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Cardiovascular autonomic modulation by nitric oxide synthases accounts for the augmented enalapril-evoked hypotension in ethanol-fed female rats

Mahmoud M. El-Mas [1], Abdel A. Abdel-Rahman*

Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA

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ABSTRACT

In this study, we investigated the role of nitric oxide synthase (NOS) isoforms in the enhanced enalapril-evoked hypotension in ethanol-fed female rats by examining the effect of the selective inhibitors of eNOS [N^5-(1-iminoethyl)-L-ornithine; L-NIO], nNOS (N^{ω}-propyl-L-arginine; NPLA), or iNOS (1400W) inhibition on the cardiovascular effects of enalapril in ethanol- (5% w/v) fed rats and in their pair-fed controls. In liquid diet-fed control rats, enalapril- (10 mg/kg) evoked hypotension was abolished by L-NIO (20 mg/kg), but not by NPLA (1 mg/kg) or 1400W (5 mg/kg), suggesting a preferential role for eNOS in this response. Enalapril had no effect on spectral indices of hemodynamic variability or $+dP/dt_{max}$ (myocardial contractility). However, in ethanol-fed rats, the greater enalapril-evoked hypotension was associated with reductions in (i) $+dP/dt_{max}$, (ii) low-frequency/high-frequency ratio of interbeat intervals ($IBI_{LF/HF}$), suggesting cardiac parasympathetic dominance, and (iii) low-frequency spectral band of systolic blood pressure (BP), a marker of vasomotor sympathetic tone. While NPLA or 1400W attenuated the enalapril-evoked hemodynamic and autonomic responses in ethanol-fed rats, L-NIO virtually abolished the hypotensive response and was more efficacious in rectifying autonomic responses to enalapril. Together, these findings implicate NOS isoforms, particularly eNOS, in the altered cardiovascular autonomic control that leads to the augmented enalapril-evoked hypotension in ethanol-fed female rats.

Introduction

Reduction in circulating angiotensin II, due to angiotensin converting enzyme (ACE) inhibition, is the principal mechanism by which ACE inhibitors lower BP (Sepehrdad, Frishman, Stier, & Sica, 2007). The reduced cardiovascular risk and mortality in patients receiving ACE inhibitors also relates to the improved cardiac autonomic control and hemodynamic variability, which may or may not be related to the BP-lowering effect (Binkley et al., 2000; Ylitalo, Airaksinen, Sellin, & Huikuri, 1999). Notably, cardiovascular autonomic neuropathy is associated with impaired regulation of BP, heart rate and heart rate variability (HRV), and increased susceptibility to ventricular arrhythmias and sudden cardiac death (Gerritsen et al., 2001). Further, whereas reductions in cardiac parasympathetic tone predispose to sudden cardiac death (due probably to increased susceptibility to fibrillatory attacks), vagal dominance is coupled with a reduced risk of arrhythmias (Billman, 2002; Sgoifo et al., 1997). Clinical data have also established a relationship between BP variability and the severity of end-organ damage (Mancia & Parati, 2000; Parati, Ulian, Santucciu, Omboni, & Mancia, 1995).

Our recent study established the first evidence that chronic ethanol exposure potentiates the enalapril-evoked hypotensive response in female rats (El-Mas & Abdel-Rahman, 2011). The underlying molecular mechanism appears to involve ethanol enhancement of angiotensin II/bradykinin signaling because ethanol-fed rats, when compared to pair-fed control rats, exhibited significantly higher renal Ang II levels and ACE and bradykinin receptor protein expressions. Also, blockade of bradykinin B_2 receptors (bradyzide) eliminated the enhanced hypotensive response caused by ACE inhibition in ethanol-fed rats (El-Mas & Abdel-Rahman, 2011).

Notably, reported studies have shown that ethanol does not uniformly potentiate the BP response elicited by antihypertensive medications. Chronic ethanol exposure decreases centrally mediated (clonidine) and increases peripherally mediated (hydralazine) hypotension (El-Mas & Abdel-Rahman, 2003, 2004). Similarly, the mechanism of the BP-lowering effect of antihypertensive drugs determines, at least partly, whether acutely administered ethanol increases or decreases the antihypertensive response (El-Mas & Abdel-Rahman, 1997, 1999a, 1999b). It is imperative to note that all previous studies on the interaction of ethanol with antihypertensive medications were undertaken in

* Corresponding author. Tel.: +1 252 744 3470; fax: +1 252 744 3203.
E-mail address: abdelrahmana@ecu.edu (A.A. Abdel-Rahman).
[1] Dr. Mahmoud M. El-Mas is on a sabbatical leave from the Department of Pharmacology, Faculty of Pharmacy, Alexandria University, Egypt.

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male rats (El-Mas & Abdel-Rahman, 1997, 2003, 2004). Therefore, our recent observation that chronic ethanol exposure enhanced the hypotensive action of enalapril in female rats (El-Mas & Abdel-Rahman, 2011) constituted an important step for investigating the interaction of ethanol with antihypertensive therapies in the female population.

In this communication, which extends our previous work (El-Mas & Abdel-Rahman, 2011), we tested the hypothesis that the modulation of cardiovascular autonomic control by NOS mediates the enhancement of the enalapril-evoked hypotension in ethanol-fed female rats. Observations that prompted us to investigate this possibility are (i) NOS upregulation contributes to the cardiovascular effects of ethanol (El-Mas, Fan, & Abdel-Rahman, 2008, 2011; Williams, Adams, & Mustafa, 1990) or enalapril (Förstermann & Sessa, 2012; Sahach, Baziliuk, Stepanenko, Korkach, & Kotsiuruba, 2007), and (ii) NOS/NO (nitric oxide) signaling regulates cardiovascular autonomic activity (Heaton et al., 2005; Herring & Paterson, 2001). The present studies were conducted in telemetered female rats at the conclusion of chronic ethanol (5% w/v) or isocaloric liquid diet feeding, described in our recent study (El-Mas et al., 2011), to investigate the effect of selective inhibition of constitutive and inducible NOS on the enalapril-evoked changes in BP, $+dP/dt_{max}$, and spectral indices of hemodynamic variability. Spectral indices of hemodynamic variability are categorized into low-frequency interbeat intervals (IBI_{LF}; 0.25–0.75 Hz; reflect the sympathetic drive) and high-frequency interbeat intervals (IBI_{HF}; 0.75–3 Hz; reflect the cardiac vagal control), along with the ratio of LF to HF interbeat intervals ($IBI_{LF/HF}$), which is a measure of the sympathovagal balance of the heart (El-Mas & Abdel-Rahman, 2012; Thomas, 2011). The ethanol (5% w/v, 8 weeks) or isocaloric liquid diet was provided using a pair-feeding paradigm to ensure similar fluid and nutrient intakes as in our previous studies (El-Mas & Abdel-Rahman, 2004; El-Mas et al., 2011). This ethanol paradigm produced blood–ethanol concentrations of 100–130 mg/dL (El-Mas & Abdel-Rahman, 2011; El-Mas et al., 2011), which are comparable to those attained following mild to moderate human consumption of ethanol (Eddleston, Gunnell, von Meyer, & Eyer, 2009; Ireland, Vandongen, Davidson, Beilin, & Rouse, 1984; Schaller et al., 2010).

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 rat chow and were maintained on a 12:12-h light–dark cycle with lights off at 4:00 PM. Room temperature was maintained at 22 ± 1 °C. After 1 week of acclimatization, rats were fed a standard Lieber and DeCarli high-protein liquid diet (Dyets Inc., Bethlehem, PA) for another week before starting the ethanol regimen. Rats received the diet daily at 3:30 PM before the start of the dark cycle. All experiments were approved by the Institutional Animal Care and Use Committee of East Carolina University and were 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 ($n = 7$) were provided a standard Lieber and DeCarli high-protein liquid diet containing 5% w/v ethanol (36% of total caloric intake) for 8 weeks as described in our previous study (El-Mas & Abdel-Rahman, 2011). The other group of rats received an isocaloric liquid diet and served as controls ($n = 6$). 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 to approximately 8–9 g/kg. Control rats were pair-fed and received an isocaloric amount of dextrin/maltose (89.6 g/L) in place of ethanol, which allowed nutrient intake and fluid consumption similar to that of ethanol-fed rats. Fresh diets were prepared every other day and refrigerated until dispensed.

Hemodynamic effects of enalapril in absence or presence of constitutive or inducible NOS inhibitor

At the conclusion of the ethanol/liquid diet feeding described in our recent study (El-Mas et al., 2011), each rat in the ethanol ($n = 7$) or control ($n = 6$) group received 5 different i.p. injections at 3-day intervals: (i) saline (1 mL/kg), (ii) enalapril (10 mg/kg), (iii) the eNOS inhibitor L-NIO (20 mg/kg) + enalapril (10 mg/kg), (iv) the nNOS inhibitor NPLA (1 mg/kg) + enalapril (10 mg/kg), and (v) the iNOS inhibitor 1400W (5 mg/kg) + enalapril (10 mg/kg). NOS inhibitors were administered 10 min prior to enalapril. All injections were done at 9:00 AM and hemodynamic monitoring continued for the following 5 h. The chosen doses of the NOS inhibitors were based on published reports (El-Mas et al., 2008, 2009). Rats were maintained on ethanol or control diet for the duration of the study.

Telemetry transmitter implantation, data acquisition and analysis

The methods used for telemetry transmitter implantation (Data Sciences Int., St. Paul, MN) and the chronic ethanol-feeding regimen are detailed in our previous studies (El-Mas & Abdel-Rahman, 2000, 2003, 2011). Data were 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 s every 10 min. The maximum rate of rise of BP waves ($+dP/dt_{max}$), which represents myocardial contractility (van den Buuse, 2003), was computed by Data Sciences software. 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 & 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, which are quantitative indices of cardiovascular autonomic control (El-Mas & Abdel-Rahman, 2011; Stein, Bosner, Kleiger, & Conger, 1994), were used to detect 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 & Tarassenko, 2004; El-Mas & 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 were interpolated to obtain equally spaced samples with an effective sampling frequency of 10 Hz (0.1 s duration). A second-order quadratic equation was employed to fit a smooth curve to the existing data points and produce a smoother