Endotoxemia-Mediated Induction of Cardiac Inducible Nitric-Oxide Synthase Expression Accounts for the Hypotensive Effect of Ethanol in Female Rats

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ABSTRACT

We have recently shown that intragastric (i.g.) ethanol lowers blood pressure (BP) in conscious female rats via a reduction in cardiac output (CO). However, the mechanisms implicated in these hemodynamic effects of ethanol are not known. Therefore, we tested the hypothesis that ethanol-evoked endotoxemia mediates the reduction in CO via enhanced myocardial inducible nitric-oxide synthase (iNOS) expression. Immunoblot (myocardial iNOS), biochemical (plasma endotoxin and nitrite/nitrate), and integrative [BP, heart rate, CO, stroke volume (SV), and total peripheral resistance (TPR)] studies were conducted in conscious female rats that received i.g. ethanol (1 g/kg) in the absence or presence of 1400W (N-(3-[aminomethyl]benzyl)acetamidine) or ampicillin to selectively inhibit iNOS and to eliminate endogenous endotoxin, respectively. Ethanol-evoked hypotension coincided with reductions in CO and SV and increases in: 1) TPR, 2) plasma endotoxin and nitrite/nitrate, and 3) myocardial iNOS expression. These effects of ethanol were virtually abolished in rats pretreated with ampicillin (200 mg/kg/day for 2 days by gavage) or with 1400W (5 mg/kg i.p.) except for the increase in plasma endotoxin, which persisted in 1400W-pretreated rats. These findings yield insight into the mechanistic role of endotoxin-myocardial iNOS signaling in the cardiodepressive action of ethanol, which accounts for its hypotensive effect in conscious female rats.

Ethanol elicits hypotension in female, but not in age-matched male, rats (El-Mas and Abdel-Rahman, 1999a). Furthermore, the hypotensive effect of ethanol is estrogen-dependent (El-Mas and Abdel-Rahman, 1999b). It is imperative also to note that moderate ethanol consumption is associated with lower BP in young but not in old women (Klatsky, 1990), which highlights the clinical relevance of the reported experimental findings. The precise mechanism by which ethanol elicits hypotension in female rats is not known. It is noteworthy that there are similarities between the cardiovascular effects of ethanol and estrogen, which include inhibition of calcium influx (Turlapaty et al., 1979; Zhang et al., 1994), enhancement of NOS activity (Weiner et al., 1994; Wang and Abdel-Rahman, 2005; Zhang et al., 2005), and reduction of α-adrenergic receptor responsiveness (Abdel-Rahman et al., 1985; Sudhir et al., 1997). Indeed, the NOS-derived NO seems to be the major mediator of the estrogen-dependent hypotensive effect of ethanol because NO is directly or indirectly linked to vasodilation and reduces cardiac contractility (Yeh et al., 2005). It is possible, therefore, that ethanol may interact synergistically with estrogen to produce vascular and/or cardiac changes that might lead to hypotension.

Built on the premise that ethanol enhances iNOS signaling (Durante et al., 1995), we demonstrated in a recent study the ability of ethanol to increase vascular (aortic) iNOS expression in female rats (El-Mas et al., 2006). Such a response could not be mechanistically linked to the hypotensive effect of ethanol because the latter is a result of a reduction in cardiac output (CO) [mainly due to reduction in stroke volume (SV)] (El-Mas and Abdel-Rahman, 1999a,b). Together, these findings raised the interesting possibility that the myocardium might be the logical target for mediating the iNOS-dependent hypotensive effect of ethanol in female rats. Furthermore, the mechanism by which ethanol elicits enhancement of iNOS expression in our recent study (El-Mas et al., 2006) has not been investigated. Endotoxin, a bacterial lipopolysaccharide derived from the gut gram-negative bacteria (Tamai et al., 2000), has been shown to enhance iNOS expression and to cause myocardial depression and hypotension (Forstermann et al., 1998; Wolfard et al., 2000; Isobe et al., 2001; El-Mas et al., 2006). It is noteworthy that ethanol increases intestinal endotoxin absorption (Tamai et al., 2000) and plasma endotoxin levels (Rivera et al., 1998; Urbansek et al., 2001).

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ABBREVIATIONS: BP, blood pressure; iNOS, inducible nitric-oxide synthase; CO, cardiac output; SV, stroke volume; i.g., intragastric(ally); HR, heart rate; TPR, total peripheral resistance; 1400W, N-(3-[aminomethyl]benzyl) acetamidine; MAP, mean arterial pressure; eNOS, endothelial nitric-oxide synthase.
In the present study, we tested the hypothesis that endotoxemia induced by ethanol mediates the iNOS-dependent hemodynamic actions of ethanol in female rats. To achieve our goals, we undertook hemodynamic, Western blot, and biochemical studies, which comprised investigation of the effect of i.g. ethanol on BP, heart rate (HR), CO, SV, and total peripheral resistance (TPR) along with myocardial iNOS expression and serum endotoxin and nitrite/nitrate levels. Given the dependence of the hypotensive effect of ethanol on estrogen (El-Mas and Abdel-Rahman, 1999b), the studies were undertaken during the proestrus phase, which exhibits the highest plasma estrogen levels (Marcordes et al., 2001). Pharmacological interventions were employed to investigate the impact of endogenous endotoxin elimination by ampicillin or iNOS inhibition by the highly selective iNOS inhibitor, 1400W (Boer et al., 2000), on the hemodynamic, cellular, and biochemical effects of ethanol in conscious female rats.

Materials and Methods

Female Sprague-Dawley rats (12–13 weeks old; Charles River, Raleigh, NC) were employed. All experiments were approved by the institutional animal care and use committee and carried out in accordance with the Declaration of Helsinki and with the Institute of Laboratory Animal Resources (1996).

Intravascular and Intragastric Cannulations. The method described in our previous studies (El-Mas and Abdel-Rahman, 1999a,b) was adopted. In brief, the rats were anesthetized with pentobarbital (50 mg/kg i.p.). Catheters were placed into the abdominal aorta and vena cava via the left femoral vessels for measurement of arterial pressure and i.v. injections, respectively. Intragastric catheterization was performed as reported (El-Mas and Abdel-Rahman, 1999a,b).

Measurement of Cardiac Output. The thermodilution technique described in our previous studies (El-Mas and Abdel-Rahman, 1999a,b) was employed for the measurement of CO (ml/min) and SV (ml/beat); TPR (mean arterial pressure [MAP]/CO, mm Hg/ml/min) was calculated as in our previous studies (El-Mas and Abdel-Rahman, 1999a,b). The catheters and the thermostir were tunneled s.c. and exteriorized at the back of the neck. Vascular and nasogastric catheters were flushed with heparin (100 U/ml) and water, respectively, and plugged with stainless steel pins. Each rat received buprenorphine hydrochloride (Buprenex; 30 μg/kg i.m.) and penicillin G benzathine and penicillin G procaine (Durapen, 100,000 U/kg) and was housed in a separate cage. The experiments were performed 2 to 3 days later in conscious freely moving rats as in our previous studies (El-Mas and Abdel-Rahman, 1999a,b).

Western Blotting. For the determination of myocardial iNOS protein expression, the rat ventricle was homogenized on ice in a homogenization buffer [50 mM Tris, pH 7.5, 0.1 mM EGTA, 0.1 M EDTA, 2 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride, 0.1% (v/v) Nonidet P-40, 0.1% SDS, and 0.1% deoxycholate]. After centrifugation (12,000 × g for 10 min), protein in the supernatant was diluted in water) or equal volume of water was administered intragastrically as in our previous studies (El-Mas and Abdel-Rahman, 1999a,b). The iNOS inhibitor 1400W (5 mg/kg i.p.) or equal volume of saline (1 ml/kg i.p.) was administered 30 min before receiving ethanol or water. Two doses of ampicillin (200 mg/kg/day by gavage) were administered on 2 consecutive days as reported (Evans and Whittle, 2001) with the second dose given 1 h before ethanol or water. Hemodynamic monitoring continued for a period of 90 min after ethanol or water.

Four arterial blood samples (0.3 ml each) were drawn at 15, 30, 60, and 90 min after ethanol or water, and the plasma was stored at −80°C until analyzed. At the conclusion of the experiment, the rats were anesthetized with pentobarbital sodium (50 mg/kg), and heparinized blood was collected from the portal vein using strict pyrogen-free techniques for the measurement of endotoxin levels. Blood was centrifuged at 150g for 10 min, and the collected platelet-rich plasma was stored at −80°C. Rats were then euthanized with an overdose of pentobarbital sodium (100 mg/kg), and the hearts were harvested and stored at −80°C for later measurement of iNOS protein expression in ventricular tissues. It is noteworthy that biochemical and protein expression studies were conducted on blood and tissues collected from the same animals used in the hemodynamic studies. Furthermore, the studies were conducted on individual animals during the proestrus phase.

To determine whether alterations in the rat movement contributed to the observed hemodynamic effects, spontaneous locomotor activity was monitored in four additional groups of rats (n = 7–8 each) randomly assigned to receive one of the following treatments: 1) saline + water, 2) saline + ethanol, 3) 1400W + water, or 4) 1400W + ethanol. The movement activity was monitored as described elsewhere (Matthews et al., 1986) using Autonex 2S activity meters (Columbus Instruments, Columbus, OH). Individual rat activity was measured kinetically using a chromogenic test based on the limulus amebocyte lysate assay (Kinetic-QCL; BioWhittaker, Walkersville, MD) as described elsewhere (Rivera et al., 1998; Kono et al., 2000).

Measurement of Plasma Ethanol, Nitrate/Nitrite, and Endotoxin Levels. Ethanol content was determined by the enzymatic method as in our previous studies (El-Mas and Abdel-Rahman, 1999a). Nitrite/nitrate was measured in plasma obtained from arterial blood, whereas endotoxin was measured in plasma obtained from portal vein at time of sacrifice as described below. Nitrite/nitrate level was measured using a fluorometric assay kit in accordance with the manufacturer’s instructions (Cayman Chemical Company, Ann Arbor, MI) and as detailed elsewhere (Misko et al., 1993). Endotoxin was measured kinetically using a chromogenic test based on the limulus amebocyte lysate assay (Kinetic-QCL; BioWhittaker, Walkersville, MD) as described elsewhere (Rivera et al., 1998; Kono et al., 2000).

Experimental Groups and Protocols. A total of 38 female Sprague-Dawley rats divided into six groups were used during the proestrus phase, which exhibits the highest plasma estrogen levels (Marcordes et al., 2001). The rats in each group received one of the following combinations: 1) saline + water (n = 8), 2) saline + ethanol (n = 8), 3) 1400W + water (n = 7), 4) 1400W + ethanol (n = 6), 5) ampicillin + water (n = 7), or 6) ampicillin + ethanol (n = 6). On the day of the experiment, the thermistor was connected to a Cardiomax II for measurement of CO, and the arterial catheter was connected to a pressure transducer for measurement of BP and HR as mentioned above. At least a 30-min stabilization period was allowed at the beginning of the experiment. Ethanol (1 g/kg; 10 ml/kg 13% ethanol diluted in water) or equal volume of water was administered intragastrically as in our previous studies (El-Mas and Abdel-Rahman, 1999a,b). The iNOS inhibitor 1400W (5 mg/kg i.p.) or equal volume of saline (1 ml/kg i.p.) was administered 30 min before receiving ethanol or water. Two doses of ampicillin (200 mg/kg/day by gavage) were administered on 2 consecutive days as reported (Evans and Whittle, 2001) with the second dose given 1 h before ethanol or water. Hemodynamic monitoring continued for a period of 90 min after ethanol or water.

Equivalent sample loading was confirmed by stripping membranes with blot restore membrane rejuventation solution (SignaGen Laboratories, Gaithersburg, MD) and reprobing with anti-actin antibody (Sigma Chemical Co., St. Louis, MO). Positive controls for iNOS (BD Biosciences) and actin (Sigma Chemical Co.) were also loaded. The iNOS bands were quantified by measuring the intergrated density (mean density × area) using the NIH Image software (version 1.62).

The impact of endogenous endotoxin elimination by ampicillin or iNOS inhibition by the highly selective iNOS inhibitor, 1400W (Boer et al., 2000), on the hemodynamic, cellular, and biochemical effects of ethanol in conscious female rats.
cages were placed on the top of the activity meters. The rat’s movement triggers electrical impulses within the device that are recorded as activity counts. Locomotor activity was recorded for 15 min before treatment (baseline values) in all rat groups and then over 15-min periods after vehicle or drug treatment for 90 min (i.e., six 15-min periods).

**Drugs.** Pentobarbital sodium, 1400W dihydrochloride, ampicillin trihydrate (Sigma Chemical Co.), povidone-iodine solution (Norton Co., Rockford, IL), ethanol (Midwest Grain Products Co., Weston, MO), Buprenex (Rickitt and Colman, Richmond, VA), and Durapen (Vedo, Inc., Overland Park, KS) were purchased from commercial vendors.

**Data Analysis and Statistics.** Values are presented as means ± S.E.M. MAP was calculated as diastolic pressure + one-third pulse pressure (systolic-diastolic pressures). Data were analyzed by repeated measures two-way analysis of variance followed by the Newman-Keuls post-hoc test. This test distinguishes between-subject variability from within-subject variability. These analyses were performed by SAS software release 6.04 (SAS Institute Inc., Cary, NC) as in our previous study. Probability levels less than 0.05 were considered significant.

**Results.**

Baseline values of MAP and HR were similar in all groups of ethanol and water-treated female rats used in the present study (Table 1). Changes in MAP, HR, CO, SV, and TPR observed in ethanol (1 g/kg i.g.)-treated conscious female rats in the absence or presence of the selective iNOS inhibitor 1400W are illustrated in Figs. 1 and 2. Compared with water-treated rats, ethanol caused significant (p < 0.05) reductions in MAP that continued for the duration of the study (90 min), with a maximum drop of 15.1 ± 2.3 mm Hg observed at 30 min (Fig. 1A). The hypotensive effect of ethanol coincided with significant (p < 0.05) reductions in CO (Fig. 2A) and SV (Fig. 2B) compared with control values. The peak reduction in either parameter was demonstrated at 45 min and was maintained afterward (Fig. 2, A and B). The HR was increased by ethanol during the first 50 min of the study and then returned to near-control (saline-water) values (Fig. 1B). TPR remained unchanged during the first 30 min but was significantly (p < 0.05) increased thereafter (Fig. 2C).

The inhibition of iNOS by 1400W (5 mg/kg i.p.) virtually abolished the hypotensive effect of ethanol (Fig. 1A) and attenuated the associated reductions in CO (Fig. 2A) and SV (Fig. 2B) Furthermore, the ethanol-evoked increases in HR (Fig. 1B) and TPR (Fig. 2C) were abolished in 1400W-pretreated rats. However, the spontaneous locomotor activity of rats was not affected by these drug treatments. As shown in Table 2, the pretreatment (baseline) as well as the post-treatment movement counts of rats treated with ethanol, 1400W, or their combination were not statistically different from corresponding control (saline + water) values. Similar to the effects of 1400W, pretreatment of female rats with ampicillin (200 mg/kg/day for 2 days by gavage) significantly (p < 0.05) attenuated the ethanol-evoked reductions in MAP (Fig. 3A), CO (Fig. 4A), and SV (Fig. 4B) and the associated increases in HR (Fig. 3B) and TPR (Fig. 4C).

Plasma endotoxin levels measured at the conclusion of the experiments showed a 4-fold increase in ethanol compared with water-treated female rats (90.3 ± 5.6 and 22.9 ± 1.3 pg/ml, respectively, p < 0.05). This effect of ethanol was still evident in 1400W-pretreated rats but was virtually abolished in the ampicillin-pretreated rats (Fig. 5). Likewise, ethanol elicited significant (p < 0.05) increases in myocardial iNOS protein expression (Fig. 5), and plasma nitrate/nitrite levels.

**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (beats per minute)</th>
<th>CO (milliliters per minute)</th>
<th>SV (microliters per beat)</th>
<th>TPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + water 8</td>
<td>109.1 ± 2.1</td>
<td>392.5 ± 21.3</td>
<td>87.6 ± 2.8</td>
<td>234.9 ± 13.8</td>
</tr>
<tr>
<td>Saline + ethanol 8</td>
<td>100.5 ± 3.7</td>
<td>416.0 ± 8.9</td>
<td>93.6 ± 6.1</td>
<td>226.7 ± 20.1</td>
</tr>
<tr>
<td>1400W + water 7</td>
<td>100.3 ± 3.6</td>
<td>373.9 ± 8.8</td>
<td>86.0 ± 1.8</td>
<td>230.2 ± 2.9</td>
</tr>
<tr>
<td>1400W + ethanol 6</td>
<td>99.3 ± 3.7</td>
<td>388.5 ± 9.1</td>
<td>80.5 ± 12.8</td>
<td>208.6 ± 12.8</td>
</tr>
<tr>
<td>Ampicillin + water 7</td>
<td>119.8 ± 8.6</td>
<td>372.5 ± 20.7</td>
<td>75.0 ± 3.5</td>
<td>202.3 ± 10.3</td>
</tr>
<tr>
<td>Ampicillin + ethanol 6</td>
<td>109.5 ± 8.2</td>
<td>371.7 ± 9.1</td>
<td>71.0 ± 4.4</td>
<td>191.1 ± 10.7</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M.

![Fig. 1. Changes in mean arterial pressure (ΔMAP, A) and heart rate (ΔHR, B) evoked by intragastric ethanol (1 g/kg) or equal volume of water in conscious female Sprague-Dawley rats pretreated, 30 min earlier, with 1400W (selective iNOS inhibitor, 5 mg/kg i.p.). Values are means ± S.E.M of six to eight observations. ∗ and ∗∗, p < 0.05 versus corresponding water-treated (saline + water or 1400W + water) and 1400W + ethanol values, respectively.](image-url)
Table 3). Both effects of ethanol were blunted in the 1400W-or ampicillin-pretreated rats (Fig. 5; Table 3). The baseline plasma nitrate/nitrite levels were significantly less in rats receiving 1400W compared with corresponding saline-treated animals (Table 3). In ampicillin-treated rats, baseline plasma levels of nitrate/nitrite were slightly higher than saline-treated values, but the difference was not statistically significant (Table 3). In all rat groups receiving ethanol, blood ethanol levels peaked at 30 min following i.g. ethanol administration and then showed gradual and significant declines thereafter (Table 4). Treatment with 1400W or ampicillin had no effect on the blood ethanol levels during the course of the experiments except for significantly lower levels, compared with “saline + ethanol” values, observed at 90 min (Table 4).

Discussion

The results showed that ethanol-evoked hypotension in female rats was associated with significant reductions in CO and SV and increases in blood endotoxin and nitrite/nitrate as well as myocardial iNOS expression. Selective iNOS inhibition attenuated the hemodynamic responses and abrogated the increases in myocardial iNOS expression and serum nitrite/nitrate levels. Similar effects were demonstrated after depletion of endogenous endotoxin by ampicillin, which also abrogated the 4-fold increase in serum endotoxin caused by ethanol. Together, these findings suggest a pivotal role for the endotoxia-induced modulation of CO and implicate myocardial iNOS expression in the hemodynamic effects of ethanol in female rats. The blood ethanol concentrations observed here are compatible with levels attained in humans after mild to moderate alcohol consumption (Abdel-Rahman et al., 1987).

The ethanol-evoked reduction in MAP was associated with reductions in CO and SV, whereas TPR showed delayed increases. These hemodynamic findings, which agree with our previous findings (El-Mas and Abdel-Rahman, 1999a,b), highlight an important role for CO in the hypotensive effect of ethanol in female rats. In the present study, we focused on potential cellular mechanisms in cardiac myocytes that might underlie the reduction in CO. The myocardial iNOS-NO signaling was pursued in the present study as a likely mechanism for the following reasons. First, the increased iNOS expression in the aortae of ethanol-treated rats in our recent study (El-Mas et al., 2006) might be secondary to ethanol-evoked endotoxemia reported in female rats (Kono et al., 2000; Yin et al., 2000). As discussed above, such vascular response cannot account for the hypotensive response but raises the interesting possibility that a similar increase in myocardial iNOS expression might occur. Second, although currently not considered highly selective, aminoguanidine inhibition of iNOS virtually abolished the hypotensive effect of ethanol in our recent study (El-Mas et al., 2006) might be secondary to ethanol-evoked endotoxemia reported in female rats (Kono et al., 2000; Yin et al., 2000). As discussed above, such vascular response cannot account for the hypotensive response but raises the interesting possibility that a similar increase in myocardial iNOS expression might occur. Second, although currently not considered highly selective, aminoguanidine inhibition of iNOS virtually abolished the hypotensive effect of ethanol in our recent study (El-Mas et al., 2006). Third, enhanced production of cardiac NO by iNOS mediates contractile dysfunction in heart failure (de Belder et al., 1995; Haywood et al., 1996). Therefore, it was plausible to postulate that an enhanced cardiac iNOS expression might be responsible for the reductions in CO and subsequently BP in ethanol-treated female rats.

Enhancement of cardiac (this study) and vascular (El-Mas et al., 2006) iNOS expression seem to be caused by a systemic effect of ethanol. We report a 4-fold increase in serum endotoxin level in ethanol-treated female rats, which presents the first evidence that links endotoxin to the reduction in CO, SV, and BP in our model system. It is noteworthy that reported findings highlight a role for endotoxia in triggering some of the biological and toxicological effects of ethanol in female rats such as: 1) increased eicosanoid release from Kupffer cells (Rivera et al., 1998) and 2) early liver damage caused by endotoxin-mediated activation of hepatic nuclear factor-α (Kono et al., 2000; Yin et al., 2000). Furthermore, consistent with the ability of endotoxin to facilitate cellular iNOS expression (Thiemermann, 1997), we show that the...
4-fold increase in serum endotoxin by ethanol was associated with significant increase in myocardial iNOS expression. It might be argued that the present findings may not readily permit establishing a causal relationship between the increased endotoxin and the hemodynamic responses because endotoxin was measured at the conclusion of the study when BP started to recover. It must be remembered, however, that measurement of endotoxin in portal vein blood, as in reported observations. The 4-fold increase in serum endotoxin by ethanol was associated with significant increase in myocardial iNOS expression. It might be argued that the present findings may not readily permit establishing a causal relationship between the increased endotoxin and the hemodynamic responses because endotoxin was measured at the conclusion of the study when BP started to recover. It must be remembered, however, that measurement of endotoxin in portal vein blood, as in reported studies (Kono et al., 2000), precluded time course measurements in the present study. Nonetheless, at the time of endotoxin measurement, a significant increase in myocardial iNOS expression coincided with the nadir of CO and SV. The effects of the CO and SV on BP were somewhat offset by the increased TPR. The link between ethanol-evoked hypotension and endotoxemia is further supported by: 1) ethanol elicits no hypotension in male or ovariectomized rats (El-Mas and Abdel-Rahman, 1999a,b), preparations in which the endotoxic effect of ethanol is virtually absent (Kono et al., 2000; Yin et al., 2000); 2) the hypotensive and myocardial depressant effects of ethanol in female rats observed in the present and reported (El-Mas and Abdel-Rahman, 1999,a,b) studies mimic those produced by endotoxemia (Thiemermann, 1997; El-Mas et al., 2006); and 3) ethanol aggravates myocardial injury induced by sepsis (McDonough and Henry, 1991), whereas its withdrawal protects against sepsis-induced myocardial depression (McDonough, 1995).

To more directly address the role of endotoxemia in the observed responses, ethanol was administered to female rats pretreated with ampicillin. Different broad-spectrum antibiotic regimens have been employed to reduce bacterial endotoxin output (Rivera et al., 1998; Tamai et al., 2000; Morschel et al., 2001). Ampicillin pretreatment indeed prevented the remarkable increase in plasma endotoxin levels induced by ethanol (Fig. 5). More importantly, the ethanol-evoked increases in cardiac iNOS expression (Fig. 5) and serum nitrite/nitrate (Table 3) and the reductions in BP (Fig. 3A), CO, and SV (Fig. 4, A and B) were all substantially attenuated in ampicillin-pretreated rats. Together, these findings, complemented by the ability of 1400W to attenuate the hypotensive response to endotoxin shock (Cheng et al., 2003), strongly support our hypothesis that an endotoxin-mediated facilitation of cardiac iNOS expression accounts, at least partly, for the hemodynamic effects of ethanol in female rats. It is noteworthy that the spontaneous locomotor activity of rats was not affected by ethanol, 1400W, or their combination, which precludes any possible role for behavioral factors in the antagonistic ethanol-1400W hemodynamic interaction. It is noteworthy that since ethanol-evoked hypotension is estrogen dependent (El-Mas and Abdel-Rahman, 1999b), all present studies were conducted in female rats during the proestrus phase, which exhibits the highest plasma estrogen levels (Marcondes et al., 2001). Whether the hemodynamic effects of ethanol could be altered if investigated during other phases of the cycle with lower estrogen levels (e.g., metaestrus or diestrous) is not clear and warrants further investigation. It is noteworthy that we have previously shown that ethanol-evoked hypotension is drastically attenuated in ovariectomized rats (El-Mas and Abdel-Rahman, 1999b).

Two potential arguments may be raised here regarding the ethanol-iNOS interaction. First, the increase in myocardial

**TABLE 2**

Counts of spontaneous locomotor activity before (baseline) and after vehicle or drug treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Saline + water</td>
<td>32 ± 4</td>
<td>39 ± 12</td>
</tr>
<tr>
<td>Saline + ethanol</td>
<td>35 ± 8</td>
<td>34 ± 7</td>
</tr>
<tr>
<td>1400W + water</td>
<td>37 ± 8</td>
<td>35 ± 10</td>
</tr>
<tr>
<td>1400W + ethanol</td>
<td>42 ± 8</td>
<td>34 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. of seven to eight observations.
InOS expression 90 min postethanol seems surprising, considering the relatively longer times required for appreciable changes in iNOS expression to be manifested (Durante et al., 1995). However, we recently reported similar increases by acute ethanol in vascular iNOS expression (El-Mas et al., 2006). In addition, an increase in iNOS protein was detected in Kupffer cells as early as 2 h after lipopolysaccharide challenge (Takagi et al., 2007). The rapidity, however, with which ethanol hypotension developed in the present and previous studies (El-Mas et al., 2006) may suggest a role for constitutive NOS, at least in the early phase of hypotensive response.

In support of this assumption are the observations: 1) ethanol (Wang and Abdel-Rahman, 2005; Zhang et al., 2005) or endotoxemia (Thiemermann, 1997; Forstermann et al., 1998) up-regulates eNOS/neuronal NOS activity, and 2) endotoxemia causes early and delayed activation of cardiovascular eNOS and iNOS, respectively (Forstermann et al., 1998). Remarkably, the present study showed that CO and SV remained significantly reduced in ethanol-treated rats receiving 1400W or ampicillin, despite the complete abolition of the presumably causally related increases in cardiac iNOS ex-

**TABLE 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + water</td>
<td>8.42 ± 0.72</td>
<td>9.17 ± 0.89</td>
</tr>
<tr>
<td>Saline + ethanol</td>
<td>7.82 ± 0.37</td>
<td>11.01 ± 0.63#</td>
</tr>
<tr>
<td>1400W + water</td>
<td>5.69 ± 0.33#</td>
<td>5.33 ± 0.29</td>
</tr>
<tr>
<td>1400W + ethanol</td>
<td>5.05 ± 0.94#</td>
<td>4.74 ± 0.69</td>
</tr>
<tr>
<td>Ampicillin + water</td>
<td>9.61 ± 0.66</td>
<td>9.62 ± 0.64 #</td>
</tr>
<tr>
<td>Ampicillin + ethanol</td>
<td>11.02 ± 1.09</td>
<td>10.30 ± 0.68 #</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. respective baseline (before) values in the saline-treated groups.

#p < 0.05 vs. respective "before" value.
pression (by 1400W or ampicillin) and blood endotoxin (by ampicillin). These findings may suggest a contributory role for endotoxin-independent eNOS/neuronal NOS pathways or perhaps NOS-independent mechanisms in the hemodynamic effects of ethanol. More studies are clearly needed to investigate these possibilities. It is also important to comment on the observation that 1400W treatment caused a reduction in iNOS protein expression. Although the mechanism is not presently known, this observation is consistent with previous reports, which highlighted the ability of iNOS inhibitors to suppress iNOS activity and expression (Thiemermann, 1997; Vona-Davis et al., 2002).

It is important to comment on the role of TPR in ethanol hemodynamics. The delayed increase in TPR caused by ethanol (Fig. 2C) may represent a counter-regulatory mechanism to offset the ethanol-evoked reductions in CO and BP. Remarkably, the hypodynamic state (high TPR and low CO) induced by ethanol in this study resembles that produced by lipopolysaccharide during septic shock (Cheng et al., 2003). The latter is believed to trigger a surge in plasma levels of vasconstrictors, e.g., angiotensin II, endothelin-1, and vasoressin, which increase TPR to counterbalance the BP/CO falls (Brackett et al., 1985; Gardiner et al., 1996; Mitaka et al., 1999). These findings, together with the observations that iNOS inhibition, by 1400W or by endothelin elimination, significantly lessened the increase in TPR by ethanol, further supported our hypothesis that implicates endotoxin in the hemodynamic effects of ethanol.

In summary, we present evidence that endotoxin-iNOS signaling plays a crucial role in the hemodynamic effects of ethanol. This is supported by the ability of pharmacological interventions that inhibit endogenous endotoxin (ampicillin) or iNOS inhibition (1400W) to abrogate the hemodynamic responses to ethanol. Our conclusion is bolstered by demonstrating the ability of ethanol to cause significant increases in endotoxin and nitrite/nitrate blood levels as well as myocardial iNOS. It is noteworthy that the hemodynamic, biochemical, and cellular responses elicited by ethanol were substantially reduced by pretreatment with ampicillin or 1400W. Collectively, these findings provide the first experimental evidence that implicates endothotoxin-myocardial iNOS signaling in the reduction in CO caused by ethanol, which mediates the hypotensive response observed in female rats.

Acknowledgments
We thank Kui Sun for technical assistance.

References


### TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline + ethanol (8)</td>
<td>69.8 ± 4.9</td>
<td>84.1 ± 4.5</td>
<td>62.9 ± 5.1</td>
<td>60.9 ± 4.2</td>
</tr>
<tr>
<td>1400W + ethanol (6)</td>
<td>65.9 ± 4.0</td>
<td>72.5 ± 2.8</td>
<td>56.5 ± 1.6</td>
<td>50.1 ± 2.1</td>
</tr>
<tr>
<td>Ampicillin + ethanol (6)</td>
<td>59.2 ± 4.9</td>
<td>74.8 ± 4.3</td>
<td>57.3 ± 1.9</td>
<td>46.2 ± 3.3</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. corresponding peak ethanol levels at 30 min in the same group.

*p < 0.05 vs. corresponding ‘saline + ethanol’ values at 90 min.

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### References


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