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

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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 μg/kg/dy), 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 combination 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-HFnu, 0.75–3 Hz) and decreased low-frequency (IBI-LFnu, 0.2–0.75 Hz) bands and IBILF/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 α2A adrenergic and I1 imidazoline receptors (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 α2 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,
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 SHRs 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 SHRs 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 SHRs (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 ± 1°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 SHRs (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; Windham, NH) as in previous studies (El-Mas and Abdel-Rahman, 1999a,b). 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.

**Statistical Analysis.** Data are expressed as means ± 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

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**Ethanol-Clonidine Hemodynamic Interaction in SHRs 853**

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(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 SHRs 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Ethanol</th>
<th>Clonidine</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>174 ± 3</td>
<td>178 ± 2</td>
<td>171 ± 3</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>126 ± 5</td>
<td>120 ± 5</td>
<td>125 ± 2</td>
<td>127 ± 4</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>301 ± 7</td>
<td>308 ± 2</td>
<td>310 ± 6</td>
<td>304 ± 6</td>
</tr>
<tr>
<td>dP/dt_max, mm Hg/s</td>
<td>1722 ± 62</td>
<td>1838 ± 71</td>
<td>1706 ± 57</td>
<td>1525 ± 154</td>
</tr>
<tr>
<td>IBI-LF, s²/Hz</td>
<td>0.52 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>0.49 ± 0.03</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>IBI-HF, s²/Hz</td>
<td>0.48 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>0.51 ± 0.03</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>IBI_LF/HF</td>
<td>1.09 ± 0.08</td>
<td>0.97 ± 0.08</td>
<td>0.98 ± 0.09</td>
<td>1.07 ± 0.13</td>
</tr>
<tr>
<td>SBP-LF, mm Hg²/Hz</td>
<td>0.92 ± 0.01</td>
<td>0.88 ± 0.02</td>
<td>0.92 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
</tbody>
</table>

**Fig. 1.** Averaged daily ethanol intakes (grams per kilogram per day; A) and urine ethanol (milligram percent; B) in telemetered SHRs 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 ± S.E.M. of six to seven rats.
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 μg/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). \( +\frac{dP}{dt_{\text{max}}} \) was not affected by ethanol or clonidine, except for significant \( +\frac{dP}{dt_{\text{max}}} \) reductions by clonidine at weeks 9, 10, and 12 (Fig. 5). In the ethanol/clonidine group, however, significant reductions in \( +\frac{dP}{dt_{\text{max}}} \) 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
combined treatment elicited substantial reductions in \( +dP/dt \); 3) clonidine enhancement of IBIH (index of vagal tone) and reduction of IBILF (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 SHRs.

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; Fucha et al., 2001; Stamler et al., 2002; Huntgeburth et al., 2005). Although the exact mechanism for this biphasic

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 μg/kg/day), or their combination on DBP (A) and SBP (B) arterial pressures and HR (C) in telemetered SHRs. Values are weekly means ± 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 (Hatton 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 SHRs 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 ($dP/dt_{max}$) 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 SHRs 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 SHRs. 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-
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/cloneidine-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-

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**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 μg/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 SHRs. 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; #, P < 0.05 versus clonidine 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 action 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) 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 SHRs in a previous study from our lab (Abdel-Rahman, 1994).

In conclusion, our study provides insights into the hemo-
dynamic and autonomic interactions between limited access

![Graph](image_url)

**Fig. 7.** Effect of chronic limited access (8:30 AM–4:30 PM, 3 months) paradigms of ethanol [2.5% (w/v)], clonidine (150 
\[\mu g/kg/day\]), or their combination on the FFT spectral density (0.20–0.75 Hz) of SBP in telemetered SHRs. 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.

regimens of ethanol and clonidine in conscious telemetered 

SHRs. Concurrent ethanol administration exaggerated the 

hypotensive response elicited by daytime administration of 

clonidine in SHRs. 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 etha-

nol offers a more reasonable approach to simulate ethanol 

consumption in humans. Furthermore, the intermittent manue-

ver might be a viable therapeutic opportunity to establish 

a maintained antihypertensive effect for clonidine and re-

lated imidazolines without any signs of tolerance, rebound 

hypertension, or irregularities in body fluids or electrolytes 

(El-Mas and Abdel-Rahman, 2007).

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**References**


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