Reduced Cardiac Contractile Force Due to Sympathovagal Dysfunction Mediates the Additive Hypotensive Effects of Limited-Access Regimens of Ethanol and Clonidine in Spontaneously Hypertensive Rats

Mahmoud M. El-Mas¹ and Abdel A. Abdel-Rahman

Department of Pharmacology, School of Medicine, East Carolina University, Greenville, North Carolina

Received August 3, 2010; accepted September 22, 2010

ABSTRACT

Our previous attempts to investigate the long-term hemodynamic interaction between ethanol and clonidine in telemetered spontaneously hypertensive rats (SHRs) were hampered by the lack of a sustained hypotensive response to continuous clonidine exposure. This limitation was circumvented when we adopted a limited-access clonidine (8:30 AM–4:30 PM) paradigm in a recent study. The latter paradigm was employed here to evaluate the ethanol-clonidine interaction and possible roles of myocardial function and autonomic control in this interaction. Changes in blood pressure (BP), heart rate, maximum rate of rise in BP wave ($+dP/dt_{max}$), and spectral cardiovascular autonomic profiles were measured by radiotelemetry in pair-fed SHRs receiving clonidine (150 μ g/kg/day), ethanol [2.5% (w/v)], or their combination during the day for 12 weeks. Ethanol or clonidine elicited long-term decreases in BP, and their combi-

nation caused additive hypotensive response. Significant reductions in $+dP/dt_{max}$ were observed upon concurrent treatment with ethanol and clonidine, in contrast to no effect for individual treatment. In addition, the combined treatment increased the high-frequency (HF) spectral band of interbeat interval (IBI-HF_{nu}, 0.75–3 Hz) and decreased low-frequency (IBI-LF_{nu}, 0.2–0.75 Hz) bands and IBI_{LF/HF} ratios. Clonidine-evoked reductions in plasma and urine norepinephrine and BP-LF spectral power (measure of vasomotor sympathetic tone) were not affected by ethanol. In conclusion, concurrent treatment with ethanol and clonidine shifts the sympathovagal balance toward parasympathetic dominance and elicits exaggerated hypotension as a result of a reduction in cardiac contractile force.

Introduction

Our previous studies have shown that ethanol counteracts the hypotensive action of centrally acting (e.g., clonidine), but not peripherally acting (e.g., hydralazine), antihypertensive agents (El-Mas and Abdel-Rahman, 1997, 1999b). The deleterious effect of ethanol on centrally mediated hypotension involves, at least in part, the central nervous system, because centrally mediated reductions in sympathetic activity and vascular resistance that mediate hypotension of central origin are also counteracted by ethanol (El-Mas and Abdel-Rahman, 1997, 1999b). Because clonidine exhibits almost similar affinities at α_{2A} adrenergic and I₁ imidazoline recep-

tors (Ernsberger et al., 1993), subsequent studies demonstrated ethanol ability to counteract the hypotensive and sympathoinhibitory effects of rilmenidine (selective I₁ agonist) but not α -methylnorepinephrine (selective α_2 agonist) (El-Mas and Abdel-Rahman, 1999a). These findings suggested a preferential interaction of ethanol with central pathways involved in I₁-receptor-mediated hypotension (El-Mas and Abdel-Rahman, 1999a) and may explain, at least in part, the uncontrolled hypertension in regular alcohol users (Puddey et al., 1987).

In a previous study, we employed radiotelemetry to determine whether the antagonistic hemodynamic interaction between ethanol and clonidine existed in spontaneously hypertensive rats (SHRs) that received a nutritionally balanced liquid diet (El-Mas and Abdel-Rahman, 2004). Clonidine was given via timed-release pellets, whereas ethanol was mixed in a liquid diet; rats were pair-fed to circumvent the effect of differences in fluid intake on hemodynamics (Bouby et al., 1990; El-Mas and Abdel-Rahman, 2004). Unexpectedly,

This work was supported by the National Institutes of Health National Institute on Alcohol Abuse and Alcoholism [Grant AA07839].

¹ Permanent affiliation: Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

doi:10.1124/jpet.110.173443.

ABBREVIATIONS: SHRs, spontaneously hypertensive rats; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; IBI, interbeat intervals; FFT, fast Fourier transformations; LF, low frequency; HF, high frequency.

clonidine lowered blood pressure (BP) only during the first 2 days of the 4-week observation period that followed pellet implantation (El-Mas and Abdel-Rahman, 2004). Short-lived hypotension was also seen in our previous studies when clonidine was served in a liquid diet (our unpublished data). The short-lived hypotensive action of clonidine in previous studies clearly hampered the investigation of long-term ethanol-clonidine interaction in telemetered rats.

Considering that tolerance usually develops upon continuous exposure to pharmacological interventions (Portenoy and Savage, 1997), we investigated whether tolerance to clonidine-evoked hypotension in SHR could be averted when an intermittent delivery strategy is implemented (El-Mas and Abdel-Rahman, 2007). It is noteworthy that uniform and long-term (12-week) decreases in BP were observed when clonidine administration was restricted to daytime periods (8:30 AM–4:30 PM) (El-Mas and Abdel-Rahman, 2007). The clinical significance of this observation is warranted because tolerance to the antihypertensive effect of imidazolines constitutes a considerable obstacle to their medical use (Parkin et al., 2003; Monassier et al., 2004). Accordingly, the limited-access model of long-term clonidine hypotension (El-Mas and Abdel-Rahman, 2007) was employed in the current study to evaluate the long-term hemodynamic consequences to concurrent exposure to ethanol and clonidine. Experiments were undertaken in radiotelemetered SHR that received a liquid diet containing ethanol, clonidine, or their combination during the daytime. Changes in BP, heart rate (HR), $+dP/dt_{\max}$, and spectral profiles of cardiovascular autonomic control were monitored for 12 weeks. Finally, we investigated the effect of individual and combined drug treatment on urine and plasma norepinephrine (measure of vasomotor sympathetic activity).

Materials and Methods

Animals. Male SHR (9–10 weeks, 225–275 g; Harlan, Indianapolis, IN) were used. Upon arrival, rats were housed individually in standard plastic cages and allowed free access to water and Purina chow. Rats were maintained on a 12-h light/dark cycle with lights off at 7:00 PM, and the room temperature was maintained at $22 \pm 1^\circ\text{C}$. 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* (Institute of Laboratory Animal Resources, 1996).

Telemetry Transmitter Implantation. The description of the telemetry system (Data Sciences International, St. Paul, MN) and the method used for the telemetry transmitter implantation are detailed in our previous studies (El-Mas and Abdel-Rahman, 2000a; Rekik et al., 2002). After surgery, rats were housed individually.

Experimental Groups. Four groups of telemetered SHR ($n = 6$ –7 each) were used to investigate the hemodynamic and autonomic effects of limited access regimens of ethanol, clonidine, or their combination. One week after transmitter implantation, rats were transferred and housed individually in metabolic cages (Nalgene; Nalge Nunc International, Rochester, NY) for urine collection and fed a Lieber-DeCarli liquid diet (Dyets Inc., Bethlehem, PA). Two weeks after transmitter implantation, rats were provided a diet containing 2.5% (w/v) ethanol (18% of total caloric intake), clonidine ($150 \mu\text{g/kg/day}$, $n = 7$), or their combination ($n = 7$). The fourth group of SHR (controls, $n = 6$) was pair-fed and received an isocaloric amount of dextrin/maltose (89.6 g/l). The ethanol or clonidine diet was provided for only 8 h (8:30 AM–4:30 PM), and this was followed by a control diet until the next morning. Rats in all groups were

pair-fed to ensure similar fluid and nutrient intakes in our previous studies (El-Mas and Abdel-Rahman, 2000a; Rekik et al., 2002). Each rat in a given group received 40 and 20 ml of liquid diet during morning and overnight periods, respectively.

Data Acquisition and Analysis. Individual radio receivers were placed on the tops of the metabolic cages, and data were collected using a computerized data acquisition system (Dataquest A.R.T. 2.3; Data Sciences International). Measurements of systolic (SBP) and diastolic (DBP) blood pressures and HR started immediately after transmitter implantation to ensure proper operation of the system. BP waveforms were sampled at a rate of 500 Hz for 10 s; every 10-min interbeat interval (IBI) was calculated from BP waveforms. The maximum rate of rise of BP waves ($+dP/dt_{\max}$) (van den Buuse, 2003) was computed with the use of Data Sciences software.

Spectral Analysis of Hemodynamic Variability. Spectral hemodynamic fluctuations, quantitative indices of cardiovascular autonomic control (Stein et al., 1994; El-Mas and Abdel-Rahman, 2007), were used to reflect changes in sympathetic and vagal outflows. Hemodynamic variability was analyzed in the frequency domain using fast Fourier transformation (FFT) algorithms of SBP, DBP, and IBI data series. Data Sciences software (Dataquest A.R.T. 2.3) uses the FFT algorithm 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). A second-order interpolation was employed to fit a smooth curve to existing data points and produce a smoother visual representation of data. The evenly spaced (equidistant) sampling allowed direct spectral analysis with the use of FFT algorithms. Spectra were integrated into two specific frequency bands, LF (0.20–0.75 Hz) and HF (0.75–3 Hz). Daily 8-h values of spectral parameters were obtained by averaging 48 10-s data points (six measurements per hour). Values of each 7 consecutive days were averaged to get weekly values.

Urine and Plasma Collection Schedules. Urine was collected, under light mineral oil (Young et al., 2002), starting 1 week before clonidine or ethanol feeding (baseline) and then every other week. For any particular week, urine was collected every other day during the 8-h daytime treatment period. Urine was kept frozen at -20°C until analyzed. Blood samples were taken from rats at the study conclusion (week 12) for determination of plasma ethanol and norepinephrine levels.

Measurement of Ethanol Concentration. The ethanol content of the collected urine (every other week) and plasma (week 12) samples was measured by the enzymatic method described by Bernt and Gutmann (1974) and used in our previous studies (El-Mas and Abdel-Rahman, 1999a,b).

Radioimmunoassay of Norepinephrine. Urinary and plasma norepinephrine (nanograms per milliliter) was measured by a commercially available ^{125}I -radioimmunoassay (ALPCO Diagnostics, Windham, NH) as in previous studies (El-Mas and Abdel-Rahman, 2007).

Drugs. Ethanol (Midwest Grain Products Co., Weston, MO) and clonidine hydrochloride (Sigma-Aldrich, St. Louis, MO) were purchased from commercial vendors.

Statistical Analysis. Data are expressed as means $\pm$ S.E.M. All parameters were averaged over a 7-day period for weekly values. The repeated measures analysis of variance followed by a Newman-Keuls post hoc test was used to test for statistical significance. Probability levels lower than 0.05 were considered significant.

Results

Baseline Hemodynamic Data and Blood and Urine Analyses. Baseline hemodynamic values (week 0) of all rat groups during the 8-h daytime period of the study before any drug treatment were similar (Table 1). The average weekly ethanol intake (Fig. 1A), biweekly urinary ethanol content

TABLE 1

Baseline (week 0) hemodynamic values in SHR assigned to receive ethanol, clonidine, or their combination

Values are means $\pm$ S.E.M.

Parameter	Control	Ethanol	Clonidine	Combination
SBP, mm Hg	174 $\pm$ 3	178 $\pm$ 2	171 $\pm$ 3	168 $\pm$ 4
DBP, mm Hg	126 $\pm$ 5	120 $\pm$ 5	125 $\pm$ 2	127 $\pm$ 4
HR, beats/min	301 $\pm$ 7	308 $\pm$ 2	310 $\pm$ 6	304 $\pm$ 6
dP/dt _{max} , mm Hg/s	1722 $\pm$ 62	1838 $\pm$ 71	1706 $\pm$ 57	1525 $\pm$ 154
IBI-LF _{nu} , s ² /Hz	0.52 $\pm$ 0.02	0.48 $\pm$ 0.01	0.49 $\pm$ 0.03	0.51 $\pm$ 0.03
IBI-HF _{nu} , s ² /Hz	0.48 $\pm$ 0.02	0.52 $\pm$ 0.01	0.51 $\pm$ 0.03	0.49 $\pm$ 0.03
IBI _{LF/HF}	1.09 $\pm$ 0.08	0.97 $\pm$ 0.08	0.98 $\pm$ 0.09	1.07 $\pm$ 0.13
SBP-LF, mm Hg ² /Hz	0.92 $\pm$ 0.01	0.88 $\pm$ 0.02	0.92 $\pm$ 0.01	0.92 $\pm$ 0.01

Fig. 1. Averaged daily ethanol intakes (grams per kilogram per day; A) and urine ethanol (milligram percent; B) in telemetered SHR rats receiving ethanol [2.5% (w/v)] over 8-h periods (8:30 AM–4:30 PM). Plasma ethanol measured at the conclusion of the study (week 12) is also shown in C. Values are means $\pm$ S.E.M. of six to seven rats.

(Fig. 1B) and week-12 blood ethanol concentration (week 12; Fig. 1C) measured in rats treated with ethanol or the ethanol plus clonidine combination were similar. Compared with control diet, clonidine or ethanol plus clonidine diets caused similar and significant decreases in urinary (weeks 2–12; Fig. 2A) and plasma (week 12; Fig. 2B) norepinephrine levels.

No changes in urinary or plasma norepinephrine were observed in SHR rats receiving the ethanol diet alone (Fig. 2).

Hemodynamic Interactions between Ethanol and Clonidine. Changes in the averaged weekly values of hemodynamic variables during the 8-h (8:30 AM–4:30 PM) exposure periods to ethanol, clonidine, or their combination are

Fig. 2. Urinary norepinephrine levels (A) in telemetered SHR receiving limited-access regimen (8:30 AM–4:30 PM, 3 months) of ethanol [2.5% (w/v)], clonidine (150 μ g/kg/day), or their combination. Plasma norepinephrine measured at the conclusion of the study (week 12) is also shown in B. Values are means $\pm$ S.E.M. of six to seven rats. *, $P < 0.05$ versus corresponding control values.

illustrated in Figs. 3, 4, and 5. Compared with respective control values, ethanol (2.5%) caused significant reductions in DBP (Fig. 3A) and SBP (Fig. 3B). The hypotensive effect of ethanol started to diminish after week 6 and returned to near-control values by the conclusion of the study (Fig. 3, A and B). On the other hand, decreases caused by clonidine (150 μ g/kg/8 h) in DBP (Fig. 3A) and SBP (Fig. 3B) were maintained through the duration of the study. In rats fed an ethanol/clonidine diet, dramatic decreases in BP were observed that were significantly greater than the hypotension caused by the individual treatments (Fig. 3, A and B). The hypotensive effect of the combined treatment remained steady until the end of the study (Fig. 3, A and B). In addition, Fig. 4 shows that unlike the effects of individual treatments, the reductions in SBP caused by the ethanol/clonidine regimen were of significantly greater magnitude than the associated reductions in DBP. Compared with control values, HR was reduced by clonidine alone but not by ethanol or the ethanol/clonidine combination (Fig. 3C). $+dP/dt_{\max}$ was not affected by ethanol or clonidine, except for significant $+dP/dt_{\max}$ reductions by clonidine at weeks 9, 10, and 12 (Fig. 5). In the ethanol/clonidine group, however, significant reductions in $+dP/dt_{\max}$ were observed during the first week of treatment and continued thereafter (Fig. 5).

Autonomic Interactions between Ethanol and Clonidine.

Figures 6 and 7 illustrate the effects of individual or combined treatment with ethanol and clonidine on spectral FFT

measures of cardiovascular autonomic control. Compared with control values, LF (0.20–0.75 Hz; Fig. 6A) and HF (0.75–3 Hz; Fig. 6B) oscillations of IBI were not affected by ethanol, whereas they showed significant decreases and increases, respectively, during the early (1–3) and middle (7–9) weeks of the study in clonidine-fed rats. These effects of clonidine on IBI_{LF} and IBI_{HF} oscillations were accentuated and lasted longer upon concurrent administration of ethanol (Fig. 6, A and B). The LF/HF_{IBI} ratio, a measure of cardiac sympathovagal balance, was reduced by clonidine and by ethanol plus clonidine; however, the reductions caused by the latter treatment were significantly greater (Fig. 6C). FFT analysis of SBP spectra showed that clonidine significantly reduced the LF power, in contrast to no effect for ethanol (Fig. 7). The effect of clonidine on SBP-LF remained unaltered in the presence of ethanol (Fig. 7).

Discussion

The strategy of intermittent drug delivery was employed in this study to assess the hemodynamic interaction between ethanol and clonidine. The simultaneous exposure to ethanol and clonidine produced additive decreases in BP. The underlying mechanism seems to be of cardiac rather than vascular origin because 1) the hypotensive effect of ethanol/clonidine was more pronounced during systole than diastole; 2) in contrast to no or little effect for ethanol or clonidine, the

Fig. 3. Effect of long-term limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 μ g/kg/day), or their combination on DBP (A) and SBP (B) arterial pressures and HR (C) in telemetered SHR. Values are weekly means $\pm$ S.E.M. of measurements taken from rats during the 8-h periods of drug exposures ($n = 6$ –7 each). *, $P < 0.05$ versus control values; +, $P < 0.05$ versus ethanol values; #, $P < 0.05$ versus clonidine values.

combined treatment elicited substantial reductions in Δ DP/dt; 3) clonidine enhancement of IBI_{HF} (index of vagal tone) and reduction of IBI_{LF} (measure of cardiac sympathetic tone) were accentuated by ethanol; and 4) clonidine-evoked reductions in LF oscillations of SBP (index of vasomotor sympathetic tone) and plasma and urine norepinephrine levels were not affected by ethanol. Together, these findings suggest a pivotal role for cardiac vagal overactivity and subsequent myocardial depression in the enhanced hypotensive response elicited by concurrent exposure to ethanol and clonidine in telemetered SHR.

The cardiovascular effects of ethanol are complex and depend on the dose and duration, as well as pattern of alcohol consumption. The current finding that ethanol feeding to

SHRs produced significant decreases in BP is consistent with our earlier reports and others (Howe et al., 1989; El-Mas and Abdel-Rahman, 2000b; Rekik et al., 2002), which established a dose-hypotensive effect relationship for ethanol in this rat model. The 2.5% ethanol strength was employed here because it was found in this and previous studies (Rekik et al., 2002) to produce mild decreases in BP and to achieve blood ethanol concentrations comparable with those attained in humans after consumption of mild to moderate amounts of ethanol (Ireland et al., 1984). It is noteworthy that mild and severe ethanol consumption produce opposite effects, decreases and increases, respectively, on BP (He and Bazzano, 2000; Fuchs et al., 2001; Stamler et al., 2002; Huntgeburth et al., 2005). Although the exact mechanism for this biphasic

Fig. 4. Comparisons of the effects of long-term limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 μ g/kg/day), or their combination on DBP (A) and SBP (B) arterial pressures in telemetered SHR. Values are weekly means $\pm$ S.E.M. of measurements taken from rats during the 8-h periods of drug exposures ($n = 6-7$ each). *, $P < 0.05$ versus DBP values.

Fig. 5. Effect of long-term limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 μ g/kg/day), or their combination on the maximum rate of rise of the blood pressure wave (dP/dt_{max}) in telemetered SHR. Values are weekly means $\pm$ S.E.M. of measurements taken from rats during the 8-h periods of drug exposures ($n = 6-7$ each). *, $P < 0.05$ versus control values; +, $P < 0.05$ versus ethanol values; #, $P < 0.05$ versus clonidine values.

dose-dependent BP effect of ethanol is not clear, it has been proposed that when consumed in large quantities, the initial vasodilator effect of ethanol might be overshadowed by the sympathetic and vasopressor reactions to excess ethanol intake (Huntgeburth et al., 2005). Experimental studies also showed that ethanol feeding characteristics affect the net BP response to long-term ethanol consumption. Hypotension developed when ethanol was administered in liquid diet (Hutton et al., 1992; El-Mas and Abdel-Rahman, 2000b, 2005) compared with hypertension when drinking water was used as a vehicle (Chan et al., 1985).

Because BP was reduced in SHR treated with ethanol or clonidine, the observation that exaggerated hypotension appeared when the two drugs were combined was not entirely unpredicted. Therefore, we focused in this study on determining whether alterations in the cardiac contractile force and/or autonomic activity accounts for the ethanol-clonidine interaction. Among other parameters, cardiac function is typically reflected by electrocardiographic or echocardiographic measurements of left ventricular developed pressure (dP/dt) and left ventricular end-diastolic pressure (Carroll et al., 2006; Adeyemi et al., 2009). We employed an important feature of the radiotelemetry technique that permitted computation of the maximum rate of pressure rise across the BP wave form ($+dP/dt_{max}$), which serves as an indirect measure of the contractile force of the heart. The finding that $+dP/dt_{max}$ was reduced in SHR receiving the combined but not the individual treatment argues for a key role for diminished cardiac contractile force in the additive hypotensive response caused by the combined treatment. Indeed, the examination of Figs. 3 and 5 revealed a temporal relationship between reductions in blood pressure and $+dP/dt_{max}$.

Further evidence for the importance of cardiac contractile force in the ethanol-clonidine interaction emerged from the observation that remarkably greater reductions in systolic than diastolic pressures were seen in ethanol/clonidine-treated SHR. According to the Frank-Starling phenomenon, systolic blood pressure proportionally relates to left ventricular pressure and contractile force (Duncker and Bache, 2008; Truijen et al., 2010). Therefore, one plausible explanation for the greater reduction in systolic pressure is the reduction in venous return and subsequently lesser stretch of the heart during diastole (Truijen et al., 2010) and dimin-

Fig. 6. Effect of long-term limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 $\mu\text{g/kg/day}$), or their combination on the FFT spectral density of IBI in the low-frequency (IBI-LF, 0.20–0.75 Hz; A) and high-frequency (IBI-HF, 0.75–3 Hz; B) ranges as well as on the LF/HF ratio of IBI (C) in telemetered SHR rats. Values are means $\pm$ S.E.M. of measurements taken from rats during each successive 3-week period ($n = 6$ –7 each). *, $P < 0.05$ versus control values; +, $P < 0.05$ versus ethanol values; #, $P < 0.05$ versus clonidine values.

ished stroke volume. Although neither cardiac output nor stroke volume was measured in the present study, a causal relationship has been established in other rat models between reductions in stroke volume and the hypotension caused by ethanol on the one hand (El-Mas and Abdel-Rahman, 1999c; Ren and Wold, 2008), and clonidine on the other (El-Mas et al., 1994). The clonidine-evoked diminution of CO may be attributed to the sympathoinhibition-induced relaxation of capacitance vessels (venodilatation), which is followed by reductions in venous return (Berne and Levy, 1988). It is noteworthy that HR is unlikely to contribute to the cardiac interaction between ethanol and clonidine, because HRs of ethanol/clonidine-treated rats were not different from those of control rats. Moreover, the reductions in HR caused by clonidine disappeared rather than intensified after concurrent treatment with ethanol. Nonetheless, future echocardiographic and electrocardiographic studies are needed to

gain insight into the roles of cardiac dynamics such as end-diastolic and end-systolic volumes, stroke volume, and ejection fraction in the ethanol-clonidine interaction.

Spectral analysis of cardiovascular data produced important information that suggests a key role for cardiovascular autonomic control in the hemodynamic profile caused by the ethanol/clonidine combination. The reciprocal changes caused by the ethanol/clonidine in the LF (decreases) and HF (increases) powers of IBI, which reflect cardiac vagal and sympathetic activities, respectively (Stein et al., 1994; El-Mas and Abdel-Rahman, 2007), represented an autonomic phenotype that is consistent with increased parasympathetic dominance of the heart (Danson et al., 2009). The latter may conceivably account for the reduced cardiac contractility and possibly hypotension seen in ethanol/clonidine-treated rats. Furthermore, biochemical and spectral data of our study do not support a role for vascular mechanisms in the ethanol-

Fig. 7. Effect of chronic limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 µg/kg/day), or their combination on the FFT spectral density (0.20–0.75 Hz) of SBP in telemetered SHR. Values are means ± S.E.M. of measurements taken from rats during each successive 3-week period ($n = 6-7$ each). *, $P < 0.05$ versus control values; +, $P < 0.05$ versus ethanol values.

clonidine hemodynamic interaction. Indeed, concurrent ethanol administration had no effect on clonidine-evoked reductions in plasma or urine norepinephrine and LF oscillations of SBP (index of vasomotor sympathetic activity). It is noteworthy that the ability of ethanol to potentiate the cardiac, and not vascular, sympathoinhibitory action of clonidine infers selectivity of the ethanol-clonidine interaction on regional sympathetic activity.

Finally, three important comments should be made regarding the clinical relevance of the current data. First, results of present and previous studies illustrate quite clearly that short- and long-term ethanol regimens exert directionally opposite effects on the hypotensive action of centrally drugs of the clonidine type. Whereas short-term ethanol administration reversed the hypotensive and sympathoinhibitory of clonidine and related imidazolines (El-Mas and Abdel-Rahman, 1997, 1999a,b), the same effects of clonidine were facilitated by concurrent long-term ethanol administration (this study). Although either type of interaction is clinically important, irregularities in the dose and duration of ethanol consumption in clinical settings may make it difficult to predict the magnitude or direction of the interaction of ethanol with antihypertensive medications. The second comment pertains to the notion that the establishment of an autonomic profile characterized by decreased sympathetic and increased parasympathetic activity provides a good prognosis for cardiovascular disease (Stein et al., 1994; Danson et al., 2009). Although this same profile of parasympathetic dominance was achieved in rats receiving the long-term ethanol/clonidine regimen, whether this constitutes a favorable cardiovascular outcome cannot be ascertained from the current study and needs further investigation. Clinical studies suggest that light-to-moderate alcohol consumption is associated with reduced risk of coronary heart disease and myocardial infarction. This contrasts with the long-term effects of large amounts of alcohol, which cause cardiomyopathy and increased incidence of arrhythmias and sudden death (Piano, 2002; Cheng et al., 2006). Third, the type of diet (nutritional balance) also influences the outcome because, contrary to the present findings with a liquid diet, the addition of ethanol and clonidine to drinking water resulted in abrogation of the hypotensive effect of clonidine in SHR in a previous study from our lab (Abdel-Rahman, 1994).

In conclusion, our study provides insights into the hemodynamic and autonomic interactions between limited access

regimens of ethanol and clonidine in conscious telemetered SHR. Concurrent ethanol administration exaggerated the hypotensive response elicited by daytime administration of clonidine in SHR. The underlying mechanism involves a reduction in myocardial contractile force, possibly because of a shift in cardiac autonomic control toward parasympathetic dominance. Obviously, the limited-access regimen for ethanol offers a more reasonable approach to simulate ethanol consumption in humans. Furthermore, the intermittent maneuver might be a viable therapeutic opportunity to establish a maintained antihypertensive effect for clonidine and related imidazolines without any signs of tolerance, rebound hypertension, or irregularities in body fluids or electrolytes (El-Mas and Abdel-Rahman, 2007).

Acknowledgments

We thank Kui Sun for technical assistance.

References

- Abdel-Rahman AA (1994) Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. *Hypertension* **24**:802–807.
- Adeyemi O, Roberts S, Harris J, West H, Shome S, and Dewhurst M (2009) QA interval as an indirect measure of cardiac contractility in the conscious telemetered rat: model optimisation and evaluation. *J Pharmacol Toxicol Methods* **60**:159–166.
- Berne RM and Levy MN (1988) Control of cardiac output coupling of heart and blood vessels, in *Physiology*, 2nd ed, pp 525–539, C.V. Mosby Co., St. Louis.
- Bernt E and Gutmann I (1974) Ethanol: determination with alcohol dehydrogenase and NAD, in *Methods of Enzymatic Analysis* (Bergmeyer HU ed) vol 3, 2nd ed, pp 1499–1502, Academic Press, New York.
- Bouby N, Bachmann S, Bichet D, and Bankir L (1990) Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. *Am J Physiol* **258**:F973–F979.
- Carroll JF, Zenebe WJ, and Strange TB (2006) Cardiovascular function in a rat model of diet-induced obesity. *Hypertension* **48**:65–72.
- Chan TC, Wall RA, and Sutter MC (1985) Chronic ethanol consumption, stress, and hypertension. *Hypertension* **7**:519–524.
- Cheng CP, Cheng HJ, Cunningham C, Shihabi ZK, Sane DC, Wannenburg T, and Little WC (2006) Angiotensin II type 1 receptor blockade prevents alcoholic cardiomyopathy. *Circulation* **114**:226–236.
- Danson EJ, Li D, Wang L, Dawson TA, and Paterson DJ (2009) Targeting cardiac sympatho-vagal imbalance using gene transfer of nitric oxide synthase. *J Mol Cell Cardiol* **46**:482–489.
- Dunker DJ and Bache RJ (2008) Regulation of coronary blood flow during exercise. *Physiol Rev* **88**:1009–1086.
- El-Mas MM and Abdel-Rahman AA (1997) Role of cardiac output in ethanol-evoked attenuation of centrally mediated hypotension in conscious rats. *Hypertension* **30**:288–294.
- El-Mas MM and Abdel-Rahman AA (1999a) Ethanol counteraction of I1-imidazoline but not alpha-2 adrenergic receptor-mediated reduction in vascular resistance in conscious spontaneously hypertensive rats. *J Pharmacol Exp Ther* **288**:455–462.
- El-Mas MM and Abdel-Rahman AA (1999b) Role of the sympathetic control of vascular resistance in ethanol-clonidine hemodynamic interaction in SHR. *J Cardiovasc Pharmacol* **34**:589–596.
- El-Mas MM and Abdel-Rahman AA (1999c) Sexually dimorphic hemodynamic effects of intragastric ethanol in conscious rats. *Clin Exp Hypertens* **21**:1429–1445.

- El-Mas MM and Abdel-Rahman AA (2000a) Ovariectomy alters the chronic hemodynamic and sympathetic effects of ethanol in radiotelemetered female rats. *Clin Exp Hypertens* **22**:109–126.
- El-Mas MM and Abdel-Rahman AA (2000b) Radiotelemetric evaluation of hemodynamic effects of long-term ethanol in spontaneously hypertensive and Wistar-Kyoto rats. *J Pharmacol Exp Ther* **292**:944–951.
- El-Mas MM and Abdel-Rahman AA (2004) Chronic ethanol-clonidine hemodynamic interaction in telemetered spontaneously hypertensive rats. *Vascul Pharmacol* **41**:107–113.
- El-Mas MM and Abdel-Rahman AA (2005) Autonomic modulation of altered diurnal hemodynamic profiles in ethanol-fed hypertensive rats. *Alcohol Clin Exp Res* **29**:499–508.
- El-Mas MM and Abdel-Rahman AA (2007) Intermittent clonidine regimen abolishes tolerance to its antihypertensive effect: a spectral study. *J Cardiovasc Pharmacol* **49**:174–181.
- El-Mas MM, Carroll RG, and Abdel-Rahman AA (1994) Centrally mediated reduction in cardiac output elicits the enhanced hypotensive effect of clonidine in conscious aortic barodenervated rats. *J Cardiovasc Pharmacol* **24**:184–193.
- Ernsberger P, Damon TH, Graff LM, Schäfer SG, and Christen MO (1993) Moxonidine, a centrally acting antihypertensive agent, is a selective ligand for I₁-imidazoline sites. *J Pharmacol Exp Ther* **264**:172–182.
- Fuchs FD, Chambless LE, Whelton PK, Nieto FJ, and Heiss G (2001) Alcohol consumption and the incidence of hypertension: The Atherosclerosis Risk in Communities Study. *Hypertension* **37**:1242–1250.
- Hatton DC, Bukoski RD, Edgar S, and McCarron DA (1992) Chronic alcohol consumption lowers blood pressure but enhances vascular contractility in Wistar rats. *J Hypertens* **10**:529–537.
- He J and Bazzano LA (2000) Effects of lifestyle modification on treatment and prevention of hypertension. *Curr Opin Nephrol Hypertens* **9**:267–271.
- Howe PR, Rogers PF, and Smith RM (1989) Effects of chronic alcohol consumption and alcohol withdrawal on blood pressure in stroke-prone spontaneously hypertensive rats. *J Hypertens* **7**:387–393.
- Huntgeburth M, Ten Freyhaus H, and Rosenkranz S (2005) Alcohol consumption and hypertension. *Curr Hypertens Rep* **7**:180–185.
- Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals*, 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.
- Ireland MA, Vandongen R, Davidson L, Beilin LJ, and Rouse IL (1984) Acute effects of moderate alcohol consumption on blood pressure and plasma catecholamines. *Clin Sci* **66**:643–648.
- Monassier L, Grenay H, Thomas L, and Bousquet P (2004) Chronic treatment with rilmenidine in spontaneously hypertensive rats: differences between two schedules of administration. *J Cardiovasc Pharmacol* **43**:394–401.
- Parkin ML, Godwin SJ, and Head GA (2003) Importance of imidazoline-preferring receptors in the cardiovascular actions of chronically administered moxonidine, rilmenidine and clonidine in conscious rabbits. *J Hypertens* **21**:167–178.
- Piano MR (2002) Alcoholic cardiomyopathy: incidence, clinical characteristics, and pathophysiology. *Chest* **121**:1638–1650.
- Portenoy RK and Savage SR (1997) Clinical realities and economic considerations: special therapeutic issues in intrathecal therapy—tolerance and addiction. *J Pain Symptom Manage* **14** (3 Suppl):S27–S35.
- Puddey IB, Beilin LJ, and Vandongen R (1987) Regular alcohol use raises blood pressure in treated hypertensive subjects. A randomised controlled trial. *Lancet* **1**:647–651.
- Rekik M, El-Mas MM, Mustafa JS, and Abdel-Rahman AA (2002) Role of endothelial adenosine receptor-mediated vasorelaxation in ethanol-induced hypotension in hypertensive rats. *Eur J Pharmacol* **452**:205–214.
- Ren J and Wold LE (2008) Mechanisms of alcoholic heart disease. *Ther Adv Cardiovasc Dis* **2**:497–506.
- Stamler J, Liu K, Ruth KJ, Pryer J, and Greenland P (2002) Eight-year blood pressure change in middle-aged men: relationship to multiple nutrients. *Hypertension* **39**:1000–1006.
- Stein PK, Bosner MS, Kleiger RE, and Conger BM (1994) Heart rate variability: a measure of cardiac autonomic tone. *Am Heart J* **127**:1376–1381.
- Truijien J, Bundgaard-Nielsen M, and van Lieshout JJ (2010) A definition of normovolaemia and consequences for cardiovascular control during orthostatic and environmental stress. *Eur J Appl Physiol* **109**:141–157.
- van den Buuse M (2003) Acute effects of antipsychotic drugs on cardiovascular responses to stress. *Eur J Pharmacol* **464**:55–62.
- Young JB, Weiss J, and Boufath N (2002) Effects of rearing temperature on sympathoadrenal activity in young adult rats. *Am J Physiol Regul Integr Comp Physiol* **283**:R1198–R1209.

Address correspondence to: Dr. Abdel A. Abdel-Rahman, Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27834. E-mail: abdelrahmana@ecu.edu