Published in final edited form as:

J Cardiovasc Pharmacol. 2011 January ; 57(1): 72–78. doi:10.1097/FJC.0b013e3181fef9e8.

Bradykinin B₂ receptor-dependent enhancement of enalapril-evoked hypotension in ethanol-fed female rats

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Abstract

Our previous studies showed that chronic ethanol feeding attenuates centrally (clonidine)- and potentiates peripherally (hydralazine)-evoked hypotension in female rats. In this study, we investigated whether chronic ethanol (8 weeks, 5% w/v) alters hemodynamic responses elicited by angiotensin converting enzyme (ACE) inhibition (enalapril) in telemetered female rats. Given the intimate interaction between ACE and bradykinin, studies were extended to investigate the role of bradykinin receptor (B₂R) in ethanol-enalapril interaction. Compared with pair-fed controls, ethanol-fed female rats exhibited: (i) higher renal expressions of ACE and B₂R proteins and angiotensin II levels, and (ii) lower blood pressure (BP). Pharmacological inhibition of ACE and B₂R support functional role for the higher levels of these two proteins in ethanol-fed rats because enalapril (10 mg/kg i.p.) caused significantly greater hypotensive response in ethanol-fed rats than in control rats. Further, blockade of B₂R with bradyzide (2 mg/kg i.p.) abrogated the enhanced hypotensive effect of enalapril in ethanol-fed rats but had no effect on enalapril-evoked hypotension in control rats. Finally, enalapril enhancement of spontaneous baroreflex sensitivity (BRS) in control was absent in ethanol-fed rats. These findings demonstrate that chronic ethanol produces B₂R-dependent enhancement of the hypotensive response elicited by enalapril and abrogates enalapril-evoked enhancement of spontaneous baroreflex response in female rats.

Keywords

Blood pressure; angiotensin converting enzyme; bradyzide; enalapril; arterial baroreceptors

Introduction

The renin angiotensin system (RAS) plays important role in the preservation of hemodynamic stability. Increased RAS activity is associated with the development of hypertension, atherosclerosis, left ventricular hypertrophy and other cardiovascular events (1). ACE inhibitors constitute an important therapeutic approach for management of hypertension and heart failure. Importantly, ACE inhibitors have also been used in normotensive individuals to protect against acute coronary syndrome and (2) to attenuate

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decline in renal function. The main mechanism of action of ACE inhibitors involves the inhibition of ACE, a carboxydiptidase that synthesizes Ang II from its decapeptide precursor Ang I (3). The reduced cardiovascular risk and mortality in patients receiving ACE inhibitors might also be a consequence of the improved cardiac autonomic control and hemodynamic variability, which may or may not be related to the BP lowering effect (4,5). The inhibition of bradykinin degradation and subsequent increase in nitric oxide and prostacyclin release is another mechanism for the hypotensive action of ACE inhibitors (1). ACE inhibition increases blood levels of bradykinin and kallidin (6) and the blockade of B₂R reduces the hypotensive response elicited by ACE inhibitors (7).

These findings demonstrate that long-term ethanol feeding elicits divergent effects on centrally and peripherally evoked hypotension in female rats (8). In the current study, we conducted pharmacological and cellular studies to investigate the effect of chronic ethanol feeding on RAS activity and hemodynamic responses elicited by the ACE inhibitor enalapril. To our knowledge, while RAS activity has been shown to increase in response to chronic ethanol administration in male animals (9–11), no reports are currently available on the chronic effects of ethanol on the RAS or drugs that inhibit its activity in female rats. In the present study, RAS activity was determined by measuring Ang II levels and ACE protein expression in renal homogenates. We focused on renal RAS because in addition to regulating glomerular blood flow, tubular sodium reabsorption, and renin secretion, the renal RAS also amplifies circulating Ang II actions and positively correlates with BP (12,13). Given that ACE inhibitors confer cardiovascular protection, in part, via enhancement of cardiac reflexes (5), we also investigated the effect of chronic ethanol on this beneficial cardiovascular effect of ACE inhibition. Because preliminary findings showed that enalapril-evoked hypotension was enhanced by ethanol feeding, we tested the hypothesis that upregulation of B₂R, which is linked to ACE via a feedback mechanism (14), might contribute to the ethanol-enalapril interaction. This was achieved by demonstrating, for the first time, higher renal B₂R expression in ethanol-fed rats and by investigating the effect of bradyzide, a blocker of B₂R, on hemodynamic responses elicited by enalapril in ethanol-fed rats and their controls. Ethanol (5% w/v, 8 weeks) or isocaloric liquid diet was provided using pair-feeding paradigm to ensure similar fluid and nutrient intakes as in our previous studies (8,15).

Materials and Methods

Female Sprague-Dawley rats (9–10 weeks; 190–225 g; Harlan, Indianapolis, IN) were used in the present study. Upon arrival, the 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±1°C. After one-week acclimatization, rats were fed a standard Lieber-DeCarli high protein liquid diet (Dyets Inc., Bethlehem, PA) for another week before ethanol feeding. 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.

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 (8,15). Rats were left for 3 additional weeks before starting the experiment during which the rats continued to receive ethanol and control diets.

Telemetry data acquisition and analysis

Individual rat cages were placed on the top of the radio receivers and 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 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 20-min blocks (i.e. the average of two successive measurements) for analysis as in our previous studies (8,15). 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.

Spontaneous BRS (index α)

was assessed by the frequency domain analysis of periodogram function of systolic blood pressure (SBP) and IBI data series as in previous studies including our own (16,17). 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 sec). 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. The index α , square roots of the ratio of IBI and SBP powers at LF (LF α), was calculated to reflect changes in spontaneous BRS as in our previous studies (17).

Western blotting

For the determination of renal ACE and B₂R protein expressions, the kidney was homogenized on ice in a homogenization buffer [50 mM Tris (pH 7.5), 0.1 mM EGTA, 0.1 mM EDTA, 2 μ M 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 quantified (Bio-Rad protein assay system; Bio-Rad, Hercules, CA). Protein extracts (20 and 60 μ g per lane for ACE and B₂R, respectively) were run on a 4 to 12% SDS-polyacrylamide gel electrophoresis gel (Invitrogen, Carlsbad, CA) and electroblotted to nitrocellulose membranes. Blots were blocked for 60 min at room temperature in tris-buffered saline/tween20 (TBS/T) buffer (100 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) containing 5% non-fat milk. They were then incubated overnight at 4 °C with rabbit polyclonal antibodies to ACE (Ser1417; 1:200; Santa Cruz Biotech, Santa Cruz, CA) or mouse monoclonal antibodies to B₂R (1:500, BD Biosciences, San Jose, CA) in TBS/T buffer containing 5% BSA. After 3 washes with TBS/T buffer, the blots were incubated for 60 min at room temperature with the appropriate horseradish peroxidase-linked species-specific anti-IgG to ACE (GE Healthcare BIO-Sciences Corp, Piscataway, NJ) or B₂R (Bio-Rad, Hercules, CA). After 3 washes with the TBS/T buffer, the blots were detected by enhanced chemiluminescence system and exposed to an X-ray film. Equivalent sample loading was confirmed by stripping membranes with blot restore membrane rejuvenation solution (SignaGen Laboratories, Gaithersburg, MD) and reprobing with anti-actin antibody (Sigma). Protein bands were quantified by measuring the integrated density (mean density $\times$ area) using the NIH Image software (version 1.62); data was normalized in relation to actin, and expressed as a percent of control as in our previous studies (18).

Measurement of renal Ang II

Tissue preparation was performed as described above under Western blot analysis. The levels of Ang II in the kidney were measured by the enzyme immunoassay (EIA) using EIA

kit in accordance with manufacturer's instructions (SP1-bio, Cambrex Bio Science Walkersville, Inc, Walkersville, MD).

Blood analyses

Blood samples were withdrawn from the tail vein of ethanol-fed and control rats one day before starting acute studies (i.e. 8 weeks after feeding ethanol or control diet) for the determination of plasma estrogen and ethanol levels by the radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX) and enzymatic methods, respectively, as described in our previous studies (17,19).

Protocols and experimental groups

Four groups of female rats matched for body weight were used in two experiments. In two of the four groups, telemetry transmitters were implanted as detailed above to permit continuous blood pressure recording throughout the study. One of the telemetry-implanted (n=7) and one of the non-telemetry implanted groups (n=7) received ethanol as described below. The other two groups received isocaloric liquid diet and served as controls (telemetry group, n=6; non-telemetry group, n=8). Rats in the ethanol groups 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 (8,15). To acclimate the rats to the ethanol diet, ethanol (Midwest Grain Products Co., Weston, MO) was first provided as half strength (2.5% w/v, 18% of calories intake) and then increased to 5% w/v after 3 days. The daily ethanol intake amounted approximately to 8–9 g/kg body weight. The 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.

Effect of ethanol on renal ACE and B₂R expression and angiotensin II level

In this experiment we investigated the effect of chronic ethanol administration on indices of the RAS activity in our model system. The kidney was chosen because of its relatively high content of ACE and Ang II. At the end of the 8-week treatment period, the rats in the ethanol (n=7) and control (n=8) groups were sacrificed. The kidneys were stored in a –80°C freezer until use for measurement of renal ACE and B₂R expression and Ang II level as described above.

Hemodynamic consequences of ACE inhibition in the absence or presence of bradykinin B₂R blockade in ethanol-fed rats

The objective of this experiment was to investigate the influence of ethanol feeding on chronic hemodynamic and baroreflex effects caused by enalapril in the absence or presence bradyzide, a selective B₂R blocker. After 8 weeks of ethanol feeding, each rat in the ethanol (n=7) and control (n=6) groups received 4 different i.p. injections (saline 1 ml/kg, enalapril 10 mg/kg, bradyzide 2 mg/kg, or bradyzide 15 min before enalapril); a period of 3 days was allowed between subsequent injections. All injections were given at 9:00 am and hemodynamic variables were monitored for the following 5 hr. The chosen doses for enalapril and bradyzide are based on published reports (20,21). Rats were maintained on ethanol or control diet for the duration of the study and the i.p injections had no significant impact on daily ethanol/liquid diet intake.

Drugs

Enalapril maleate, bradyzide di(trifluoroacetate) salt hydrate (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 areas under the curves (AUC) of the changes in BP and spontaneous BRS caused by enalapril, bradyzide, or their combination were calculated for individual experiments using trapezoidal integration (Graph pad prism, version 3.0). The unpaired Student's t-test was used for the analysis of the effect of ethanol on ACE and B₂R protein expressions. 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 enalapril, bradyzide, or their combination. 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

Chronic effects of ethanol on BP and renal ACE and B₂R expression and Ang II levels

Compared with control rats, 8-week ethanol feeding caused modest but significant reductions in BP while HR and spontaneous BRS were not affected by ethanol (Table 1). Western blotting showed that 8-week ethanol feeding caused significant increases in the renal expression of ACE and B₂R (Fig. 1A). Renal levels of Ang II were also increased by ethanol feeding compared with pair-fed control rats (5.39 ± 0.35 vs. 2.49 ± 0.33 pg/mg protein). Plasma estradiol levels in ethanol-fed and control rats were similar (20 ± 3 and 21 ± 2 pg/ml). In ethanol-fed rats, plasma ethanol levels averaged 130 ± 10 mg/dl. Baseline body weights of rats before starting ethanol or control diet feeding were similar (200 ± 3 vs. 201 ± 2 g). The body weight of rats that received ethanol diet for 8 weeks was significantly lower than that of control rats (281 ± 5 vs. 301 ± 6 g).

Hemodynamic consequences of ACE inhibition in the absence or presence of bradykinin B₂R blockade in ethanol-fed rats

The pretreatment hemodynamic values obtained in ethanol-fed and control rats prior to the acute administration of enalapril, bradyzide, or bradyzide plus enalapril or their vehicle (saline) are shown in Table 1. Figures 2 and 3 illustrate the time-course effects of i.p. saline, enalapril (10 mg/kg), bradyzide (2 mg/kg), or enalapril/bradyzide combination on BP and HR in telemetered rats treated chronically with ethanol (5% w/v, 8 weeks) or isocaloric liquid diet. The i.p. administration of saline caused slight decrease in BP in ethanol-treated (Fig. 2B) but not in control rats (Fig. 2A); however, differences between the changes in DBP were not statistically significant. Compared with corresponding vehicle (saline) values, enalapril elicited significant reduction in BP in liquid diet fed (control) rats that reached a nadir (-19 ± 3 mmHg) at 60 min and was sustained during the 5-hr observation period of the study (Fig. 2A). HR (Fig. 3A) was not affected by enalapril. In ethanol-fed rats, the maximal hypotensive effect of enalapril (-28 ± 2 mmHg, Fig. 2B) as well as its duration were substantially augmented. This was demonstrated by the area under the curve (AUC), which measured the cumulative falls in BP over the entire 5 hr period that followed enalapril administration. AUC was almost doubled (6215 ± 653 vs. 3888 ± 521 mmHg.min, $P<0.05$) in ethanol-fed rats compared with corresponding value in liquid diet pair-fed controls. The substantially greater hypotensive response in ethanol-fed following ACE inhibition (enalapril) was abrogated by prior blockade of bradykinin B₂R with bradyzide (Fig. 2B). By contrast, the hypotension caused by enalapril in liquid diet fed (control) rats was not influenced by bradyzide (Fig. 2A). Further, in the presence of bradyzide, the AUC values, which reflect the magnitude and duration of enalapril hypotension, were not statistically

different in ethanol-fed vs. liquid diet fed control rats (2830 ± 762 vs. 3141 ± 600 mmHg.min). The administration of enalapril in bradyzide-pretreated rats elicited significant increases in HR (Fig. 3) in both ethanol-fed and control rats.

In liquid diet fed control rats, enalapril treatment significantly ($P < 0.05$) increased the AUC of the spectral index of spontaneous BRS (index α) measured during the 5-hr observation period of the study (Fig. 4). Although blockade of bradykinin B_2R (bradyzide) had no effect on spontaneous BRS (Fig. 4), bradyzide abrogated the enhancement of BRS caused by subsequently administered enalapril (Fig. 4). By contrast, enalapril failed to enhance spontaneous BRS in ethanol-fed rats in the presence or absence of bradyzide (Fig. 4).

Discussion

The present study is the first to report on the hemodynamic interaction between ethanol and the RAS-bradykinin pathway in female rats. The most important findings are: (i) Compared with pair-fed control rats, ethanol-fed female rats exhibited enhanced RAS activity, exemplified by significantly higher renal Ang II level and ACE and B_2R protein expressions. (ii) Pharmacological evidence supported enhanced RAS activity in ethanol-fed rats because ACE inhibition (enalapril) caused significantly greater hypotensive response in ethanol-fed compared with control rats. (iii) Prior blockade of bradykinin B_2R with bradyzide abrogated the enhanced hypotensive response caused by ACE inhibition in ethanol-fed rats but had no effect on enalapril-evoked hypotension in control rats. Bradykinin B_2R signaling appears to contribute to the augmented hypotensive effect of enalapril in ethanol-fed rats. Finally, enalapril enhanced spontaneous BRS in control, but not in ethanol-fed, rats and such effect was virtually abolished by bradyzide. Together, cellular and pharmacological data suggest that upregulation of ACE activity and B_2R signaling underlies, at least partly, the substantially enhanced hypotensive response elicited by ACE inhibition in ethanol-fed female rats. However, the well-documented ethanol inhibitory action on baroreflexes seems to offset the B_2R -dependent enhancement of BRS caused by enalapril in the present study.

Results of the present study show, for the first time, that compared with liquid diet fed control rats; enalapril produced substantially greater hypotension in rats treated chronically with ethanol despite the presence of modestly but significantly lower blood pressure in these rats. Remarkably, the AUC of the hypotension caused by enalapril in ethanol-fed rats over the entire 5 hr observation period was approximately 2-fold the AUC achieved in their control counterparts. Because ethanol-fed rats exhibited elevated levels of Ang II and ACE protein expression in renal tissues, it is conceivable that the exaggerated hypotensive response of enalapril in ethanol-fed rats might be related to the heightened RAS activity in these rats. As mentioned earlier, local RAS activity in the kidney plays important role in BP regulation and reinforces vascular responsiveness to circulating Ang II (12,13). Notably, previous studies undertaken in male animals demonstrated that chronic ethanol administration enhances RAS activity (9–11). Our similar findings in female rats, therefore, indicate that the enhancement of RAS activity by ethanol does not seem to be gender specific. However, it remains to be determined, in future studies, whether the facilitatory effect of chronic ethanol on the hypotensive response elicited by ACE inhibition could be replicated in male rats.

Considering the established role of bradykinin in the hemodynamic responses elicited by ACE inhibition (6,7), we reasoned that facilitation of bradykinin B_2 signaling might contribute to the enhanced hypotensive response elicited by ACE inhibition in ethanol-fed female rats. We demonstrated significant increase in B_2R expression in the kidney of ethanol-fed compared with control rats. Pharmacological studies also showed that prior

blockade of bradykinin B₂R (bradyzide) abrogated the increase in enalapril-evoked hypotension in ethanol-fed rats. By contrast, the hypotensive effect of enalapril in the absence of ethanol in control rats was not significantly affected by bradyzide. In effect, in bradyzide-pretreated ethanol-fed or isocaloric diet-fed rats, the overall hypotensive response (AUC) elicited by enalapril was very similar. Interestingly, the upregulation of B₂R might also be responsible for the increase in ACE expression caused by ethanol, perhaps via a feedback mechanism (14). Alternatively, the B₂R-dependent enhancement of enalapril-evoked hypotension in ethanol-fed rats could be attributed to the facilitation of bradykinin signaling in the presence of ethanol. Consistent with this view, ethanol augments the NO-dependent vasodilator effect of bradykinin via enhancing the activity of NOS in endothelial cells and in blood vessels (22–24). Ethanol also increases kallikrein activity and potentiates the bradykinin-induced increase in vascular permeability (25). Taken together, present findings suggest a primary role for B₂R upregulation in the increases caused by chronic ethanol in the hypotensive effect of enalapril, and probably RAS activity (14).

The improvement of cardiac autonomic control and hemodynamic variability has been implicated in the reduced cardiovascular risk and mortality in patients receiving ACE inhibitors (4,5). The present study yield insights into whether this favorable autonomic consequence of ACE inhibition could be compromised by long-term ethanol administration in female rats. In agreement with previous reports (5), spectral analysis of hemodynamic variability undertaken in the present study showed that enalapril increased the spontaneous BRS (index α) in control rats. B₂R signaling appears to be involved in the facilitatory baroreflex effect of enalapril because the latter was abolished by prior B₂R blockade (bradyzide). ACE inhibition increases blood levels of bradykinin (6), which is believed to sensitize both vagal and sympathetic afferent fibers involved in reflex control of cardiac autonomic activity (26,27). With that said, the lack of an effect of enalapril on baroreflex activity in ethanol-fed rats despite concomitant increase in B₂R activity seems surprising. One possible explanation for this paradox may relate to the ability of ethanol to interfere with other baroreflex regulating mechanisms, which act to offset anticipated facilitation of the baroreflex response to enalapril in ethanol-fed rats. Indeed, we (28) and others (29) have reported that ethanol disrupts central glutamatergic (inhibition) and GABAergic (facilitation) pathways, which are crucial for the central processing of baroreceptor information.

Reported effects of ACE inhibition on HR are variable and depend on the dose and duration of drug regimen (30,31). In this study, enalapril had no effect on HR when administered alone but caused sustained tachycardia in bradyzide-pretreated control rats. Interestingly, the increase in HR caused by enalapril was ethanol-independent since it was similarly demonstrated in bradyzide-treated ethanol-fed rats. While the mechanism is not clear, it is likely that the tachycardia associated with enalapril becomes evident in presence of interventions (bradyzide or ethanol) that abrogate the baroreflex facilitatory effect of enalapril (see Fig. 4). Indeed, the interruption of baroreflex gain has been shown to be accompanied by acceleration of the heart due to increased and decreased sympathetic and vagal drives, respectively (32). More studies are needed to investigate this possibility.

It is imperative to comment on the possibility that the enhanced RAS activity serves a counterbalancing role against ethanol-evoked hypotension. If this assumption is correct, then the modest hypotensive response in ethanol-fed rats in the present and reported (19,33) studies constitutes the algebraic sum between ethanol-evoked hypotension on the one hand and enhancement of RAS activity on the other. Notably, our previous studies implicated reduced myocardial contractile force and cardiac output, perhaps due to increased myocardial iNOS expression, in the hypotensive effect of ethanol (17–19,33). Therefore, our present results highlight a possible counterbalancing role for RAS against the hypotensive

action of ethanol. In effect, the observation that pharmacologic ACE inhibition by enalapril elicited more hypotension in ethanol-fed rats supports this assumption. It is also important to comment on the possibility that enhanced B₂R expression might reflect involvement of bradykinin in the hypotensive effect of ethanol. However, the lack of effect of B₂R blockade (bradyzide) on BP in ethanol-fed rats despite the concomitant upregulation of B₂R apparently argues against the involvement of B₂R in ethanol-evoked hypotension. More studies are needed, however, to clarify the precise role of RAS/bradykinin signaling in the cardiovascular responses elicited by ethanol in female rats. It is also imperative that the impact of sex/gender on these responses be investigated in future studies given the significantly greater, estrogen-dependent, hypotensive response elicited by chronic ethanol administration in normotensive female, compared with age-matched male, rats (33,34).

In summary, the present study established the first experimental evidence that chronic ethanol potentiates the hypotensive response elicited by ACE inhibition in female rats due, at least partly, to increased RAS activity. The facilitation of B₂R signaling seems also to contribute to the ethanol-enalapril interaction because: (i) chronic ethanol significantly increased B₂R expression in renal tissues, and (ii) prior blockade of bradykinin B₂R by bradyzide abrogated the exaggerated hypotensive effect of enalapril in ethanol-treated rats. It is also noteworthy that ethanol virtually abolished the B₂R-dependent favorable effect of enalapril on baroreflex responsiveness. Clinically, alcohol use should be avoided or at least minimized to guard against excessive falls in BP when ACE inhibitors are used in normotensive women as cardioprotective or to attenuate the decline in renal function (2). Further, given the widespread use of ACE inhibitors for the management of hypertension, future studies are warranted to determine whether the antihypertensive effect of ACE inhibitors is also unduly enhanced by alcohol in hypertensive animals. Notably, the dose of ethanol used in the present study produced blood ethanol concentrations comparable to those attained in humans following consumption of mild to moderate amounts of ethanol (35).

Acknowledgments

Supported by Grant R01 AA014441 from the National Institute on Alcohol Abuse and Alcoholism. The authors thank Ms. Kui Sun for her technical assistance.

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Figure 1.

Effect of 8-week feeding of 5% w/v ethanol (n=7) or isocaloric control liquid diet (n=8) on renal expression of angiotensin converting enzyme (ACE) and bradykinin B₂ receptor (B₂R) in female rats. Illustrative gels depicting renal ACE and B₂R protein expressions are shown.

*P<0.05 vs. control values

Figure 2.

Effect of 8-week feeding of 5% w/v ethanol (bottom panel, n=7) or control liquid diet (top panel, n=6) on changes in mean arterial pressure (MAP) evoked by i.p. enalapril (10 mg/kg), bradyzide (2 mg/kg), or their combination in conscious telemetered female rats. Values are means $\pm$ S.E.M. * and $^+P<0.05$ versus corresponding vehicle (saline) and bradyzide+enalapril values, respectively.

Figure 3.

Effect of 8-week feeding of 5% w/v ethanol (bottom panel, n=7) or control liquid diet (top panel, n=6) on changes in heart rate (HR) evoked by i.p. enalapril (10 mg/kg), bradyzide (2 mg/kg), or their combination in conscious telemetered female rats. Values are means $\pm$ S.E.M. * and +P<0.05 versus corresponding vehicle (saline) and bradyzide+enalapril values, respectively.

Figure 4.

Bar graphs showing the area under the curve (AUC) of the effect of i.p. enalapril (10 mg/kg), bradyzide (2 mg/kg), or their combination on the spectral index of spontaneous baroreflex gain (index α) in ethanol-fed (5% w/v for 8 weeks, n=7) or liquid diet fed (n=6) telemetered female rats. Values are means $\pm$ S.E.M. * and +P<0.05 versus corresponding vehicle (saline) and bradyzide+enalapril values, respectively.

Table 1

Mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min), and index α (sec/mmHg) obtained 8 weeks after ethanol or isocaloric liquid diet (control) feeding and prior to acute pharmacological interventions.

Parameter	Pre-saline	Pre-enalapril	Pre-bradyzide	Pre-enalapril/bradyzide
MAP				
Liquid diet	104.5±3.6	104.8±1.8	99.8±2.0	102.0±2.1
Ethanol	96.7±2.5*	96.8±3.0*	91.7±1.8*	91.7±2.3*
HR				
Liquid diet	345.7±11.2	380.7±8.1	364.4±13.0	340.4±4.8
Ethanol	331.5±11.1	382.3±9.4	345.5±9.7	326.4±14.1
Index α				
Liquid diet	0.696±0.076	0.790±0.084	0.719±0.079	0.672±0.081
Ethanol	0.787±0.094	0.865±0.079	0.872±0.068	0.883±0.099

Values are means±SEM of 7 (ethanol) or 6 (control) observations.

* P<0.05 vs. corresponding values in control (liquid diet) rats.