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Combined Catalase and ADH Inhibition Ameliorates Ethanol-induced Myocardial Dysfunction Despite Causing Oxidative Stress in Conscious Female Rats

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Abstract

Background—Ethanol-evoked oxidative stress, which contributes to myocardial dysfunction in proestrus rats, is mediated by increases in NADPH oxidase activity, malondialdehyde (MDA) and ERK1/2 phosphorylation. Whether these biochemical responses, which are triggered by alcohol-derived acetaldehyde in non-cardiac tissues, occur in proestrus rats' hearts remains unknown. Therefore, we elucidated the roles of alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1) and catalase, which catalyze alcohol oxidation to acetaldehyde, in these alcohol-evoked biochemical and hemodynamic responses in proestrus rats.

Method—Conscious proestrus rats prepared for measurements of left ventricular (LV) function and blood pressure (BP) received ethanol (1.5 g/kg, i.v infusion over 30 min) or saline 30 min after an ADH and CYP2E1 inhibitor, 4-MP (82 mg/kg, i.p), a catalase inhibitor, 3-AT (0.5 g/kg, i.v), their combination or vehicle. LV function and BP were monitored for additional 60 min after ethanol or saline infusion before collecting the hearts for *ex vivo* measurements of LV reactive oxygen species (ROS), NADPH oxidase activity, MDA, and ERK1/2 phosphorylation.

Results—Ethanol reduced LV function (dP/dt_{max} and LVDP) and BP, and increased cardiac NADPH oxidase activity, ROS and MDA levels, and ERK1/2 phosphorylation. Either inhibitor partially, and their combination significantly, attenuated these responses despite the substantially higher blood ethanol level, and the increased cardiac oxidative stress and reduced BP caused by 3-AT alone or with 4-MP. The inhibitors reduced cardiac MDA level and reversed ethanol effect on cardiac and plasma MDA.

Conclusions—Ethanol oxidative metabolism plays a pivotal role in the ethanol-evoked LV oxidative stress and dysfunction in proestrous rats. Notably, catalase inhibition (3-AT) caused cardiac oxidative stress and hypotension.

Keywords

Ethanol; Myocardial Dysfunction; Alcohol Dehydrogenase; Cytochrome P4502E1; Catalase; Oxidative Stress

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INTRODUCTION

Alcohol metabolism plays an important role in organ injury, particularly through the generation of reactive oxygen species (ROS) (Zakhari, 2006). Hepatic alcohol dehydrogenase (ADH) along with cytochrome P4502E1 (CYP2E1) and catalase, in other tissues such as brain (Zakhari, 2006), are major enzymes that catalyze ethanol oxidation to a highly reactive and toxic metabolite, acetaldehyde. While ethanol-evoked oxidative stress is implicated in heart injury (Matyas et al., 2016, Piano and Phillips, 2014, Piano, 2002), this evidence is based on chronic exposure to ethanol. It is noteworthy that our studies unraveled oxidative stress-related myocardial dysfunction caused by acute ethanol only in the presence of endogenous estrogen (E_2) (Ibrahim et al., 2014, El-Mas and Abdel-Rahman, 2014, Yao and Abdel-Rahman, 2016) or exogenous E_2 administration to OVX (ovariectomized) (El-Mas and Abdel-Rahman, 2000) or male (El-Mas and Abdel-Rahman, 2015) rats. However, the role of these metabolizing enzymes in the alcohol-evoked myocardial oxidative stress/dysfunction in proestrus rats remains unknown.

Ethanol-evoked increase in ADH catalytic activity is associated with increases in malondialdehyde (MDA) and ROS levels (Kalaz et al., 2012, El-Mas and Abdel-Rahman, 2014, Hoek et al., 2002, Haorah et al., 2005). Notably, ROS is a product of oxidation of polyunsaturated fatty acids, and MDA level reflects lipid peroxidation (Grotto et al., 2009). ADH inhibition abrogated ethanol-evoked oxidative stresses (Nishitani and Matsumoto, 2006), and protected hepatocytes from ethanol-induced apoptosis (Bailey and Cunningham, 1998). Whether ADH inhibition protects against the acute ethanol-evoked myocardial dysfunction in female rats remains unknown. Besides ADH, the CYP2E1 plays an important role in ethanol oxidation in liver or other organs (Zimatkin et al., 2006, Zimatkin and Deitrich, 1997). CYP2E1 activity is induced by chronic (Takahashi et al., 1993) or acute (Haorah et al., 2005) alcohol administration. Further, CYP2E1 inhibition, knockdown or knockout attenuated oxidative stress (Jin et al., 2013, Lu et al., 2008, Bardag-Gorce et al., 2000), and myocardial contractile dysfunction (Zhang et al., 2013) caused by alcohol. Nonetheless, dual pharmacological inhibition of ADH and CYP2E1 by 4-MP (Plumlee et al., 2005) may not be sufficient to fully inhibit ethanol oxidative metabolism in the presence of E_2.

Catalase, the third enzyme that catalyzes ethanol oxidation to acetaldehyde (Zimatkin and Deitrich, 1997), also serves an antioxidant role in many tissues including heart (Chelikani et al., 2004). The latter role is consistent with the ability of catalase overexpression to protect against ethanol-evoked dysfunction of myocytes isolated from male mice (Zhang et al., 2003). By marked contrast, we showed that acute ethanol-evoked increase in cardiac catalase activity was associated with myocardia oxidative stress/dysfunction in E_2 replete rats (Ibrahim et al., 2014, El-Mas and Abdel-Rahman, 2014, Yao and Abdel-Rahman, 2016). Based on these contradictory findings it remains unknown whether catalase protects against or contributes to ethanol-evoked myocardial dysfunction in our model system. While sex difference and approaches (isolated myocytes vs. intact animal LV function studies) must be considered, it is notable that pharmacological catalase inhibition reduced oxidation of alcohol (Thurman and Handler, 1989), and attenuated ethanol-induced behavioral effects (Koechling and Amit, 1994, Pastor and Aragon, 2008). Whether a similar pharmacological

approach exacerbates or attenuates the E_2-dependent myocardial oxidative stress/dysfunction in female rats has not been investigated.

The aim of this study was to elucidate the mechanistic roles of the ethanol metabolizing enzymes, ADH, CYP2E1 and catalase, in the E_2-dependent myocardial oxidative stress and dysfunction caused by acute ethanol in female rats.

MATERIAL AND METHODS

Animals

Female SD rats (200–250 g, Charles River, Raleigh, NC) were kept in the university animal care center at temperature of 23 ± 1°C, humidity of 50 ± 10%, and a 12-h light/dark cycle and with access free to food (Prolab Rodent Chow, Granville Milling, Creedmoor, NC) and water. The surgical and animal care procedures 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).

Catheterization

The animals were anesthetized by the mixture of ketamine (90 mg/kg) and xylazine (10 mg/kg i.p). Vascular and left ventricular (LV) catheterizations were performed as detailed in our recent study (Yao and Abdel-Rahman, 2016, Yao and Abdel-Rahman, 2017) under sterile conditions. Briefly, heparinized arterial/venous catheters (Konigsberg Instruments Inc., Pasadena, CA) were placed into the abdominal aorta/vena cava via the femoral artery/vein for monitoring blood pressure (BP) and administering the pharmacological intervention (ethanol metabolizing enzyme inhibitors, ethanol or saline), respectively. The rats received buprenorphine (0.03 mg/kg; s.c) for postoperative analgesia.

Hemodynamic recording

LV function and BP measurements were conducted in conscious freely moving rats as detailed in our previous studies (Yao and Abdel-Rahman, 2016, Ibrahim et al., 2014, Yao and Abdel-Rahman, 2017). BP and LV indices were simultaneously recorded by ML870 (PowerLab 8/30; Colorado Springs, CO) via Gould-Statham pressure transducers (Oxnard-CA).

Plasma ethanol concentration

The whole blood was centrifuged and the plasma was collected for measurement of ethanol concentration as descripted in our previous studies (Yao and Abdel-Rahman, 2016, El-Mas et al., 2008, Yao and Abdel-Rahman, 2017). The reaction between ethanol and ADH took place in a 96-well plate. An Infinite® 200 PRO multimode microplate reader (Tencan Group Ltd., Männedorf, Schweiz) was used to detect the absorbance at 340 nm.

ROS level

We followed the methods in our previous studies (Yao and Abdel-Rahman, 2016, McGee and Abdel-Rahman, 2012, Yao and Abdel-Rahman, 2017). The supernatant of the homogenized myocardial tissue was used to measure the ROS formation by a fluorometric

assay using DCFH-DA (Molecular Probes-Thermo Fisher Scientific Inc., Raleigh, NC). The fluorescence intensities were read at 485/530 nm (excitation/emission) with Infinite® 200 PRO multimode microplate readers (Tencan Group Ltd., Männedorf, Schweiz) at 37°C.

NADPH oxidase (Nox) activity

As described in a previous study (La Favor et al., 2013), 20 μl supernatant of the homogenized LV tissue (100 μg protein)/standard was incubated with 10 μM Amplex Red (Molecular Probes, OR), 2.0 U/ml horseradish peroxidase, 30 U/ml superoxide dismutase and 100 μM NADPH (Sigma Aldrich, St. Louis, MO) for 90 min at 37°C. Fluorescence intensity (530 nM ex/590 nM em) was measured.

MDA level

As described in our previous studies (Yao and Abdel-Rahman, 2016, Yao and Abdel-Rahman, 2017), TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) was used for measuring MDA levels. The MDA-TBA adduct was measured colorimetrically at 530–540 nm.

Western blot

We followed the protocols described in our studies (Yao and Abdel-Rahman, 2016, Ibrahim et al., 2014, Yao and Abdel-Rahman, 2017). The supernatant (40 μg of protein) of the homogenized LV tissue was separated in a 4–12% gel electrophoresis (Novex Tis-Glycine gel, Life Technologies, CA), and transferred to nitrocellulose membrane, and then incubated with a primary antibody (mouse anti-p-ERK1/2 and rabbit anti ERK1/2; 1:500, Cell Signaling, Danvers, MA). Thereafter, the membrane was incubated with a secondary antibody (IRDye680-conjugated goat anti-mouse and IRDye800-conjugated goat anti-rabbit, 1:15000, LI-COR Biosciences, Lincoln, NE), and the protein bands were detected and quantified by Odyssey Infrared Imager (LI-COR Biosciences, Lincoln, NE).

Experimental protocols

Experiment 1. Effect of inhibiting ADH, CYP2E1 or catalase on ethanol-evoked myocardial dysfunction and hypotension in proestrus rats—Our previous findings documented that acute ethanol administration causes myocardial dysfunction (lower dP/dt_{max} and LVDP), at least partly, via oxidative stress (higher ROS and MDA levels) (Ibrahim et al., 2014, El-Mas and Abdel-Rahman, 2014, Yao and Abdel-Rahman, 2016). Here, we investigated the effect of the ADH and CYP2E1 inhibitor 4-methylpyrazole (4-MP) (Knoester et al., 2002), the catalase inhibitor 3-Amino-1,2,4-triazole (3-AT) (Correa et al., 2008) or their combination on the ethanol-evoked myocardial dysfunction in proestrus rats. The proestrus phase, determined by vaginal smear (Marcondes et al., 2001), coincides with the highest endogenous plasma E_2 levels (El-Mas and Abdel-Rahman, 2014). Fifty-two female SD rats, divided into 8 groups (5–8 rats/group), were used in this experiment. The LV function and BP were measured in conscious rats 2 days after LV and femoral vascular catheterization. At least 30 min was allowed for stabilization of the measured variables. The 8 groups were divided into 4 pairs with each pair receiving 4-MP (82 mg/kg, i.p), 3-AT (0.5 g/kg, i.v), their combination or saline 30 min before i.v infusion of ethanol (1.5 g/kg),

delivered as 0.39 ml of 50% ethanol per 100 g body weight over 30 min, or equal volume of saline. The dose of 4-MP (Waller et al., 1982, Jamal et al., 2007) and 3-AT (Correa et al., 2008) was based on reported studies. The ethanol regimen was based on our studies (El-Mas and Abdel-Rahman, 2014, El-Mas and Abdel-Rahman, 2015). Mean arterial pressure (MAP), LV developed pressure (LVDP) and maximum rate of LV pressure rise (dP/dt_{max}) were recorded for 90 min. Blood samples were collected before any treatment and at 30, 60 and 90 min after ethanol or saline infusion. At the end of the hemodynamic measurements, all rats were euthanized, and the hearts were collected for conducting the ex vivo biochemical studies.

Experiment 2. Effect of ADH, CYP2E1 or catalase inhibition on blood ethanol level and mediators of myocardial oxidative stress caused by alcohol—In these ex vivo biochemical studies, we measured the time-course changes in blood ethanol concentration in ethanol-treated rats in the absence or presence of the enzyme inhibitors, 4-MP, 3-AT, or their combination. Further, we measured ROS, MDA, Nox activity, ERK1/2 phosphorylation level in the hearts of the treatment and control groups used in the study under experiment 1, above.

Drugs

Ethanol was purchased from Midwest Grain Products Co. (Weston, MO) and diluted in saline. 3-AT and 4-MP (Sigma Aldrich, St. Louis, MO) were dissolved in saline.

Data analysis and statistics

The hemodynamic responses caused by pretreatment with the inhibitor(s) of ethanol metabolizing enzymes were analyzed by two-tailed t-test vs. their vehicle, saline (Table 1). The areas under the curve for hemodynamic indices and the biochemical data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison and Bonferroni correction when the pairwise t-tests were performed. The statistical analysis was conducted with Prism version 5 (GraphPad Software, Inc. La Jolla, CA). Values are presented as mean ± SEM. $p < 0.05$ was considered significant.

RESULTS

Inhibition of ADH and CYP2E1 (4-MP), catalase (3-AT) or their combination mitigated ethanol-evoked myocardial dysfunction in proestrus rats

Inhibition of ADH and CYP2E1 by 4-MP alone had no effect on all hemodynamic indices. However, catalase inhibition alone (3-AT) or in combination with 4-MP, reduced ($p < 0.05$) myocardial function (dP/dt_{max}, LVDP) and MAP (Table 1). In saline (vehicle for enzyme inhibitors) pretreated rats, ethanol (1.5 g/kg) reduced ($p < 0.05$) dP/dt_{max}, LVDP and MAP (Figs. 1–3). Pretreatment with 4-MP or 3-AT partially, while their combination significantly ($p < 0.05$), attenuated these adverse hemodynamic effects of ethanol (Fig. 1E and F).

The effect of 4-MP, 3-AT or their combination on blood ethanol

Blood ethanol concentration (BAC) reached 131 ± 8.2 mg/dl at 30 min after ethanol (1.5 g/kg) infusion, and then dropped to 104.8 ± 8.7 and 98.3 ± 5.9 mg/dl at 60 and 90 min,

respectively (Fig. 4A). However, BAC was higher ($p < 0.05$) in rats pretreated with 4-MP or 3-AT and synergistically increased by their combination (Fig. 4).

Inhibition of ADH and CYP2E1 (4-MP), catalase (3-AT) or their combination abrogated ethanol-evoked myocardial oxidative stress in proestrus rats

Ethanol increased ($p < 0.05$) ROS level (Fig. 5A), Nox activity (Fig. 5B), ERK1/2 phosphorylation (Fig. 6), and MDA level (Fig. 7A) in the hearts as well as MDA level in plasma (Fig. 7B) of proestrus rats. These ethanol-evoked biochemical responses, which reflect oxidative stress, were abrogated in rats pretreated with 4-MP, 3-AT or their combination (Figs. 5 and 7). Notably, catalase inhibition (3-AT) increased ($p < 0.05$) myocardial ROS level, Nox activity, and ERK1/2 phosphorylation as well as plasma MDA levels (Figs. 5–7). However, 4-MP, 3-AT or their combination reduced ($p < 0.05$) myocardial MDA levels (Fig. 7A). Despite this significant ($p < 0.05$) reduction in cardiac or elevation in plasma MDA levels, caused by combining 4-MP and 3-AT, subsequent ethanol administration reduced ($p < 0.05$) cardiac (Fig. 7A) and plasma (Fig. 7B) MDA levels. Collectively, the attenuation of the ethanol-evoked increases in mediators of oxidative stress by 4-MP, 3-AT or their combination (Figs. 5–7) paralleled the abrogation of reductions in myocardial function in the same rats (Figs. 1 and 2).

DISCUSSION

The present study generated important data on the roles of three ethanol metabolizing enzymes, ADH, CYP2E1 and catalase, in myocardial oxidative stress/dysfunction caused by acute ethanol in E_2 replete female rats. We show that ADH and CYP2E1 (4-MP) or catalase (3-AT) inhibition partially, while their combination drastically, attenuated the ethanol-evoked deleterious cardiac effects. The results of our ex vivo biochemical studies suggest an important role for the first oxidative metabolite of ethanol in triggering mediators of myocardial oxidative stress. The latter is a major contributor to the E_2-dependent myocardial dysfunction caused by alcohol. It is imperative to be cognizant of the induction of cardiac oxidative stress and the hypotension caused by 3-AT when interpreting the present findings.

We documented a pivotal role for myocardial oxidative stress in the sex/E_2 dependent myocardial dysfunction caused by acute ethanol administration in proestrus rats (Ibrahim et al., 2014, El-Mas and Abdel-Rahman, 2014, Yao and Abdel-Rahman, 2016). Realizing that acetaldehyde, the first ethanol oxidative metabolite, causes MAPK-dependent oxidative stress in many tissues (Yan et al., 2016, Ku et al., 2007), it was important to elucidate the role of this metabolic pathway in ethanol-evoked myocardial dysfunction. However, we needed to consider the possibility that this metabolic pathway is not involved in this deleterious cardiac effect of ethanol because acetaldehyde generation does not explain some effects of ethanol in other tissues (Haber et al., 2004, Manautou et al., 1992, Czech and Hartleb, 2002), and catalase overexpression protects against ethanol evoked dysfunction of isolated cardiac myocytes (Yao et al., 2015). Therefore, it was important to study, for the first time, the consequences of inhibiting ADH, CYP2E1 and catalase, which catalyze ethanol metabolism to acetaldehyde (Zakhari, 2006) in our model system. We administered 4-MP, an inhibitor of both ADH and CYP2E1 (Knoester et al., 2002), 3-AT, an inhibitor of

catalase (Correa et al., 2008), alone or in combination. This approach permitted evaluation of the contribution of the three ethanol metabolizing enzymes to the ethanol-evoked myocardial dysfunction.

In support of our hypothesis, ethanol-evoked myocardial dysfunction was attenuated by prior inhibition of ADH and CYP2E (4-MP) or catalase (3-AT) and virtually abolished by their combination (Figs. 1–3). The parallel attenuation of ethanol-evoked increases in oxidative stress (ROS) and related molecular events (MDA; ERK1/2 phosphorylation) (Figs. 5–7) suggest mechanistic roles for these biochemical effects in ethanol-evoked myocardial dysfunction and its rescue following the inhibition of ethanol oxidative metabolism. This protective effect resembles protection against ethanol-evoked apoptosis in hepatocytes (Bailey and Cunningham, 1998) by ADH inhibition (Nishitani and Matsumoto, 2006) via reducing ethanol-elicited oxidative stresses (Bailey and Cunningham, 1998). Further, genetic and pharmacological CYP2E1 inhibition attenuated chronic ethanol-induced myocardial contractile dysfunction in mice (Zhang et al., 2013), but these studies were conducted in vitro on myocytes isolated from male mice. Finally, pharmacological inhibition of catalase depressed oxidation of alcohol (Thurman and Handler, 1989), prevented ethanol-induced behavioral stimulation (Pastor and Aragon, 2008) and motivation for alcohol consumption (Koechling and Amit, 1994). However, the effects of catalase inhibition on the E_2-dependent myocardial oxidative stress and dysfunction have not been investigated.

The present study further supports the role of catalase in our model system because reported studies, including ours, show that E_2 enhances catalase catalytic activity in the heart of female rats (Ibrahim et al., 2014, El-Mas and Abdel-Rahman, 2014, Yao and Abdel-Rahman, 2016, Campos et al., 2014). Further, acute ethanol causes additional increase in cardiac catalase activity in the presence of E_2 receptor-alpha activation along with myocardial oxidative stress and dysfunction (Yao and Abdel-Rahman, 2017). The ability of catalase inhibition (3-AT) to attenuate ethanol-evoked LV dysfunction (Figs. 1–2) and oxidative stress (Figs. 5 and 7) supports a contributory role for catalase in these adverse effects. The partial attenuation of the ethanol-evoked myocardial dysfunction (dP/dt_{max} and LVDP) by 3-AT or 4-MP (Figs. 1–2), might be explained by: (i) the ability of the available enzyme(s) to compensate for the inhibited enzyme(s) or (ii) the higher blood alcohol concentration achieved (Fig. 4) because alcohol itself exerts direct cardiotoxic effect (Awtry and Philippides, 2010). Notably, the accumulation of non-oxidative ethanol metabolites such as fatty acid ethyl ester in blood (Doyle et al., 1994) and heart (Laposata and Lange, 1986) is increased following inhibition of ethanol oxidative metabolism (Werner et al., 2002). The present findings argue against the contribution of these alternative mechanisms because combined enzyme inhibition (3-AT plus 4-MP) conferred much better hemodynamic protection (Fig. 2). Interestingly, in these rats, the synergistically higher BAC (Fig. 5) correlated with substantial reductions in cardiac and plasma MDA levels (Fig. 7). While MDA reduction might explain the improved cardiac function, it remains to be determined how ethanol-evoked increase in MDA observed here and in our previous studies (Ibrahim et al., 2014) is reversed in the presence of combined ADH, CYP2E1 and catalase inhibition (Fig. 7).

The present study provides physiologically relevant data with catalase inhibition in the absence of ethanol. Consistent with its antioxidant role (Chelikani et al., 2004), and the ability of catalase inhibition to increase ROS production in isolated ventricular myocytes (Boles et al., 2014), 3-AT caused myocardial oxidative stress (higher ROS) level (Fig. 5A), at least partly, via enhanced Nox activity (Fig. 5B) and ERK1/2 phosphorylation (Fig. 6). Further, a central mechanism might play a role in the reduced BP because injection of 3-AT into the fourth ventricle (Valenti et al., 2010) or nucleus tractus solitarius (Cardoso et al., 2009) decreases BP in conscious rats. We also showed that 3-AT significantly increased plasma MDA level (Fig. 7B), which is consistent with higher plasma MDA in patients with lower catalase activity (Begenik et al., 2013). Notably, the inconsistency between lower myocardial and higher plasma MDA levels following 4-MP, 3-AT or their combination (Fig. 7A) also exists between lower liver and higher plasma MDA levels in reported studies (El-Bassiouni et al., 1998).

It is imperative to consider the impact of the biochemical and hemodynamic effects of 3-AT, given alone or with 4-MP, on data interpretation. First, while statistically nonsignificant, the reductions in LVDP and dP/dt_{max} caused by 3-AT might limit, at least partly, the ethanol-evoked LV dysfunction. This possibility is supported by the inability of ethanol to reduce BP when BP was significantly reduced in rats pretreated with 3-AT alone or in combination with 4-MP. Second, the 3-AT evoked increases in ROS and MDA levels, Nox activity and ERK1/2 phosphorylation might have limited the ethanol-evoked increases in these mediators of oxidative stress. Notably, the abrogation of the ethanol-evoked increases in ROS level and Nox activity despite a lack of change in their basal levels, in 4-MP pretreated rats, argues against this possibility. Further, we observed unexplained reductions in cardiac MDA caused by 4-MP, 3-AT and their combination and an additional reduction in rats treated with both inhibitors and ethanol (Fig. 7). Further studies are needed to elucidate the mechanisms of these biochemical effects caused by these enzyme inhibitors and ethanol.

The current study yields a novel insight into the role of ethanol oxidative metabolism in myocardial dysfunction caused by alcohol via accumulation of cardiotoxic molecules (ROS and MDA). While inhibiting ADH and CYP2E1 (4-MP), catalase (3-AT) or their combination substantially increased BAC (Fig. 4), this pharmacological approach abrogated the ethanol-evoked increases in cardiac Nox activity, ROS and MDA levels, as well as ERK1/2 phosphorylation. The findings support the contribution of these molecules, generated via oxidative metabolism of ethanol, to ethanol-evoked myocardial dysfunction in female rats. Nonetheless, at the doses used in behavioral studies, the enzyme inhibitors (3-AT and 4-MP) exerted biochemical and cardiovascular effects that might have influenced data interpretation.

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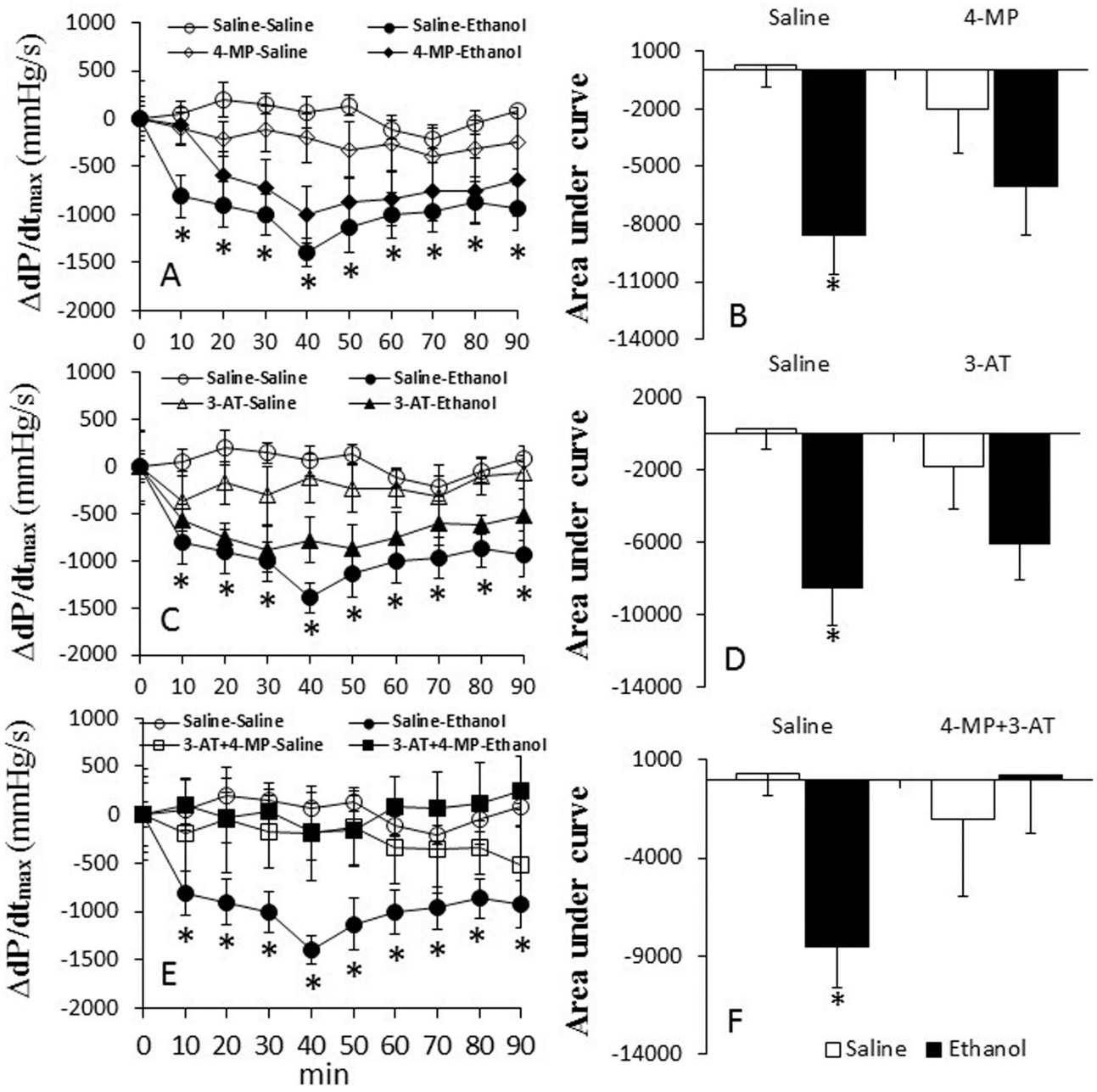

Fig. 1.

Prior inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination (30 min) ameliorates ethanol-evoked myocardial dysfunction in conscious female rats. The line graphs show the time course of the changes in the maximum rate of rise of left ventricular pressure (dP/dt_{max}) caused by ethanol in the absence or presence of 4-MP **(A)**, 3-AT **(C)** or their combination **(E)**. The bar graphs (**B, D and F**) represent the areas under the curves in these groups, respectively. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus saline + saline. #$p < 0.05$, versus saline-ethanol.

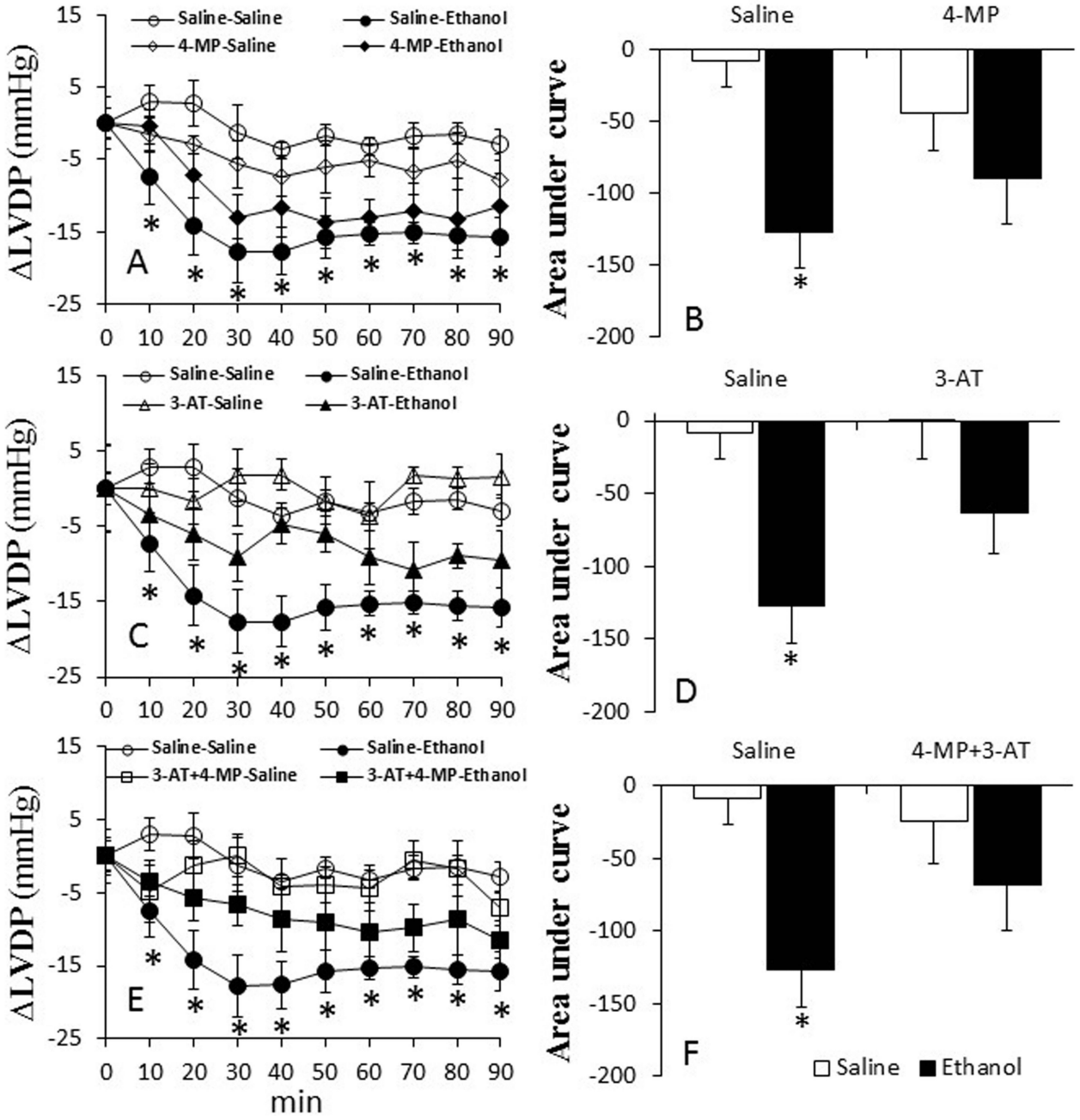

Fig. 2.

Prior inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination (30 min) ameliorates ethanol-evoked myocardial dysfunction in conscious female rats. The line graphs show the time course of the changes in left ventricular developed pressure (LVDP) caused by ethanol in the absence or presence of 4-MP **(A)**, 3-AT **(C)** or their combination **(E)**. The bar graphs (**B, D and F**) represent the areas under curves in these groups, respectively. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus saline-saline.

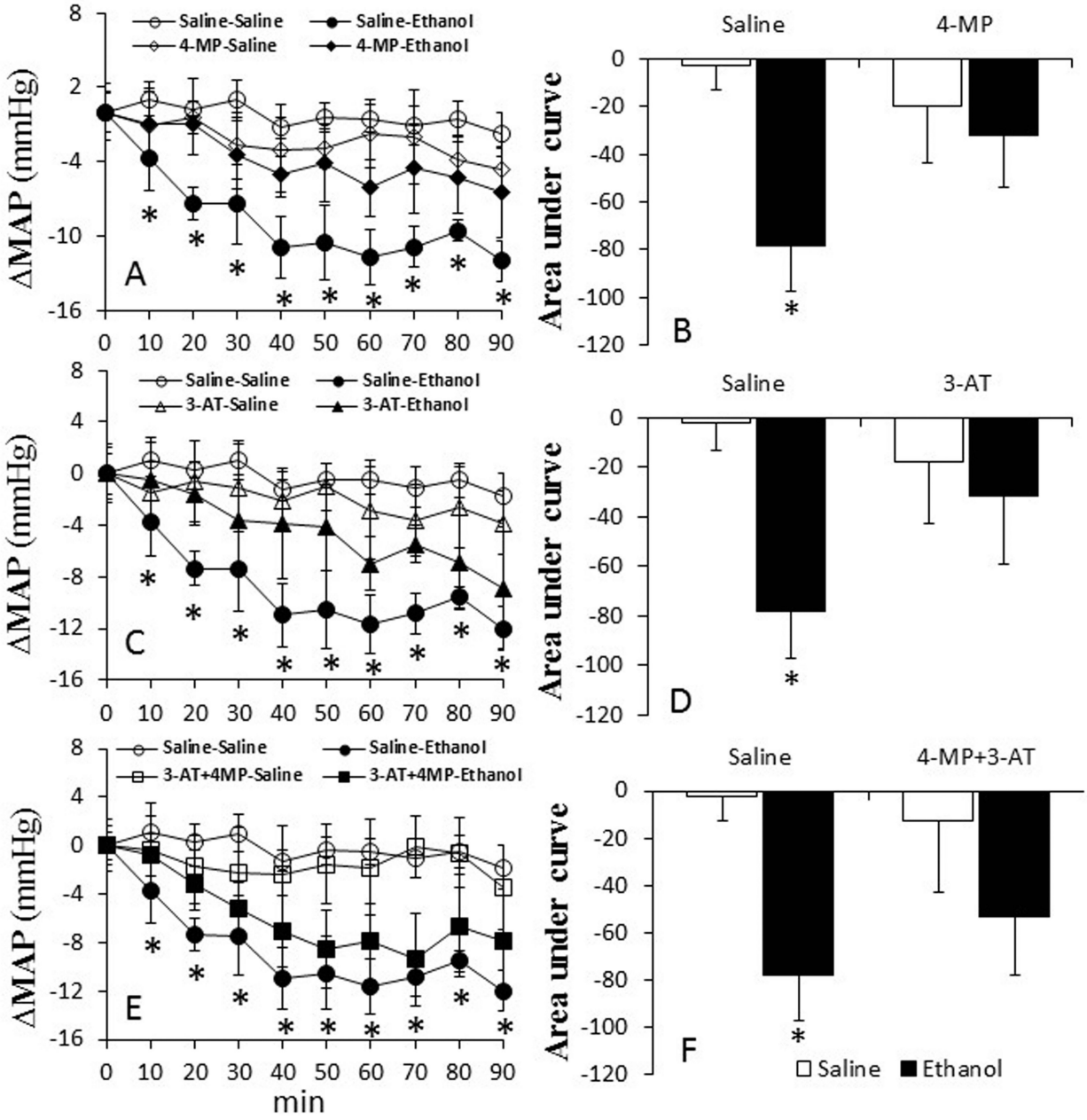

Fig. 3.

Prior inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination (30 min) ameliorates ethanol-evoked myocardial dysfunction in conscious female rats. The line graphs show the time course of the changes in mean arterial pressure (MAP) caused by ethanol in the absence or presence of 4-MP **(A)**, 3-AT **(C)** or their combination **(E)**. The bar graphs (**B, D and F**) represent the areas under curves in these groups, respectively. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by post-hoc Tukey's t-test comparison. *$p < 0.05$, versus saline-saline.

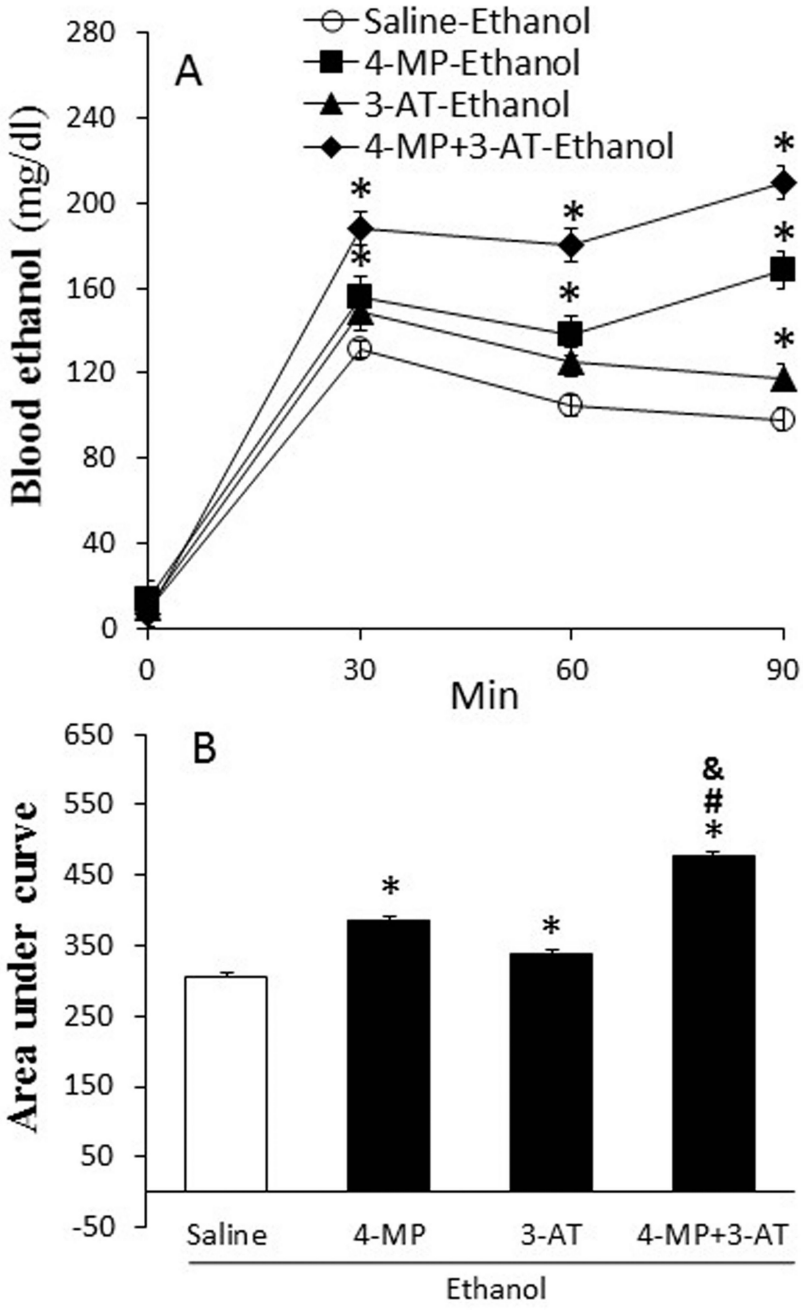

Fig. 4.

Ethanol metabolism was suppressed by inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination. Panel **A** shows the time curse of blood ethanol concentration at 30, 60, and 90 min after ethanol infusion (1.5 g/kg; i.v). The bar graph **(B)** represents the areas under curves in these groups. Values are mean ± SEM. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus saline. #$p < 0.05$, versus 4-MP. &$p < 0.05$, versus 4-MP+3-AT.

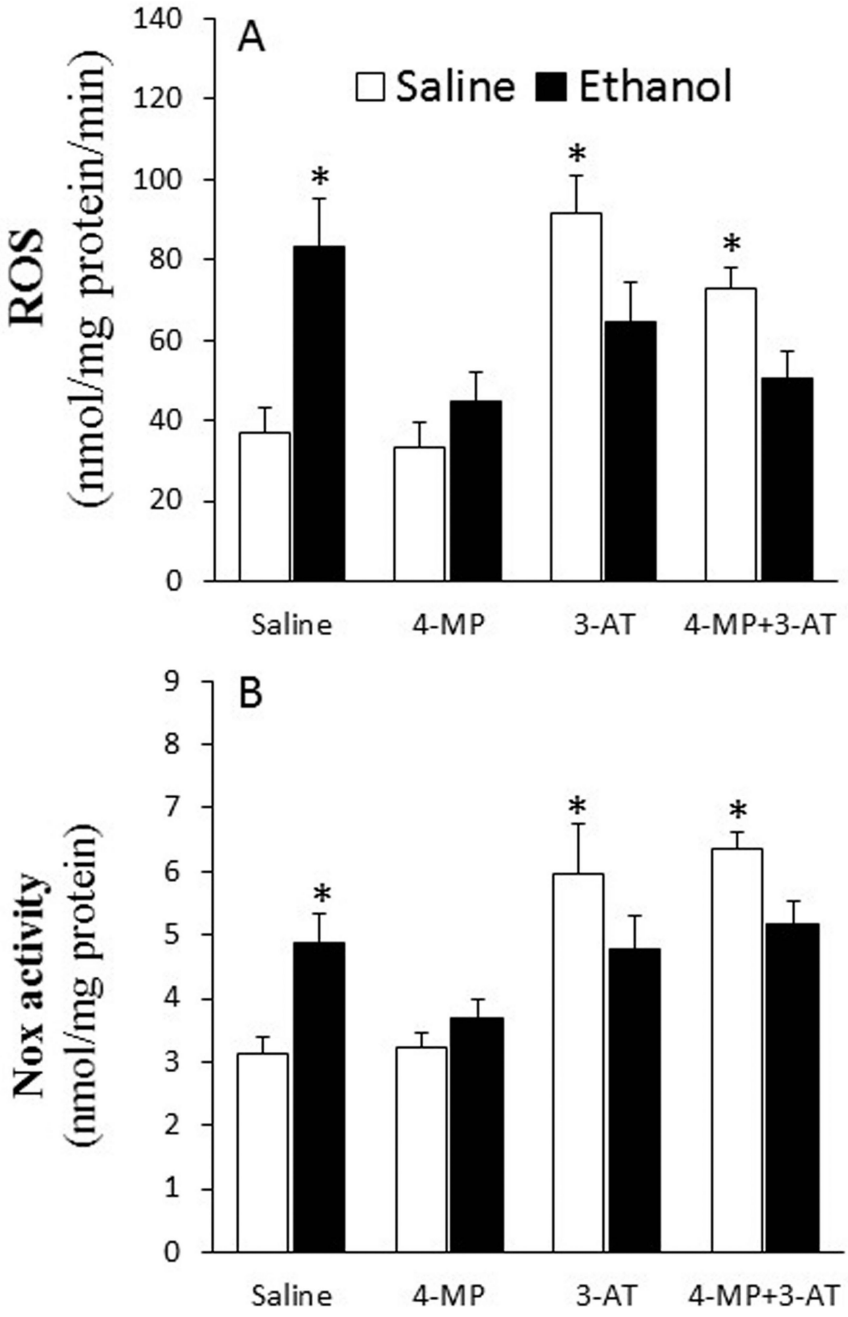

Fig. 5.

Effect of the inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination on myocardial ROS formation (**A**) and NADPH oxidase (Nox, **B**) activity. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus saline-saline.

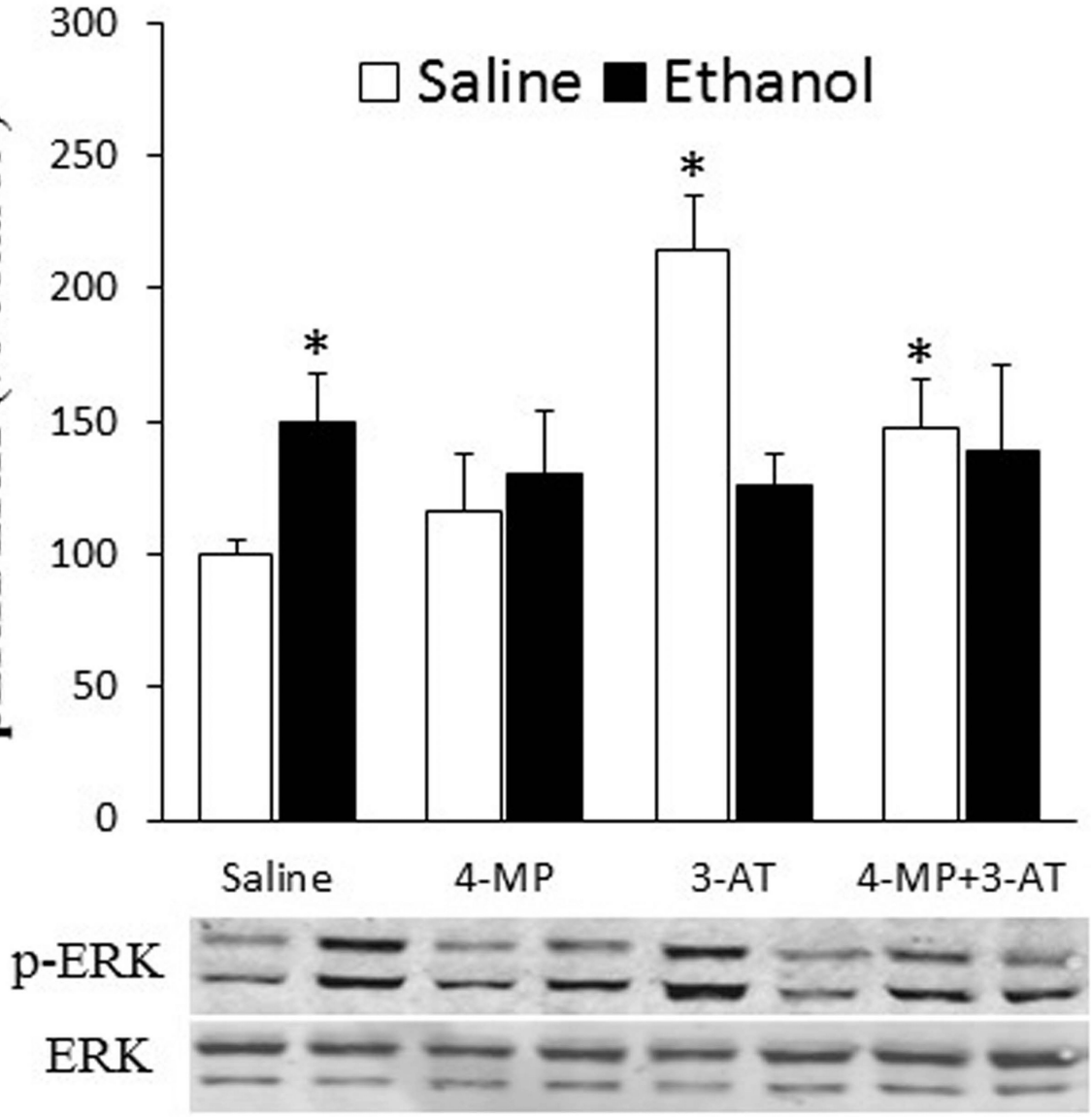

Fig. 6.

Effect of the inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination on myocardial ERK1/2 phosphorylation. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus saline-saline. #$p < 0.05$, versus 3-AT-saline.

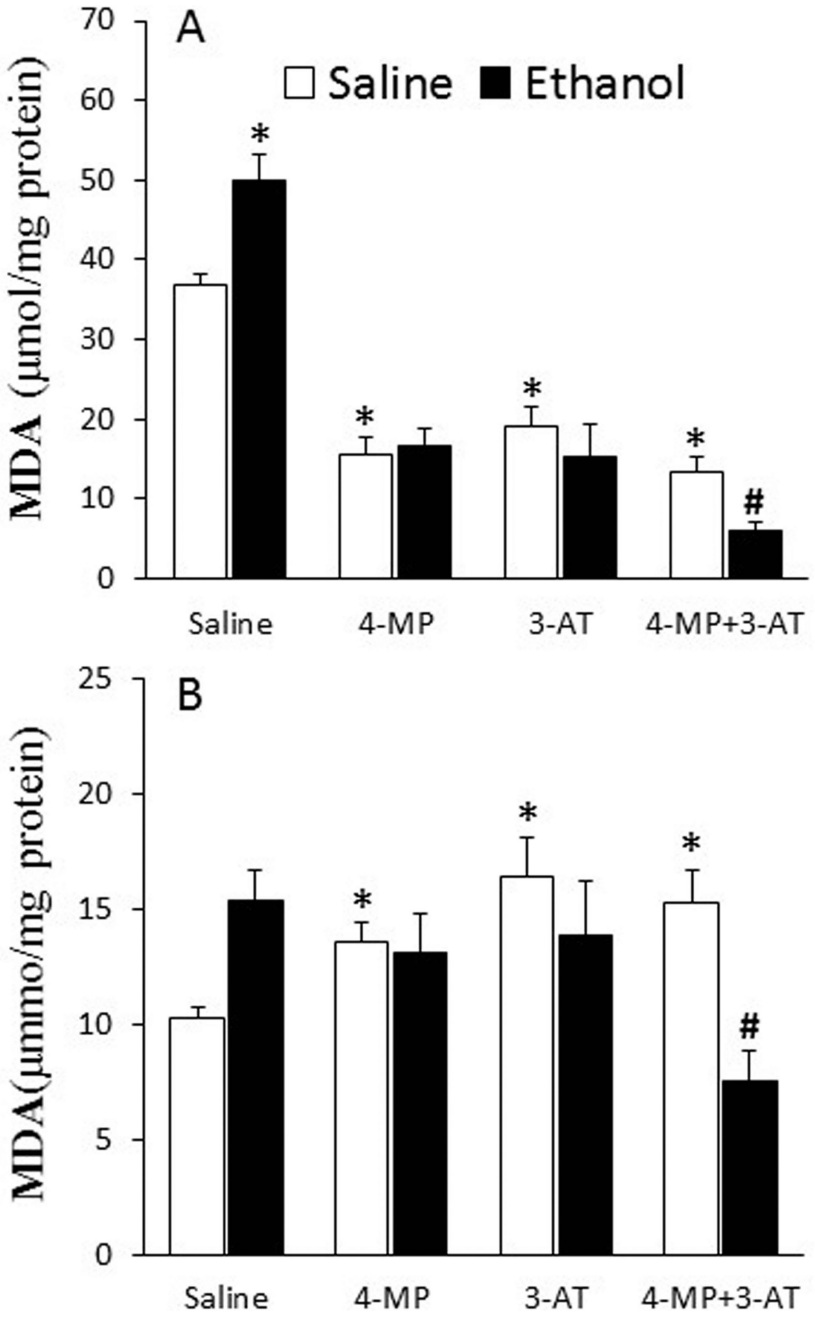

Fig. 7.

Effect of the inhibition of ADH, CYP2E1 and catalase by 4-MP, 3-AT or their combination on myocardial (**A**) and plasma (**B**) malondialdehyde (MDA) levels. Data were analyzed by one-way ANOVA followed by Tukey's Multiple Comparison Test. *$p < 0.05$, versus saline-saline. #$p < 0.05$, versus 4-MP+3-AT-saline.

Table 1

Mean arterial pressure (MAP), left ventricular developed pressure (LVDP) and maximum rate of left ventricular pressure rise (dP/dt_{max}) values before (baseline) and 30 min after pretreatment with 4-MP, 3-AT, their combination or vehicle (saline). These rat groups subsequently received ethanol or its vehicle (saline). Values are mean± SEM.

Pretreatment	Group	N	Hemodynamic variable	Baseline	After pretreatment
Saline	Saline	5	MAP (mmHg)	99.7±1.7	103.0±2.7
			LVDP (mmHg)	161.7±5.1	162.3±5.8
			dP/dt_{max} (mmHg/s)	6870±1032	6647±1066
	Ethanol	6	MAP (mmHg)	101.4±8.1	99.4±5.9
			LVDP (mmHg)	160.3±11.1	160.5±11.2
			dP/dt_{max} (mmHg/s)	7227±1236	6952±1300
4-MP	Saline	7	MAP (mmHg)	100.1±5.2	95.4±6.1
			LVDP (mmHg)	165.3±7.4	163.2±7.2
			dP/dt_{max} (mmHg/s)	7182±619	6859±572
	Ethanol	8	MAP (mmHg)	103.4±5.5	100.0±6.3
			LVDP (mmHg)	170.5±8.8	169.45±8.3
			dP/dt_{max} (mmHg/s)	7051±682	6589±644
3-AT	Saline	6	MAP (mmHg)	102.7±4.6	93.7±6.4
			LVDP (mmHg)	164.9±15.5	147.9±14.3
			dP/dt_{max} (mmHg/s)	7468±1465	6492±1388
	Ethanol	7	MAP (mmHg)	102.2±3.8	90.9±3.5 *#
			LVDP (mmHg)	166.3±10.3	156.6±10.2
			dP/dt_{max} (mmHg/s)	7331±393	6540±355
4-MPAT	Saline	6	MAP (mmHg)	98.6±2.8	84.6±4.4 *#
			LVDP (mmHg)	167.9±7.1	153.2±8.4
			dP/dt_{max} (mmHg/s)	7488±515	6735±422

Pretreatment	Group	N	Hemodynamic variable	Baseline	After pretreatment
			MAP (mmHg)	97.6±4.5	88.2±3.9*
	Ethanol	7	LVDP (mmHg)	159.9±7.4	149.7±9.5
			dP/dt_{max} (mmHg/s)	6908±735	5961±880

* $p < 0.05$ vs. corresponding value in saline-pretreated group;

# $p < 0.05$ vs. corresponding pretreatment (baseline) value.