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Differential modulation by vascular nitric oxide synthases of the ethanol-evoked hypotension and autonomic dysfunction in female rats

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Abstract

We recently reported that chronic exposure to ethanol lowers blood pressure (BP) via altering cardiac contractility and autonomic control in female rats. In this investigation we conducted pharmacological and molecular studies to elucidate the role of constitutive and inducible nitric oxide synthase (NOS) in these hemodynamic effects of ethanol. Changes caused by selective inhibition of eNOS [N^5 -(1-iminoethyl)-L-ornithine; L-NIO], nNOS (N^{ω} -propyl-L-arginine; NPLA), or iNOS (1400W) in BP, heart rate (HR), myocardial contractility index (dP/dt_{max}), and power spectral indices of hemodynamic variability were evaluated in telemetered female rats receiving ethanol (5%, w/v) or control liquid diet for 8 weeks. Ethanol increased plasma nitrite/nitrate (NOx) and enhanced the phosphorylation of eNOS and nNOS, but not iNOS, in the tail artery. Ethanol also reduced BP, dP/dt_{max} , low-frequency bands of interbeat intervals (IBI_{LF} , 0.25–0.75 Hz) and $IBI_{LF/HF}$ ratio while high-frequency bands (IBI_{HF} , 0.75–3 Hz) were increased, suggesting parasympathetic overactivity. L-NIO (20 mg/kg i.p.) caused greater increases in BP in control than in ethanol-fed rats but elicited similar reductions in $IBI_{LF/HF}$ and dP/dt_{max} both groups. NPLA (1 mg/kg i.p.) caused minimal effects in control rats but exacerbated the reductions in BP, dP/dt_{max} , and $IBI_{LF/HF}$ in ethanol-fed rats. No hemodynamic modifications were caused by 1400W (5 mg/kg i.p.) in either rat group. Together, these findings suggest that nNOS acts tonically to offset the detrimental cardiovascular actions of ethanol in female rats, and the enhanced vascular NO bioavailability may explain the blunted L-NIO evoked pressor response in ethanol-fed rats.

Keywords

Ethanol; blood pressure; cardiac autonomic control; nitric oxide synthases; female rats

Introduction

Experimental reports from our laboratory showed that ethanol, administered acutely (El-Mas and Abdel-Rahman, 1999a, 1999b) or chronically (El-Mas and Abdel-Rahman, 2000, 2001),

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lowers BP in female rats. The hypotensive effect of ethanol is modulated by the hormonal milieu because it was reduced in ovariectomized rats and restored to intact (sham-operated) levels after estrogen instatement, suggesting a contributory role for estrogen in the cardiovascular actions of ethanol (El-Mas and Abdel-Rahman, 1999a, 2001). These findings are clinically relevant because moderate ethanol consumption is associated with lower BP in young but not in old women (Klatsky, 1990). Although the mechanism of the estrogen-dependent hemodynamic effects of ethanol is not fully understood, accumulating evidence suggests that the two substances share similar cellular effects that might act additively or synergistically to trigger the hypotensive response. These effects include the inhibition of calcium influx (Vasdev et al., 2006; Babaei and Azarmi, 2008), promotion of NOS activity (Rekik et al., 2002; LeBlanc et al., 2009), and reduction of α -adrenoceptor responsiveness (Abdel-Rahman et al., 1985; Sudhir et al., 1997).

Alterations of myocardial dynamics are thought to mediate the BP lowering effect of ethanol because the latter is coupled with estrogen-dependent reductions in cardiac output and stroke volume in contrast to no changes in peripheral vascular resistance (El-Mas and Abdel-Rahman, 1999a, 1999b). In subsequent studies, we opted to implicate myocardial NOS in the hypotensive action of ethanol; however, the recruitment of individual NOS isoforms seems to depend primarily on the ethanol regimen (acute vs. chronic). Whereas myocardial iNOS is similarly facilitated by either regimen, myocardial constitutive NOS isoforms are variably increased by acute (nNOS) and chronic (eNOS) ethanol (El-Mas et al., 2008, 2009, 2011). The ethanol dose and tissue type are other factors that may influence the effect of ethanol on constitutive and inducible NOS (Tirapelli et al., 2008; El-Mas et al., 2006, 2011). The suggested cascade of events that leads to the hypotensive response includes the ability of ethanol to increase NO bioavailability, presumably due to endotoxemia-evoked upregulation of cardiac iNOS and PI3K/Akt/cNOS signaling, which reduces myocardial contractility, and subsequently cardiac output (El-Mas et al., 2008, 2009, 2011). The altered cardiac parasympathetic (increased) and sympathetic (decreased) activities, as evidenced by power spectral analysis of HR variability, may also contribute to the upregulation of cardiac NOS by ethanol and the related reductions in myocardial contractility and hypotension (El-Mas et al., 2011) because NOS-derived NO modulates cardiac vagal (Herring et al., 2001) and adrenergic (Heaton et al., 2005) neurotransmission. Perhaps for these reasons, previous studies including our own have focused on cardiac NOS signaling as a molecular mechanism for the estrogen-dependent hemodynamic effects of ethanol. Nonetheless, the possibility that vascular NOS contributes to these hemodynamic effects has not been explored. This possibility is supported by the evidence that vascular NOS contributes to myocardial depression and hypotension in pathological conditions such as circulatory shock (Thiemermann, 1997).

The main goal of the present study was to test the hypothesis that cardiovascular and autonomic actions elicited by chronic ethanol in female rats are modulated by vascular NOS. Studies were conducted in telemetered female rats to investigate the effect of chronic ethanol administration on the protein expression of phosphorylated NOS, constitutive and inducible, in the tail artery. We also investigated the effect of selective pharmacologic inhibition of inducible and constitutive NOS isoforms in ethanol-fed and control rats on blood pressure, myocardial contractility index (dp/dt_{max}), and cardiovascular autonomic control as reflected by spectral analysis of hemodynamic variability. The findings reveal contrasting modulatory roles for the constitutive NOS isoforms in the chronic cardiovascular and autonomic effects of ethanol in conscious female rats.

Materials and Methods

Female Sprague-Dawley rats (9–10 weeks; 190–225 g; Harlan, Indianapolis, IN) were used in the present study. Upon arrival, rats were housed individually in standard plastic cages and allowed free access to water and Purina chow and were maintained on a 12-12-h light-dark cycle with light off at 4:00 p.m. Room temperature was maintained at $22\pm 1^{\circ}\text{C}$. After one-week acclimatization, rats were fed a standard Lieber-DeCarli high protein liquid diet (Dyets Inc., Bethlehem, PA) for another week before starting the ethanol regimen. Rats received the diet daily before the start of the dark cycle at 3:30 p.m. 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 Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Ethanol feeding

Two groups of female rats matched for body weight were used. Telemetry transmitters were implanted as detailed below to allow BP recording. Rats in one group ($n=7$) received ethanol as described below. The other group of rats received isocaloric liquid diet and served as controls ($n=6$ each). Rats in the ethanol group were provided a standard Lieber-DeCarli high protein liquid diet containing 5% w/v ethanol (36 % of total caloric intake) as described in our previous studies (El-Mas and Abdel-Rahman, 2000, 2003). To acclimate rats to the ethanol diet, ethanol was first provided as half strength (2.5% w/v, 18% of calories intake) for 3 days and then increased to 5% w/v thereafter. The daily ethanol intake amounted approximately to 8–9 g/kg. Control rats were pair-fed and received isocaloric amount of dextrin/maltose (89.6 g/l) in place of ethanol, which allowed similar nutrient intake and fluid consumption to that of ethanol fed rats. Fresh diets were prepared every other day and stored in the refrigerator until dispensed.

Two additional groups of female rats ($n=6$ each) were used in the present study that received the same treatments as detailed above (ethanol or control diet) but without telemetry transmitter implantation. These non-telemetry rats were used for withdrawing blood from the tail vein for plasma NO_x determination and also for collecting the tail arteries for measuring the protein expressions of constitutive and inducible NOS as described below.

Telemetry transmitter implantation

The description of the telemetry system (Data Sciences Int., St. Paul, MN) and the method used for the telemetry transmitter implantation are detailed in our previous studies (El-Mas and Abdel-Rahman, 2000, 2003). Transmitter implantation was performed 5 weeks after ethanol or control diet feeding. Rats were left for 3 additional weeks before starting the experiment during which the rats continued to receive the ethanol or control diet.

Western blotting

For the measurement of the tail artery protein expression of NOS (total and phosphorylated), tissues were homogenized on ice in a homogenization buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerol phosphate, 1mM sodium orthovanadate, 0.1% leupeptin). After quantification of protein with Bio-Rad protein assay system, 75 μg of protein extract per lane was run on a 4–12% SDS-PAGE gel (Invitrogen, CA) and electroblotted to nitrocellulose membranes. Blots were blocked for 2 hr at room temperature in Odyssey blocking buffer (LI-COR Biosciences, Lincoln, NE) and then incubated overnight at 4°C in Odyssey blocking buffer with anti-rabbit phospho-iNOS (Y151) (1:1000, #ab95866, Abcam), anti-mouse eNOS (1:2000, #612393, BD Transduction Laboratories), anti-mouse nNOS (1:5000, #611853, BD Transduction Laboratories), anti-mouse iNOS (1:5000, #610328, BD Transduction

Laboratories). In addition, blots were incubated for 48 hrs at 4°C in Odyssey blocking buffer with the anti-rabbit phospho-nNOS (ser1417) (1:100, #PA1-032, Pierce, Thermo Scientific), anti-rabbit phospho-eNOS (ser1177) (C9C3) (1:100, #9570L, Cell Signaling Technology, INC. Danvers, Massachusetts). After 3 washes with PBS-T buffer, the blots were incubated for 50 min at room temperature with a goat anti-mouse secondary antibody IRDye® 800CW (# 926-32210) or a goat anti-rabbit secondary antibody IRDye® 680CW (# 926-32221, LI-COR Biosciences, Lincoln, NE). After 3 washes with PBS-T buffer, the blots were detected by Odyssey Infrared Scanning System. Protein bands were quantified by integrated intensities using Odyssey Infrared Imaging Software and the phosphorylated/total protein ratios were expressed as a percent of control values as in our previous studies (El-Mas and Abdel-Rahman, 2011; El-Mas et al., 2011; Nassar et al., 2011).

Measurement of plasma NOx

Blood samples were withdrawn from the tail vein of ethanol-fed and control rats (non-telemetry groups) one day before starting acute studies (i.e. 8 weeks after feeding ethanol or control diet) for the determination of plasma NOx fluorometrically (Cayman Chemical Company, Ann Arbor, MI) as described in our previous studies (El-Mas et al., 2011).

Hemodynamic effects of selective inhibition of constitutive and inducible NOS

In this experiment we investigated the impact of selective pharmacologic inhibition of eNOS (L-NIO), nNOS (NPLA), or iNOS (1400W) on the cardiovascular and autonomic responses elicited by ethanol feeding. After 8 weeks of ethanol/liquid diet feeding, each rat in the ethanol (n=7) or control (n=6) group received 4 different i.p. injections at 3 days intervals: (i) saline (1 ml/kg), (ii) L-NIO (20 mg/kg; Wilderman and Armstead, 1998; El-Mas et al., 2009), (iii) NPLA (1 mg/kg; El-Mas et al., 2009), and (iv) 1400W (5 mg/kg; El-Mas et al., 2008). All injections were given at 9:00 am and hemodynamic monitoring continued for the following 5 hr. Rats were maintained on ethanol or control diet for the duration of the study.

Telemetry data acquisition and analysis

Individual rat cages were placed on the top of the radio receivers and data was collected using a computerized data acquisition system (Dataquest A.R.T. 4.0, Data Sciences Int.). BP waveforms were sampled at a rate of 1000 Hz for 20 sec every 10 min. Changes in hemodynamic parameters from baseline values evoked by various drug treatments in pair-fed rats receiving liquid diet with or without ethanol (5%, w/v) were averaged in 40-min blocks (i.e. the average of 4 successive measurements) for analysis as in our previous studies (El-Mas and Abdel-Rahman, 2003, 2011). Baseline hemodynamic values were taken as the average of the 40-min period that preceded saline or drug administration. The interbeat interval (IBI) was calculated from BP waveforms.

Spectral analysis of hemodynamic variability

Spectral hemodynamic fluctuations, quantitative indices of cardiovascular autonomic control (Stein et al., 1994; El-Mas and Abdel-Rahman, 2011), were used to reflect changes in sympathetic and vagal outflows. Hemodynamic variability was assessed by the frequency domain analysis of systolic blood pressure (SBP) and interbeat interval (IBI) data series as in previous studies including our own (Clifford and Tarassenko, 2004; El-Mas and Abdel-Rahman, 2011). Data Sciences software (Dataquest A.R.T. 4.0) uses the periodogram function of the rectangular window for direct transformation of data points into power spectral density graphs. Data was interpolated to obtain equally spaced samples with an effective sampling frequency of 10 Hz (0.1 s). A second-order quadratic was employed to fit a smooth curve to the existing data points and produce a smoother visual representation of data. Spectra were integrated into 2 specific frequency bands, LF (0.25–0.75 Hz) and HF

(0.75–3 Hz) bands and expressed in normalized units (LF_{nu} and HF_{nu}). The ratio of LF to HF ($IBI_{LF/HF}$) is a measure of the sympathovagal balance of the heart. Parameters of hemodynamic variability were averaged every 40 min.

Drugs

N^{ω} -propyl-L-arginine (Tocris Bioscience, Ellisville, MO), N^5 -(1-iminoethyl)-L-ornithine (Biotium Inc., Hayward, CA), 1400W (Sigma Chemical Co., St. Louis, MO), Ketaject (ketamine), Xylaject (xylazine) (Phoenix Pharmaceuticals Inc., St Joseph, MI), Toradol (ketorolac tromethamine, Abbott Labs, Chicago, IL), Durapen (Penicillin G benzathine and penicillin G procaine, Vedco Inc., Overland Park, KS), and ethanol (Midwest Grain Products Co., Weston, MO) were purchased from commercial vendors.

Statistical analysis

All values are expressed as means $\pm$ S.E.M. The unpaired Student's t-test was used for the analysis of the effect of ethanol on plasma NOx. The repeated measures two-way analysis of variance (ANOVA) followed by a Newman-Keuls post-hoc test was used to analyze the effects of ethanol feeding on hemodynamic responses to selective NOS inhibitors. These analyses were performed by SAS software Release 6.04 (SAS Institute Inc., Cary, NC). Probability levels less than 0.05 were considered significant.

Results

Hemodynamic, autonomic, and biochemical effects of ethanol

The starting body weights (week 0) of rats subsequently receiving ethanol or control liquid diets were similar (266 ± 4 and 265 ± 5 g, respectively). After 8-week of ethanol feeding, the body weight was slightly but significantly lower (280 ± 5 g) than that of pair-fed control rats (301 ± 6 g). Compared with pair-fed control rats, female rats that received ethanol for 8 weeks exhibited significant reductions in BP, $+dP/dt_{max}$, and low-frequency bands of interbeat intervals (IBI_{LF} , 0.25–0.75 Hz) and $IBI_{LF/HF}$ ratio (Table 1). On the other hand, ethanol significantly increased high-frequency bands of interbeat intervals (IBI_{HF} , 0.75–3 Hz) (Table 1). Neither HR nor SBP spectra in the LF range was affected by ethanol (Table 1). Plasma NOx levels were significantly higher in ethanol-fed compared with control rats (38.2 ± 3.1 vs. 23.3 ± 1.9 μ M). Western blot analyses demonstrated that ethanol caused significant ($P < 0.05$) increases in the phosphorylated nNOS and eNOS expression in tail arteries, as suggested by the higher phosphorylated/total protein ratio for these NOS isoforms in ethanol-fed compared with pair-fed rats (Fig. 1). By contrast, the phosphorylated/total iNOS ratio remained unaltered by ethanol feeding (Fig. 1).

Hemodynamic and autonomic effects of selective NOS inhibitors following chronic ethanol administration

Figures 2–5 illustrate the hemodynamic and autonomic effects of selective inhibition of eNOS, nNOS, or iNOS in ethanol-fed and control rats. Compared with the corresponding saline values, the eNOS inhibitor L-NIO (20 mg/kg; i.p.) caused significant increases in BP in ethanol-fed and control (liquid diet-fed) rats (Fig 2A). The peak pressor effect of L-NIO was observed at 80 min and was of similar magnitudes (19 ± 3 vs. 23 ± 4 mmHg) in the two groups of rats (Fig. 2A). However, the pressor effect of L-NIO in control rats lasted for the whole 5-hr experimentation period in contrast to approximately 2 hr only in ethanol-fed rats (Fig. 2A). In the two groups of rats, L-NIO caused similar decreases in HR (Fig. 2B), $+dP/dt_{max}$ (Fig. 3A), IBI_{LF} (Fig. 4B), and $IBI_{LF/HF}$ (Fig. 5A) and increases in IBI_{HF} (Fig. 4A).

The inhibition of nNOS activity by NPLA (1 mg/kg; i.p.) in ethanol-fed female rats caused significant decreases in MAP (Fig. 2C), HR (Fig. 2D), $+dP/dt_{max}$ (Fig. 3B), IBI_{LF} (Fig. 4D),

IBI_{LF/HF} (Fig. 5B) and increases in IBI_{HF} (Fig. 4C). None of these parameters was affected by NPLA in control pair-fed rats (Figs. 2–5). No hemodynamic or autonomic alterations were observed in ethanol or control rats upon treatment with the iNOS inhibitor 1400W (Figs. 2–5). Moreover, none of the selective NOS inhibitors affected SBP spectra in the LF range in ethanol-fed or liquid diet-fed control rats (Fig. 6).

Discussion

Hemodynamic measurements in telemetered rats were conducted in this study to explore the potential modulatory roles of vascular (tail artery) constitutive and inducible NOS in the hemodynamic and autonomic actions of chronically administered ethanol in female rats. The most important observations can be summarized as follows. Ethanol feeding caused sustained increases in the parasympathetic dominance of the heart as reflected by the reductions in IBI_{LF/HF} ratio, which may explain the associated decreases in myocardial contractility and BP. Ethanol-fed rats exhibited enhanced phosphorylation of vascular (tail artery) eNOS and nNOS, which implicates vascular constitutive NOS in the ethanol-evoked responses. The facilitated eNOS/NO signaling might explain the attenuated pressor response caused by selective pharmacologic eNOS inhibition. On the other hand, selective nNOS inhibition exacerbated the hemodynamic and autonomic effects of ethanol, which supports a counterbalancing role for nNOS/NO signaling against the deleterious cardiovascular and autonomic actions of ethanol.

In the current study, power spectral analysis of hemodynamic variability suggests a key role for abnormalities in myocardial sympathovagal balance, increased vagal (IBI_{HF}) and decreased sympathetic (IBI_{LF}) activities, in the ethanol-induced depression of myocardial contractility and hypotension in female rats. This view is consistent with our previous findings (El-Mas et al., 2011) and with the observations that ethanol remarkably decreases cardiac output, stroke volume, cardiac contraction force, and regional blood and oxygen flow (Kelbaek, 1990; El-Mas and Abdel-Rahman, 1999a, 1999b; Choudhry et al., 2005). We recently established that facilitation of myocardial PI3K/Akt/eNOS and iNOS pathways, due possibly to ethanol-induced endotoxemia and/or increased cardiac parasympathetic dominance, is the cellular mechanism for the reduced myocardial contractility and hypotension caused by ethanol in female rats (El-Mas et al., 2011). We extended our previous findings in cardiac tissues and showed that vascular (tail artery) eNOS expression was significantly elevated in ethanol-fed rats in the current study. This new finding raised the interesting possibility that vascular NOS contributes to the hemodynamic and autonomic responses elicited by chronic ethanol administration in female rats. Although iNOS-derived NO is largely blamed for the negative cardiovascular influences of endotoxemia (Rudiger and Singer, 2007), the increased tail artery eNOS expression in the present study may complement similar increase in the myocardium (El-Mas et al., 2011) and result in additive release of NO; therefore, vascular eNOS-derived NO may contribute to the deleterious cardiac effects of ethanol in female rats. On the other hand, the increased myocardial iNOS caused by chronic ethanol (El-Mas et al., 2011) was not paralleled by any increase in vascular iNOS in the present study (Fig. 1). It is generally believed that elevated levels of cardiac NO are causally linked to cardiac contractile dysfunction (Umar and van der Laarse, 2010). In myocarditis and sepsis for example, excessive generation of NO causes myocyte necrosis, contractile dysfunction, and hypotension (Rudiger and Singer, 2007). Collectively, the increased expression of vascular eNOS may serve as another source for NO and contributes to the ethanol-evoked hemodynamic and autonomic effects in female rats. Notably, the ethanol regimen used in the current study resulted in blood ethanol concentration of 130 ± 10 mg/dl (El-Mas and Abdel-Rahman, 2011), which is achievable in humans following moderate to heavy alcohol consumption (Ireland et al., 1984; Eddleston et al., 2009; Schaller et al., 2010).

We employed the strategy of selective pharmacologic NOS inhibition in the current study to gain more insights into the roles of constitutive and inducible NOS in the hemodynamic and autonomic actions of chronic ethanol. Because ethanol increased vascular eNOS (this study) and cardiac iNOS/eNOS in our previous study (El-Mas et al., 2011), we reasoned that selective inhibition of either isoform would attenuate the hemodynamic and autonomic effects of ethanol. Here we report that selective inhibition of eNOS, but not iNOS, activity produced a pressor effect that lasted for approximately 2 hr in ethanol-fed rats. It was surprising, however, that the latter was drastically shorter than pressor effect of L-NIO in control rats (5 hr). Logically, ethanol-fed rats were anticipated to develop greater increases in BP in response to L-NIO because of the lower resting (pre-L-NIO) BP and higher vascular eNOS density seen in these rats compared with pair-fed controls. It appears, therefore, that the upregulated NOS expression and perhaps activity in ethanol-fed rats acted to compromise and counterbalance the eNOS inhibitory capacity of L-NIO. Studies showed that NOS inhibitors produce their pharmacologic activity via competition with L-arginine for a common site on the NOS molecule with the subsequent inhibition of the enzyme activity (Boucher et al., 1999; Cooper et al., 2000). It might be speculated, therefore, that under conditions of increased eNOS abundance, e.g. in ethanol-fed female rats, significant proportions of eNOS molecules may escape the inhibitory effect of L-NIO resulting in lesser than expected pressor response following inhibition of the enzyme catalytic activity. It is unlikely, though, that the discrepancy in the pressor effect of L-NIO in ethanol-fed and control rats can be accounted for by alterations in cardiac function. In fact, L-NIO produced similar alterations in myocardial contractility (decreased) and autonomic activity (decreased sympathetic and increased parasympathetic activity) in the two groups of rats. Accordingly, vascular rather than cardiac mechanisms are probably responsible for the differential BP response to L-NIO in ethanol-fed and control female rats.

The current study presents the novel observation that selective nNOS inhibition (NPLA) uncovered additional decreases in BP and myocardial contractility in ethanol-fed female rats. These effects were coupled with, and probably triggered by, further shift in the sympathovagal balance towards parasympathetic dominance as suggested by the decreases in IBI_{LF} , $IBI_{LF/HF}$ and increases in IBI_{HF} in ethanol-fed rats. Such hemodynamic and autonomic effects of NPLA are apparently ethanol-dependent because they were not evident in control rats. The lack of NPLA effect on cardiac contractility and autonomic activity in liquid diet-fed (control) rats is consistent with a similar lack of NPLA effect on BP, cardiac output and stroke volume in the same rat strain and sex in our previous study (El-Mas et al., 2009). It is conceivable, therefore, to conclude that tonic nNOS activity functions to hinder the full expression of the adverse hemodynamic and autonomic effects of chronic ethanol in female rats.

It is imperative to reconcile our seemingly contradictory pharmacologic findings following selective nNOS inhibition with the molecular findings that demonstrated, for the first time, enhancement of vascular nNOS phosphorylation in ethanol-fed rats. It is highly likely that the enhanced vascular nNOS phosphorylation guards against the deleterious reductions in cardiac contractility and BP in ethanol-fed rats caused, at least partly, via enhanced vascular eNOS/NO signaling in our model system. This view is consistent with nNOS-derived NO involvement in protection against the development of heart failure after myocardial infarction (Casadei 2006), and in a sympathetically mediated increase in BP via interaction with arterial baroreceptors (Kurihara et al., 1998). Indeed, selective nNOS inhibition uncovered a reduction in BP (Fig. 2) that paralleled the reductions in the index of cardiac contractility dP/dT_{max} (Fig. 3), and the sympathetic component of the sympathovagal balance (Fig. 4D). Together, the present molecular and pharmacological studies support a counterbalancing role for nNOS-derived NO to guard against further exacerbation of the deleterious cardiovascular effects caused by ethanol feeding. More studies are needed to

investigate the mechanisms of the protective cardiovascular and autonomic effects of nNOS-derived NO in ethanol-fed rats.

It is important to comment on two potential limitations of the present study. The first pertains to the discrepancy in the duration of the ethanol (chronic) and NOS inhibitors (acute) regimens. Typically, single dose regimens are less likely to modulate long-standing biological phenomena brought about by chronic interventions. Second, no isometric tension studies were pursued in the tail artery to assess the role of vascular endothelial activity in the BP response elicited by ethanol. Despite the clear increase in tail artery eNOS expression seen in ethanol-treated rats, the assumption that BP responses caused by ethanol in the absence or presence of NOS inhibition relate to changes in vascular reactivity cannot be ascertained from the current observations. These important issues will be addressed in future studies.

Overall, the current study provides important information regarding the modulatory roles of NOS-NO signaling in the adverse cardiovascular and autonomic responses elicited by 8-week ethanol feeding in female rats. First, a possible role for vascular NOS in the chronic hypotensive action of ethanol is suggested by the increased phosphorylated eNOS and nNOS expression in tail arteries of ethanol-fed rats. Second, the selective inhibition of eNOS in ethanol-fed rats produced lesser than expected pressor effect in ethanol-fed rats due probably to the offsetting effect of the increased expression of vascular constitutive NOS. Third, nNOS tonically suppresses the deleterious cardiovascular and autonomic effects of ethanol and the loss of this favorable function following selective nNOS inhibition could exacerbate the deleterious cardiovascular effects of ethanol.

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Figure 1.

The phosphorylated/total protein ratios of constitutive and inducible NOS isoforms in the tail artery of female Sprague-Dawley rats treated with ethanol (5% w/v) or isocaloric control diet for 8 weeks. Illustrative gels depicting vascular NOS protein contents are shown. Values are means $\pm$ S.E.M of 6–7 observations. *P<0.05 versus corresponding control values.

Figure 2.

Changes in mean arterial pressure (MAP, left panels) or heart rate (HR, right panels) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg; i.p.), nNOS (NPLA, 1 mg/kg; i.p.), or iNOS (1400W, 5 mg/kg; i.p.) or equal volume of i.p. saline in telemetered female rats fed on 5% w/v ethanol or control liquid diet for 8 weeks. * and $^+P < 0.05$ vs. saline and control pair-fed values, respectively.

Figure 3. Changes in myocardial contractility ($+dP/dt_{\max}$) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg; i.p.), nNOS (NPLA, 1 mg/kg; i.p.), or iNOS (1400W, 5 mg/kg; i.p.) or equal volume of i.p. saline in telemetered female rats fed on 5% w/v ethanol or control liquid diet for 8 weeks. * and $^+P < 0.05$ vs. saline and control pair-fed values, respectively.

Figure 4.

Changes in high-frequency (IBI-HF_{nu}, 0.75–3 Hz, right panels) and low-frequency (IBI-LF_{nu}, 0.25–0.75 Hz, left panels) spectral bands of IBI evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg; i.p.), nNOS (NPLA, 1 mg/kg; i.p.), or iNOS (1400W, 5 mg/kg; i.p.) or equal volume of i.p. saline in telemetered female rats fed on 5% w/v ethanol or control liquid diet for 8 weeks. * and +P<0.05 vs. saline and control pair-fed values, respectively.

Figure 5.

Changes in the LF/HF ratio of IBI evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg; i.p.), nNOS (NPLA, 1 mg/kg; i.p.), or iNOS (1400W, 5 mg/kg; i.p.) or equal volume of i.p. saline in telemetered female rats fed on 5% w/v ethanol or control liquid diet for 8 weeks. * and $^{+}P < 0.05$ vs. saline and control pair-fed values, respectively.

Figure 6.

Changes in low-frequency SBP spectral density ($\text{SBP-LF}_{\text{nu}}$, 0.25–0.75 Hz) evoked by selective inhibition of eNOS (L-NIO, 20 mg/kg; i.p.), nNOS (NPLA, 1 mg/kg; i.p.), or iNOS (1400W, 5 mg/kg; i.p.) or equal volume of i.p. saline in telemetered female rats fed on 5% w/v ethanol or control liquid diet for 8 weeks. * and ⁺P<0.05 vs. saline and control pair-fed values, respectively.

Table 1

Hemodynamic values after 8 weeks of ethanol or isocaloric control diet feeding.

Parameter	Control	Ethanol
MAP, mmHg	103.2±1.2	91.9±1.4 *
HR, beats/min	374.2±4.0	365.4±3.5
dP/dt _{max} , mmHg/sec	1604±105	1343±64 *
IBI-LF _{nu} , sec ² /Hz	0.678±0.011	0.565±0.032 *
IBI-HF _{nu} , sec ² /Hz	0.321±0.011	0.434±0.033 *
IBI _{LF/HF}	1.97±0.22	1.26±0.17 *
SBP-LF, mmHg ² /Hz	0.136±0.008	0.137±0.012

Values are means±SEM.

* P<0.05 vs. control values.