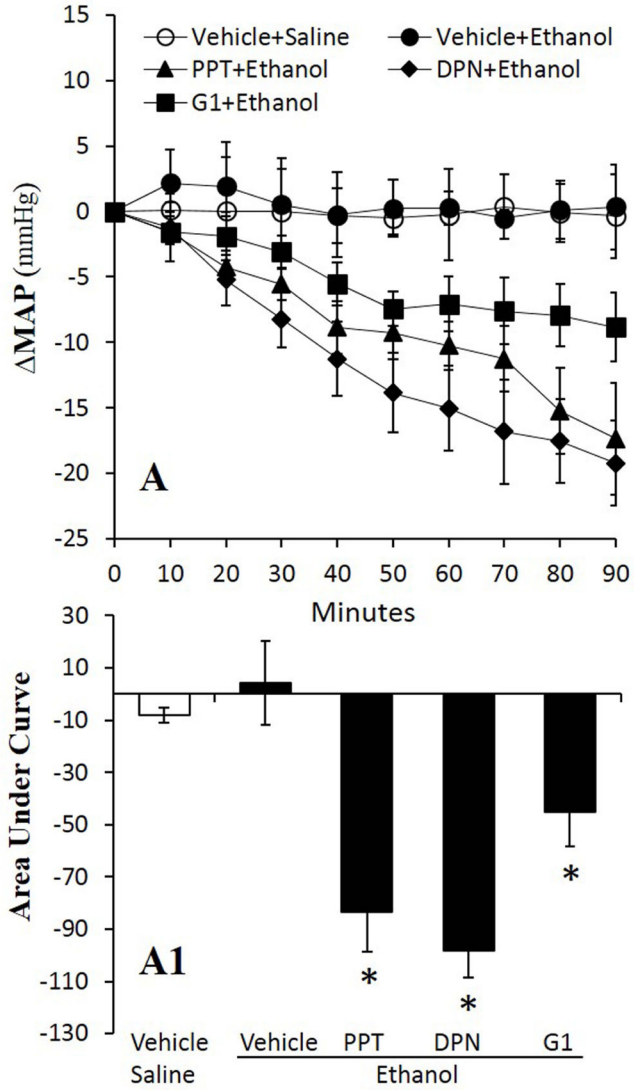

Fig. 3.

Time course of the changes in mean arterial pressure (MAP, **A**) caused by ethanol (1.0 g/kg; i.v) in the absence or presence of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1, in conscious OVX rats. Bar graph (**A1**) shows the area under curve for each treatment. Values are mean ± SEM. The data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus vehicle + ethanol.

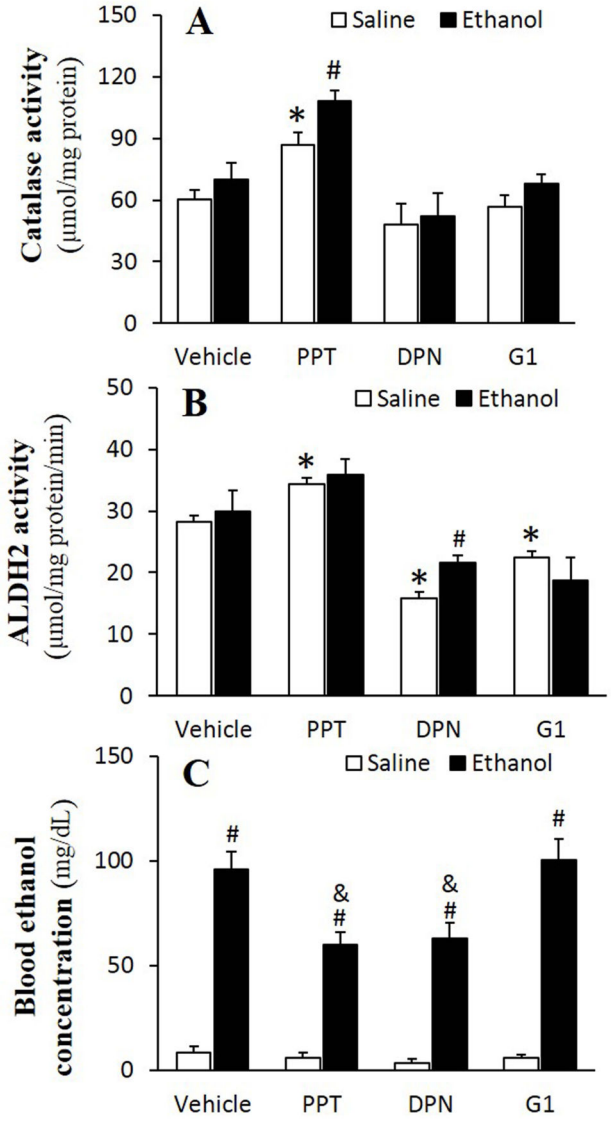

Fig. 4.

The effect of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1 on myocardial catalase (**A**) and mitochondrial aldehyde dehydrogenase (mitALDH2, **B**) activity in the absence or presence of ethanol (1.0 g/kg), and blood ethanol concentration (**C**) in different treatment groups of OVX rats. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus vehicle + saline; #$p < 0.05$, versus agonist + saline. &$p < 0.05$, versus vehicle + ethanol.

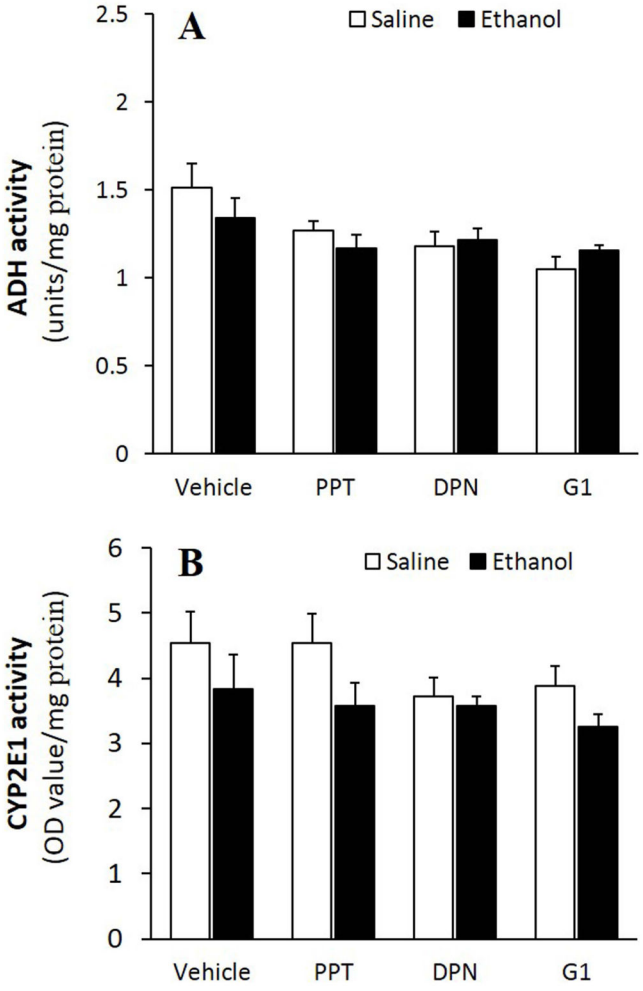

Fig. 5.

The effect of selective ERα, ERβ or GPER activation by PPT, DPN and G1, respectively, on myocardial alcohol dehydrogenase (ADH, **A**) and cytochrome P450 2E1 (CYP2E1, **B**) in OVX rats. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison.

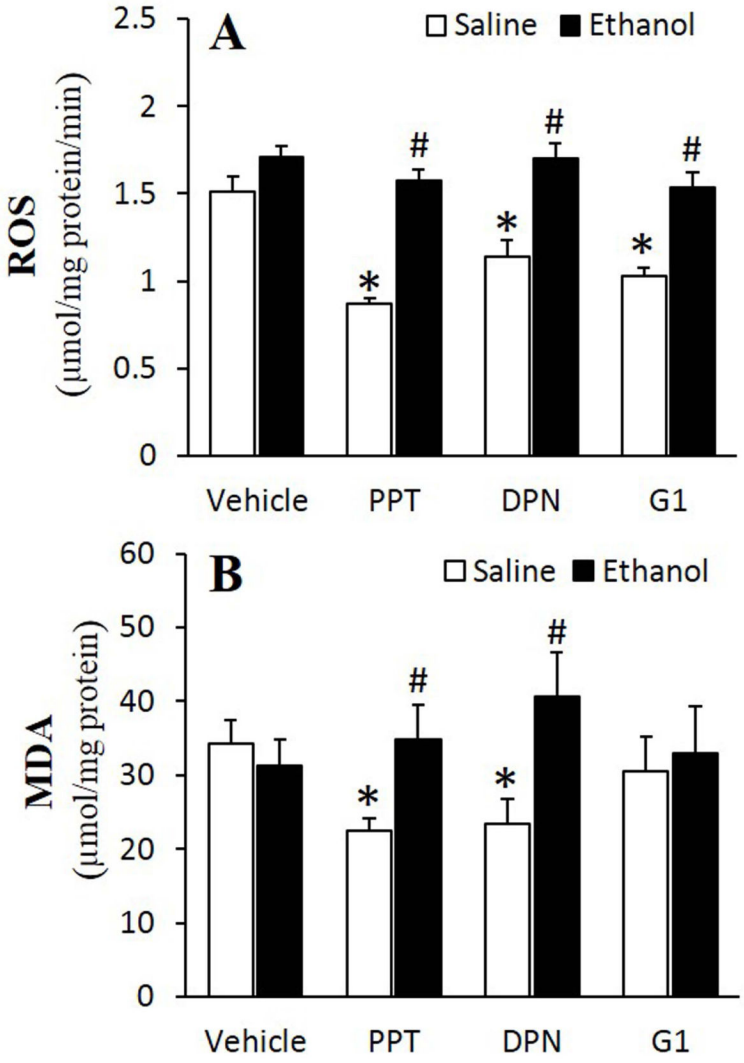

Fig. 6.

The effect of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1 on myocardial ROS level (**A**) and MDA (**B**) in the absence or presence of ethanol (1.0 g/kg) in OVX rats. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus vehicle + saline; #$p < 0.05$, versus agonist + saline.

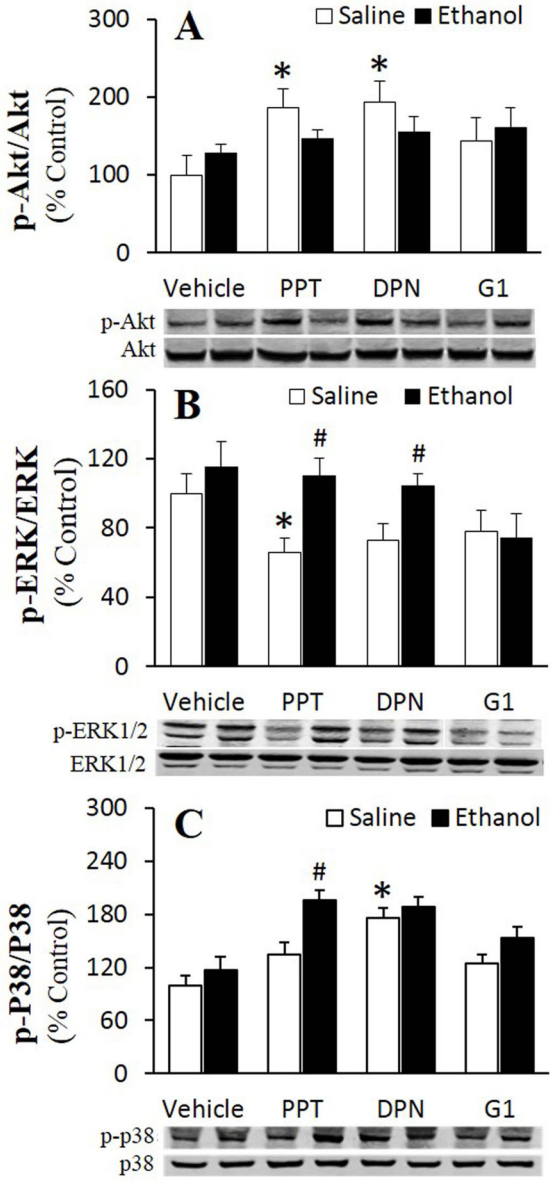

Fig. 7.

The effect of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1 on myocardial Akt (**A**), ERK1/2 (**B**) and p38 (**C**) phosphorylation in the absence or presence of ethanol (1.0 g/kg) in OVX rats. Images under the bar graphs show representative western blot bands of total and phosphorylated Akt, ERK1/2 and p38. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus vehicle + saline; #$p < 0.05$, versus agonist + saline.

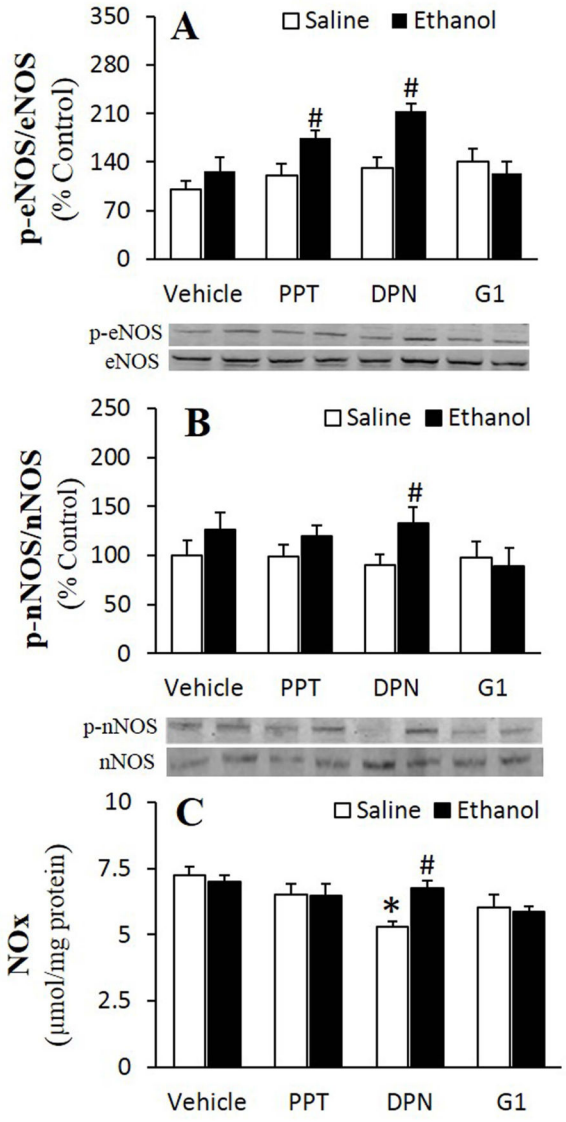

Fig. 8.

The effect of selective ERα, ERβ or GPER activation by its respective agonist PPT, DPN and G1 on myocardial endothelial nitric oxide synthase (eNOS, **A**), and neuronal nitric oxide synthase (nNOS, panel B) phosphorylation, as well as nitrite/nitrate level (NOx, **C**) in the absence or presence of ethanol (1.0 g/kg) in OVX rats. Images under the bar graphs show representative western blot bands of total and phosphorylated eNOS and nNOS. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus vehicle + saline; #$p < 0.05$, versus agonist + saline.

Table 1

Mean arterial pressure (MAP), heart rate (HR), left ventricular developed pressure (LVDP) and maximum rate the left ventricular pressure rise (dP/dt_{max}) values before (baseline) and after vehicle or ER agonist (PPT, DPN or G1) administration. Values are mean ± SEM.

Treatment	N	Hemodynamic Indices	Before	After
Vehicle+Saline	6	MAP (mmHg)	105.5±7.4	104.9±6.5
		HR (bpm)	366±18	374±16
		LVDP (mmHg)	178.1±3.6	176.8±4.4
		dP/dt_{max} (mmHg/s)	9507.5±598.2	9536.6±701.3
PPT+Saline	7	MAP (mmHg)	107.8±10.7	106.2±8.4
		HR (bpm)	348±8	352±7
		LVDP (mmHg)	168.9±10.5	166.3±7.2
		dP/dt_{max} (mmHg/s)	8866.8±717.8	8761.3±628.3
DPN+Saline	7	MAP (mmHg)	103.7±12.5	102.8±7.5
		HR (bpm)	349±26	357±23
		LVDP (mmHg)	169.0±13.9	168.3±9.1
		dP/dt_{max} (mmHg/s)	8942.9±1407.7	9123.4±1580.6
G1+Saline	7	MAP (mmHg)	105.7±4.6	103.1±5.3
		HR (bpm)	355±36	360±29
		LVDP (mmHg)	176.5±4.3	174.5±5.6
		dP/dt_{max} (mmHg/s)	9051.0±404.5	8999.3±428.1