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Chronic ethanol administration attenuates imidazoline I_1 receptor- or α_2-adrenoceptor-mediated reductions in blood pressure and hemodynamic variability in hypertensive rats

Mahmoud M. El-Mas, Abdel A. Abdel-Rahman*

Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858-4353, USA

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Abstract

Our previous studies have demonstrated that acute ethanol administration counteracts imidazoline I_1 receptor but not α_2-adrenoceptor-mediated hypotension in spontaneously hypertensive rats (SHR). In the present study, we investigated the effect of chronic ethanol administration on hypotensive responses elicited by acute administration of selective imidazoline I_1 receptor (rilmenidine) or α_2-adrenoceptor (α-methyldopa) agonist along with ethanol effects on: (i) locomotor activity and (ii) time-domain indices of variability in blood pressure (standard deviation of mean arterial pressure) and heart rate (standard deviation of beat-to-beat intervals and root mean square of successive differences in R–R intervals). Hemodynamic and locomotor responses elicited by rilmenidine or α-methyldopa were assessed in radiotelemetered ethanol-fed (2.5% or 5% w/v, 12 week) and control SHR. In control SHR, i.p. rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) significantly reduced blood pressure. Rilmenidine had no effect on heart rate whereas α-methyldopa elicited a biphasic response (tachycardia followed by bradycardia). Blood pressure and heart rate oscillations were also reduced by both drugs, which may conform to sympathoinhibition. The hypotensive effect of rilmenidine or α-methyldopa was significantly attenuated by ethanol feeding (2.5% or 5%) in a concentration-dependent manner. In addition, ethanol attenuated α-methyldopa-evoked reduction in heart rate, but not blood pressure, variability in marked contrast to attenuating rilmenidine-evoked reductions in blood pressure, but not heart rate, variability. These findings demonstrate that, unlike its acute effects, chronic ethanol attenuates both imidazoline I_1 receptor and α_2-adrenoceptor-mediated hypotension whereas its effect on hemodynamic variability depended on the nature of the hypotensive stimulus.

Keywords: Ethanol; Rilmenidine; α-Methyldopa; Hypotension; Hemodynamic variability; Time-domain analysis

1. Introduction

Experimental findings from our laboratory have shown that ethanol compromises the hypotensive effect of centrally acting antihypertensive agents such as clonidine and guanabenz (Abdel-Rahman, 1989; El-Mas et al., 1994b). These findings are clinically relevant as ethanol intake has been associated with inadequate blood pressure control in treated hypertensive patients (Volicer et al., 1978; Puddey et al., 1987). The adverse effect of ethanol on centrally mediated hypotensive responses is demonstrated in conscious aortic barodenervated (El-Mas et al., 1994b) and spontaneously hypertensive rats (SHR; Abdel-Rahman, 1989; Abdel-Rahman et al., 1992). In contrast, peripherally mediated hypotensive responses (e.g. hydralazine, nitroprusside, or hexamethonium) are not affected by ethanol (Abdel-Rahman, 1989; Abdel-Rahman et al., 1992; El-Mas and Abdel-Rahman, 1997b). These findings suggest that the adverse effect of ethanol on centrally mediated hypotensive responses involves, at least in part, the central nervous system. This view is further supported by ethanol counteraction of the centrally mediated sympathoinhibition elicited by clonidine. Ethanol counteracted clonidine-evoked reductions in plasma norepinephrine levels (Abdel-Rahman et al., 1992; El-Mas et al., 1994b) and in norepinephrine electrochemical signal in the rostral ventrolateral medulla (Mao and Abdel-Rahman, 1998). Nonetheless, the possible involvement of the peripheral hemodynamic effects of ethanol, e.g. vasodilatation of cutaneous blood vessels (Turlapaty et al.,

* Corresponding author. Tel.: +1-252-744-3470; fax: +1-252-744-3203.
E-mail address: abdelrahmana@mail.ecu.edu (A.A. Abdel-Rahman).

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1979), in its interaction with antihypertensive drugs cannot be overlooked.

Clonidine lowers blood pressure via the activation of central α_2-adrenoceptor and imidazoline I_1 receptors (Timmermans and Van Zwieten, 1982; Bousquet et al., 1992; El-Mas and Abdel-Rahman, 2001b). The role of both receptor sites (imidazoline I_1 receptor or α_2-adrenoceptor) in the deleterious effect of ethanol on centrally mediated hypotension was investigated in more recent studies from our laboratory (El-Mas and Abdel-Rahman, 1998, 1999). These studies showed that ethanol counteracts decreases in blood pressure, sympathetic activity, and peripheral vascular resistance elicited by central administration of rilmenidine (selective imidazoline I_1 receptor agonist) whereas it has little or no effect on α-methylnorepinephrine (selective α_2-adrenoceptor agonist)-mediated responses (El-Mas and Abdel-Rahman, 1998, 1999). These findings suggested a selective interaction of ethanol with central pathways involved in imidazoline I_1-receptor-mediated hypotension and sympathoinhibition (El-Mas and Abdel-Rahman, 1998, 1999). This notion gains further support from the observation that the counteraction of clonidine hypotension by ethanol is abolished in rats pretreated with efaroxan but not 2-methoxyidazoxan, selective imidazoline I_1 receptor and α_2-adrenoceptor antagonist, respectively (El-Mas and Abdel-Rahman, 2001b).

It is noteworthy that ethanol was administered acutely in our previous studies (El-Mas and Abdel-Rahman, 1998, 1999, 2001b). Whether a similar differential effect on imidazoline I_1 receptor and α_2-adrenoceptor-mediated hypotension occurs after chronic exposure to ethanol has not been explored. Therefore, we investigated in the present study the effect of chronic ethanol feeding on hypotensive responses elicited by selective activation of the imidazoline I_1 receptor or the α_2-adrenoceptor by rilmenidine and α-methyldopa, respectively, in SHR. We also investigated the chronic effects of ethanol on changes evoked by the hypotensive drugs in locomotor activity and in the variability of blood pressure and heart rate. Radiotelemetry was employed for hemodynamic measurements, which is a direct and minimally stressful procedure for continuous and simultaneous measurements of blood pressure and heart rate (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002). Further, the blood pressure or heart rate variability was measured as described elsewhere (Stein et al., 1994; Sgoifo et al., 1997; Visser et al., 2002) by computing the following time-domain parameters: (i) the standard deviation of the mean arterial pressure, (ii) the standard deviation of beat-to-beat intervals, and (iii) the root mean square of successive beat-to-beat differences in R–R interval durations. Ethanol was given as 2.5% and 5% (w/v) in Lieber–DeCarli liquid diet for 12 weeks and control rats were pair-fed isocaloric diet, which allowed similar nutrient intake and fluid consumption to that of ethanol-fed rats (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002). The amounts of ethanol used in the present study produced blood ethanol concentrations (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002) that are comparable to levels attained in humans following consumption of moderate to intoxicating amounts of ethanol (Potter and Beevers, 1984; Abdel-Rahman et al., 1987).

2. Materials and methods

Male spontaneously hypertensive rats (10–11 weeks, 250–300 g, Taconic, Germantown, NY) 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 7:00 PM. The room temperature was maintained at 22±1 °C. After 1-week acclimatization, rats were fed a regular Lieber–DeCarli liquid diet (Dyets, Bethlehem, PA) for another week before ethanol feeding. Rats received the diet daily at 8:30 AM. Experiments were performed in accordance with the European Community guidelines for the use of exper-

Table 1
Baseline values of mean arterial pressure (MAP, mm Hg), heart rate (HR, beats/min), mean arterial pressure variability (SDMAP), the standard deviation of R–R intervals (SDRR, ms), the root mean square of successive beat-to-beat differences (rMSSD, ms), and locomotor activity (counts)

Parameter	Control	Ethanol 2.5%	Ethanol 5%
MAP			
Pre-saline	156.7±4.6	142.8±3.7[a]	124.8±1.2[a]
Pre-rilmenidine	157.8±3.3	145.0±4.3[a]	128.8±2.3[a]
Pre-methyldopa	153.6±3.2	140.5±3.3[a]	126.7±1.7[a]
HR			
Pre-saline	313.6±3.1	305.5±2.9	306.4±3.1
Pre-rilmenidine	311.0±3.4	312.3±2.4	316.5±6.3
Pre-methyldopa	312.2±4.4	311.5±6.4	316.4±5.4
SDMAP			
Pre-saline	13.5±1.1	11.9±0.7	7.4±1.5[a]
Pre-rilmenidine	13.6±1.1	9.9±1.2	7.8±1.0[a]
Pre-methyldopa	13.7±1.7	11.8±0.5	9.5±1.1[a]
SDRR			
Pre-saline	19.8±2.6	17.4±2.2	15.2±2.2
Pre-rilmenidine	20.9±2.1	17.7±0.9	16.1±1.6
Pre-methyldopa	17.5±3.1	17.0±1.0	16.1±2.9
rMSSD			
Pre-saline	9.3±0.96	9.2±0.87	7.1±0.70
Pre-rilmenidine	9.3±0.59	9.2±0.42	7.7±0.52
Pre-methyldopa	8.0±0.88	8.1±0.81	6.9±1.07
Activity			
Pre-saline	2.6±0.26	1.6±0.23	2.0±0.57
Pre-rilmenidine	2.0±0.27	1.9±0.13	2.8±0.66
Pre-methyldopa	2.1±0.51	2.0±0.38	2.2±0.36

Values are means±S.E.M.
[a] $P<0.05$ vs. corresponding control values.

imental animals and were approved by the institutional ethics committee.

2.1. Ethanol feeding

Three groups of SHR matched for body weight were used in the present study. Two groups were provided a regular Lieber–DeCarli liquid diet (Lieber and DeCarli, 1982) containing 2.5% or 5% w/v ethanol (18% and 36% of total caloric intake, respectively, n=5 each) as described in our previous studies (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002). The third group of rats (controls, n=4) was 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 a refrigerator until dispensed. Rats were maintained on ethanol or control diet for 12 weeks.

2.2. Telemetry system

The telemetry system (Data Sciences Int., St. Paul, MN) used in this study has been described in our studies (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002) for the measurement of blood pressure, heart rate, and locomotor activity. The system consists of five major components: (i) implantable transmitter unit for measurement of blood pressure, (ii) radio receiver to receive telemetered signals, (iii) ambient pressure monitor to measure absolute atmospheric pressure, (iv) a consolidation matrix to multiplex multiple cage signals to the computer, and (v) a PC-based data acquisition system to process signals. The implanted sensor consisted of a fluid-filled catheter (0.7 mm diameter, 15 cm long, Model TA11PA-C40) connected to a highly stable low-conductance strain-gauge pressure transducer, which measured the absolute arterial pressure relative to a vacuum, and a radio-frequency transmitter. The tip of the

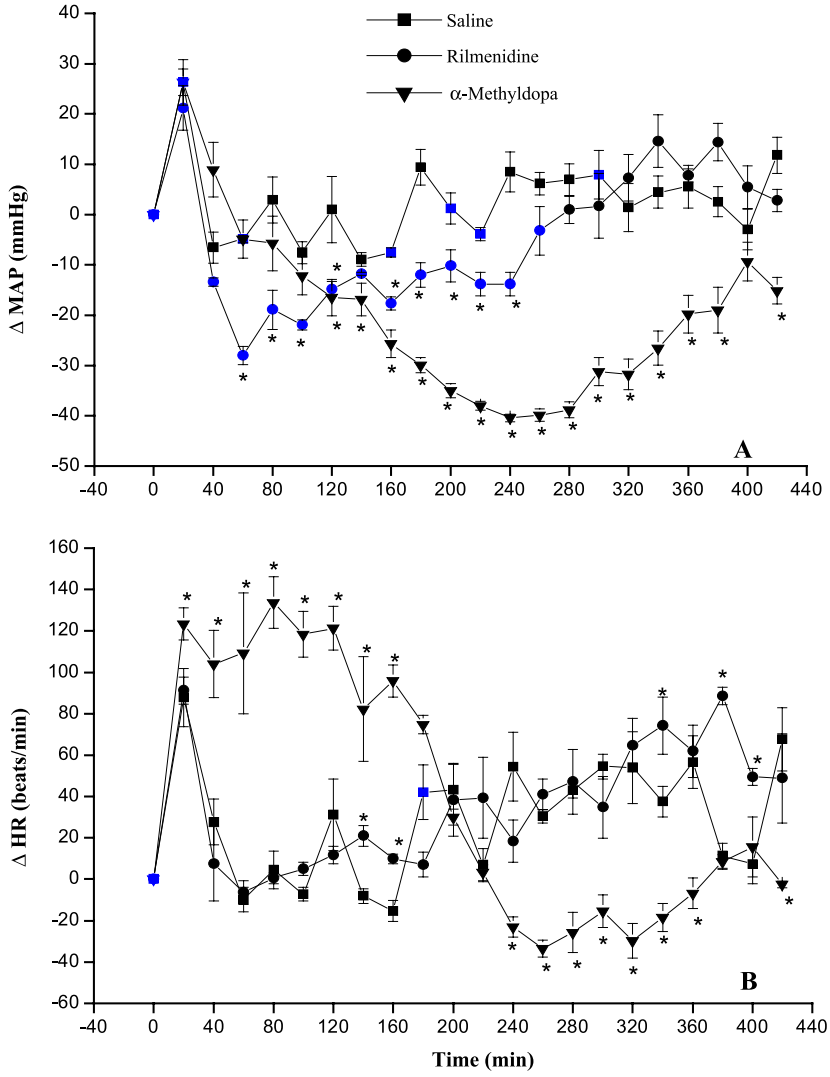

Fig. 1. Changes in mean arterial pressure (MAP) and heart rate (HR) evoked by i.p injection of rilmenidine (600 μg/kg), α-methyldopa (100 mg/kg) or equal volume of saline at 3-day intervals in conscious telemetered SHR receiving control liquid diet. Values are means±S.E.M. of four observations. *P<0.05 versus corresponding saline values.

catheter was filled with a viscous gel that prevented blood reflux and was coated with an anti-thrombogenic film to inhibit thrombus formation and maintain patency. The distal 1 cm of the catheter consisted of a thin-walled thermoplastic membrane while the remainder of the catheter was composed of a thick-walled low-compliance urethane. The implants (2.5 cm length and 1.2 cm diameter) weighed 9 g and had a typical battery life of 6 months. Implants were gas sterilized and provided precalibrated (relative to vacuum) by the manufacturer and calibrations were verified to be accurate within 3 mm Hg (Brockway et al., 1991). A radio receiver platform (RLA1010, Data Sciences Int.) connected the radio signal to digitized input that was sent to a dedicated personal computer (Compaq, Pressario 9548). Arterial pressures were calibrated by using an input from an ambient-pressure monitor (C11PR, Data Sciences Int.).

2.3. Transmitter implantation

The method described in our previous studies (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002) was adopted. The rats were anesthetized with i.p injection of a mixture of ketamine (90 mg/kg; Ketaject) and xylazine (10 mg/kg; Xyla-ject). The abdomen was opened with a midline incision (4 cm). Another incision (1.5 cm) was made along the inner thigh to expose the femoral artery. The abdominal wall was pierced with a large bore syringe needle (15 gauge) from the femoral side into the peritoneal cavity. The implant body was placed in the peritoneal cavity and the catheter (15 cm) was passed caudally through the syringe needle into the thigh area. A 5-cm portion of the catheter was inserted into the femoral artery and secured in place with sutures. The abdominal muscle was closed with non-absorbable suture incorporating the implant suture rib with alternating stitches. The skin (abdomen and thigh) was closed with surgical clips. Each rat received a subcutaneous injection of the analgesic ketorolac tromethamine (2 mg/kg; Toradol) and an intramuscular injection of 60,000 U of penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen). Individual rat cages were placed on the top of the radio receivers and all data were collected using a computerized data acquisition system (Dataquest ART, Data Sciences Int.). The system is designed to cycle from animal to animal. Transmitter implantation was performed 9 weeks after ethanol or

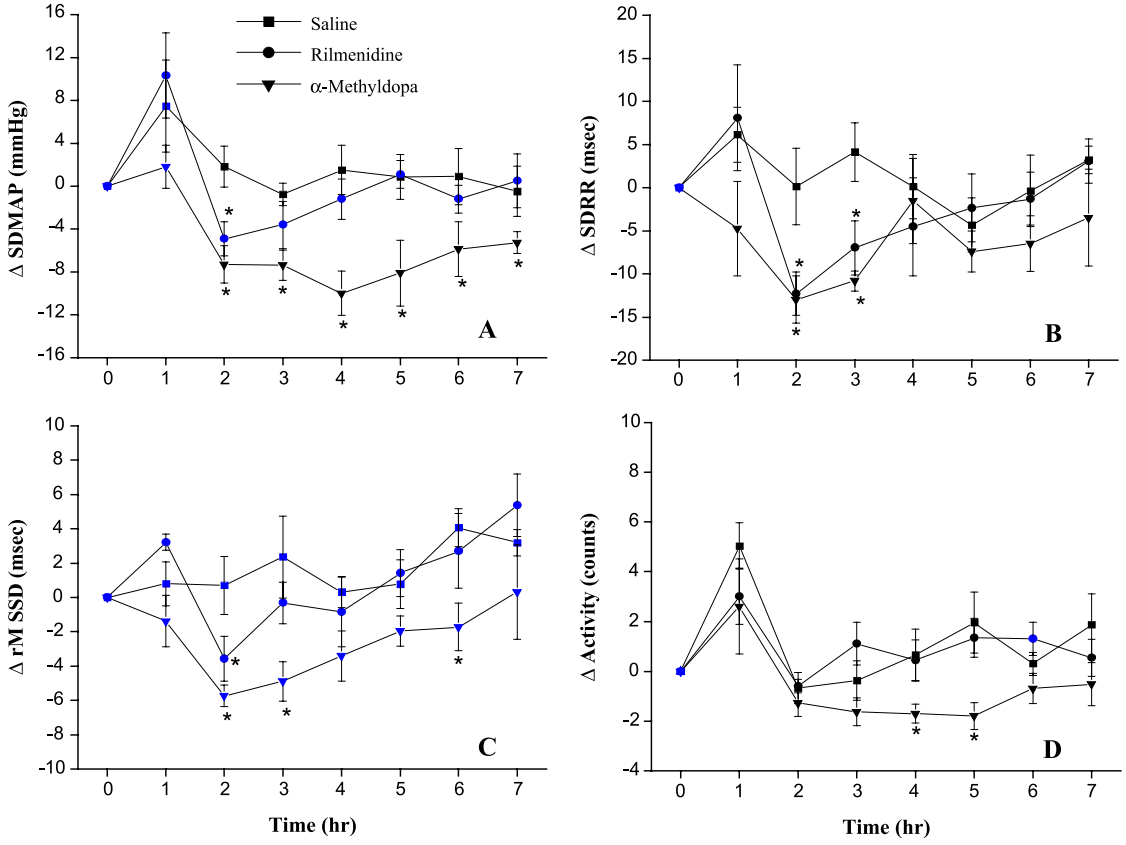

Fig. 2. Changes in the locomotor activity and variability indices of mean arterial pressure (SDMAP) and heart rate (standard deviation of beat-to-beat intervals, SDRR, and the root mean square of successive beat-to-beat differences, rMSSD) evoked by i.p injection of rilmenidine (600 μg/kg), α-methyldopa (100 mg/kg) or equal volume of saline at 3-day intervals in conscious telemetered SHR receiving control liquid diet. Values are means±S.E.M. of four observations. *P<0.05 versus corresponding saline values.

control diet feeding. Rats were left for three additional weeks before starting the experiment.

2.4. Hemodynamic measurements

This experiment investigated the influence of chronic ethanol feeding on the acute hemodynamic responses to activation of imidazoline I_1 receptors or α_2-adrenoceptors by rilmenidine and α-methyldopa, respectively, in conscious telemetered SHR. After 12 weeks of feeding ethanol (2.5% or 5%, w/v) or control liquid diet, SHR received a single i.p. injection of saline (1 ml/kg), rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) at 3-day intervals. Blood pressure and heart rate were followed for 7 h. Waveforms of blood pressure for each rat was sampled at a rate of 500 Hz for 10 s every 10 min. Changes in mean arterial pressure and heart rate from baseline values were averaged in 20-min blocks for analysis.

2.5. Time-domain analysis

As described in previous studies (Stein et al., 1994; Sgoifo et al., 1997; Visser et al., 2002), three time-domain parameters were computed as indices of hemodynamic variability, the standard deviation of the mean arterial pressure as a measure of blood pressure variability and the standard deviation of beat-to-beat intervals and the root mean square of successive beat-to-beat differences in R–R interval durations as measures of heart rate variability. The time-domain indices of blood pressure and heart rate variability correlate well with the frequency-domain measurements (Stein et al., 1994; Sgoifo et al., 1997; El-Mas and Abdel-Rahman, 2000). The standard deviation of beat-to-beat intervals is comparable to the total power of the spectrum of R–R variability, which measures the overall autonomic control of the heart. The variability in the adjacent interbeat intervals provides a measure of the parasympathetic input to the heart and, therefore, correlates with the high frequency power of the spectrum (Stein et al., 1994; Sgoifo et al., 1997). Changes from baseline values evoked by each treatment (saline, α-methyldopa or rilmenidine) in the short-term variability of blood pressure and heart rate and locomotor activity were calculated by averaging each 1-h values (i.e. six successive values measured at 10-min intervals) for a total of 7 h. The R–R intervals were computed from the heart rate values (i.e. the reciprocal of heart rate in ms) as in our previous study (Abdel-Rahman et al., 1987). Baseline values of the measured parameters were taken as the average of the 3-h period (9:00 AM to 12:00 PM) that preceded the administration of saline or the hypotensive agents.

2.6. Measurement of plasma ethanol concentration

A blood sample was taken from each rat at the end of the study and its ethanol content was determined by the enzymatic method described in our previous studies (El-Mas and Abdel-Rahman, 1999, 2000).

2.7. Drugs

α-Methyldopa (Sigma, St. Louis, MO), Ketaject (ketamine), Xyla-ject (xylazine) (Phoenix Pharmaceuticals, St Joseph, MI), Toradol (ketorolac tromethamine, Abbott Labs, Chicago, IL), Durapen (Penicillin G benzathine and penicillin G procaine, Vedco, Overland Park, KS), and ethanol (Midwest Grain Products, Weston, MO) were purchased from commercial vendors. Rilmenidine dihydrogen phosphate was a gift from Servier Pharmaceutical, France.

2.8. Statistics

All values are expressed as means±S.E.M. 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 rilmenidine and α-methyldopa. These analyses were performed by SAS software Release 6.04 (SAS Institute, Cary, NC) as in our previous study (El-Mas and Abdel-Rahman,

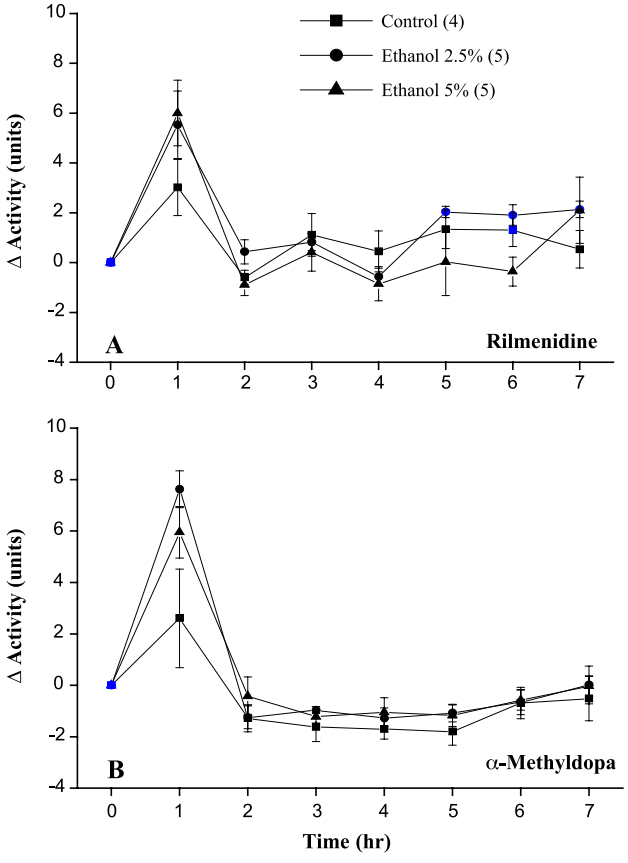

Fig. 3. Effect of chronic feeding of ethanol (2.5% and 5% w/v, 12 weeks) or control liquid diet on changes in locomotor activity evoked by rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) in conscious telemetered SHR. Values are means±S.E.M. and number of rats in each group is shown in parentheses. *P<0.05 versus corresponding control values.

1997a). Probability levels less than 0.05 were considered significant.

3. Results

3.1. Baseline data

The amount of liquid diet consumption was similar in the ethanol-fed rats and their pair-fed controls. The average daily liquid diet consumed by ethanol (2.5% or 5% w/v) and control rats during week 12 of the study amounted to 63.5±1.5, 65.6±2.1, and 64.6±1.4 ml/rat, respectively. Blood ethanol concentrations measured at the 12th week of ethanol feeding (2.5% and 5%) were 61.9±14.5 and 194.8±36.8 mg/dl, respectively. Baseline data of locomotor activity, blood pressure, heart rate, and their variability indices 12 weeks after feeding ethanol (2.5% or 5% w/v) or control liquid diet are shown in Table 1. Ethanol feeding produced significant (P<0.05) and concentration-dependent decreases in mean arterial pressure compared with control values (Table 1). Ethanol also reduced blood pressure variability and the reduction was statistically significant, compared with control values, only with the higher concentration of ethanol. The baseline locomotor activity, heart rate and the time-domain indices of heart rate variability of ethanol-fed and control rats were not significantly different (Table 1).

3.2. Hemodynamic effects of rilmenidine and α-methyldopa

Figs. 1 and 2 depict the time-course effects of i.p. rilmenidine (600 μg/kg), α-methyldopa (100 mg/kg), or equal volume of saline on locomotor activity, blood pressure, heart rate, and their variability in control SHR. Mean arterial pressure was significantly reduced after rilmenidine or α-methyldopa administration compared with the corresponding saline values (Fig. 1A). The maximum hypotensive response to rilmenidine (−28.0±1.8 mm Hg) and α-methyldopa (−40.8±0.8 mm Hg) was demonstrated at 1 and 4 h, respectively (Fig. 1A). The heart rate was not affected by rilmenidine but showed significant increases by α-methyldopa during the first 3 h followed by significant decreases over the remaining period of the study (Fig. 1B). Blood pressure variability was significantly reduced by rilmenidine only at 2 h in contrast to a longer-lasting reduction by α-methyldopa that started at 2 h and continued thereafter (Fig. 2A). The time-domain indices of heart rate variability were significantly reduced by both hypotensive drugs (Fig. 2B,C). The locomotor activity was not altered by rilmenidine but was significantly reduced by α-methyldopa at 4 and 5 h (Fig. 2D).

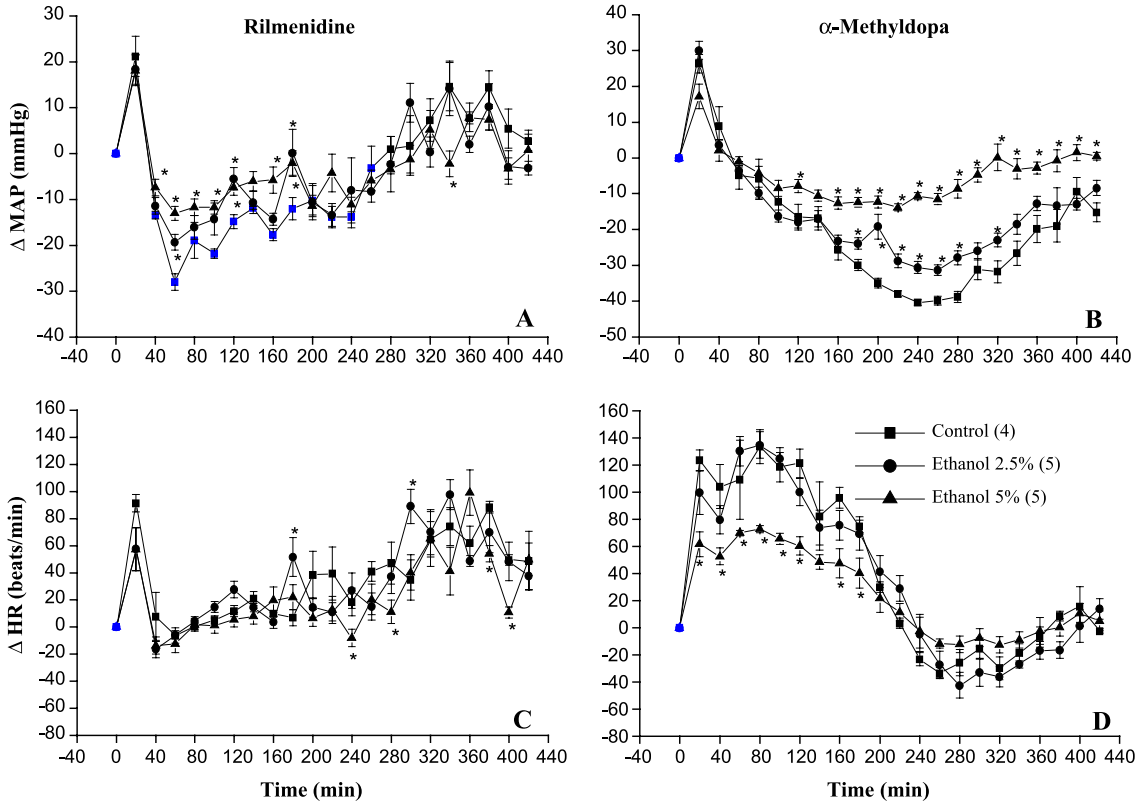

Fig. 4. Effect of chronic feeding of ethanol (2.5% or 5% w/v, 12 weeks) or control liquid diet on changes in mean arterial pressure (MAP) and heart rate (HR) evoked by rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) in conscious telemetered SHR. Values are means±S.E.M. and number of rats in each group is shown in parentheses. *P<0.05 versus corresponding control values.

3.3. Effects of ethanol on hemodynamic responses to imidazoline I_1 receptor or α_2-adrenoceptor activation

The effect of chronic ethanol feeding (2.5% or 5% w/v, 12 weeks) on the responses of locomotor activity, mean arterial pressure, heart rate, and their variability to rilmenidine or α-methyldopa are illustrated in Figs. 3–5. Ethanol feeding had no effect on the locomotor activity in SHR treated with rilmenidine (Fig. 3A) or α-methyldopa (Fig. 3B). The hypotensive effect of α-methyldopa was signifi-

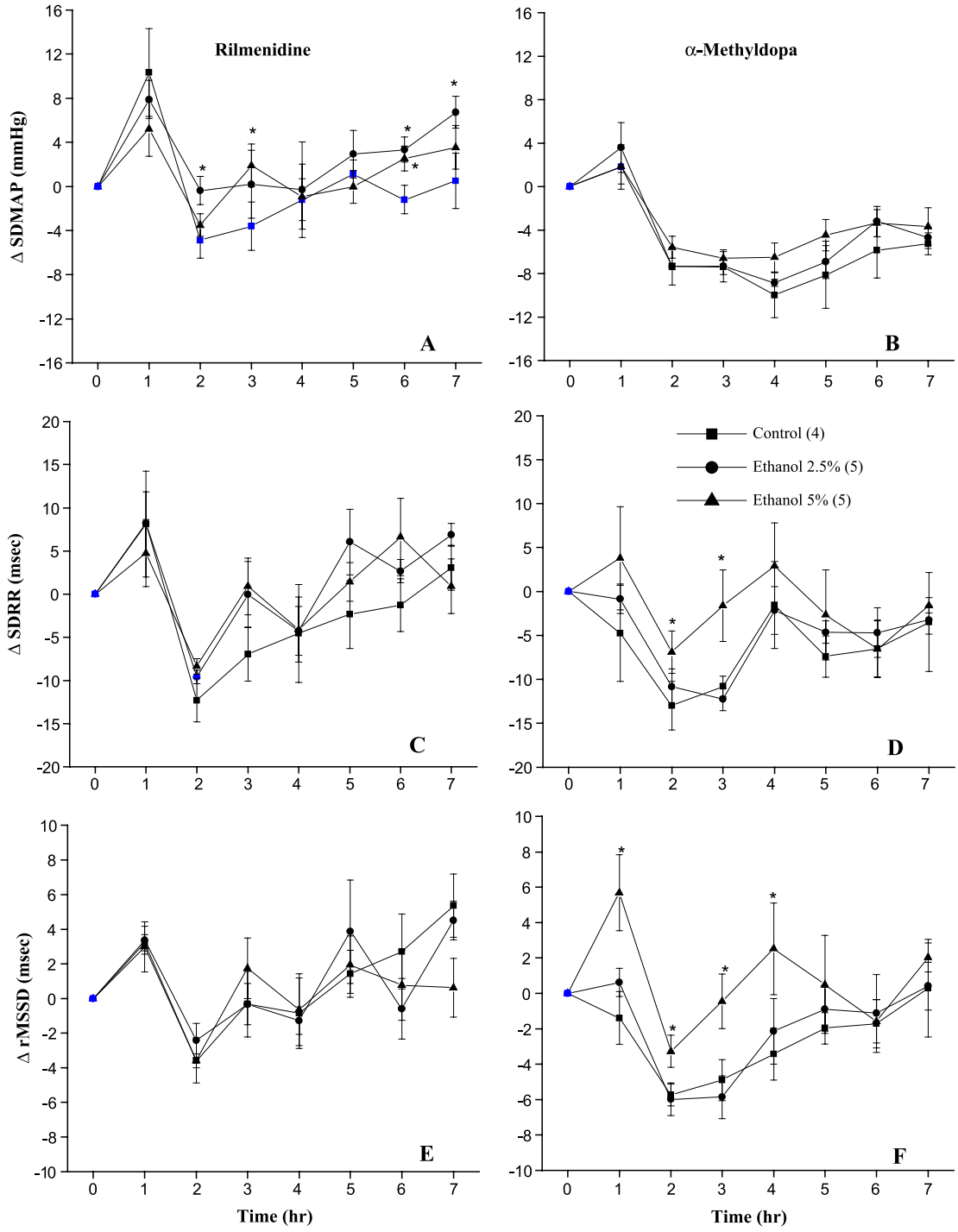

Fig. 5. Effect of chronic feeding of ethanol (2.5% or 5% w/v, 12 weeks) or control liquid diet on changes in the variability indices of mean arterial pressure (SDMAP) and heart rate (standard deviation of beat-to-beat intervals, SDRR, and the root mean square of successive beat-to-beat differences, rMSSD) evoked by rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) in conscious telemetered SHR. Values are means±S.E.M. and number of rats in each group is shown in parentheses. *P<0.05 versus corresponding control values.

cantly attenuated by ethanol in a concentration-dependent manner (Fig. 4B). The maximum hypotensive response to α-methyldopa in control rats observed after 4 h amounted to −40.4±0.8 mm Hg compared to −30.6±1.7 and −10.7±1.0 mm Hg in rats receiving 2.5% and 5% ethanol, respectively. The significant attenuation of α-methyldopa hypotension by 5% ethanol appeared at 2 h and continued for the following 5-h observation period (Fig. 4B). The hypotensive response to rilmenidine was also significantly attenuated by ethanol feeding during the first 3 h of the study (Fig. 4A). Ethanol had no effect on the heart rate response to rilmenidine (Fig. 4C) but significantly attenuated the initial tachycardic responses to α-methyldopa (Fig. 4D). Ethanol attenuated the reductions in blood pressure variability evoked by rilmenidine (Fig. 5A) but not by α-methyldopa (Fig. 5B). However, the attenuation of the rilmenidine effect on blood pressure variability was similarly demonstrated in rats receiving 2.5% and 5% ethanol (Fig. 5A). On the other hand, ethanol significantly attenuated the reductions in heart rate variability indices evoked by α-methyldopa (Fig. 5D,F) but not by rilmenidine (Fig. 5C,E).

Fig. 6 shows the changes in the measured hemodynamic parameters of all rat groups expressed as percentages of baseline values. The maximum reductions in mean arterial pressure evoked by α-methyldopa or rilmenidine at 1 and 4 h, respectively, were significantly and concentration dependently attenuated in ethanol-fed compared with control SHR (Fig. 6A). The attenuating effect of 5% ethanol on α-methyldopa hypotension was greater than its effect on rilmenidine hypotension (approximately 65% vs. 35%). In addition, ethanol (5%) significantly attenuated the percentage reductions in blood pressure variability elicited by rilmenidine (Fig. 6B) and the percentage reductions in the heart rate variability indices elicited by α-methyldopa at 3 h (Fig. 6C,D).

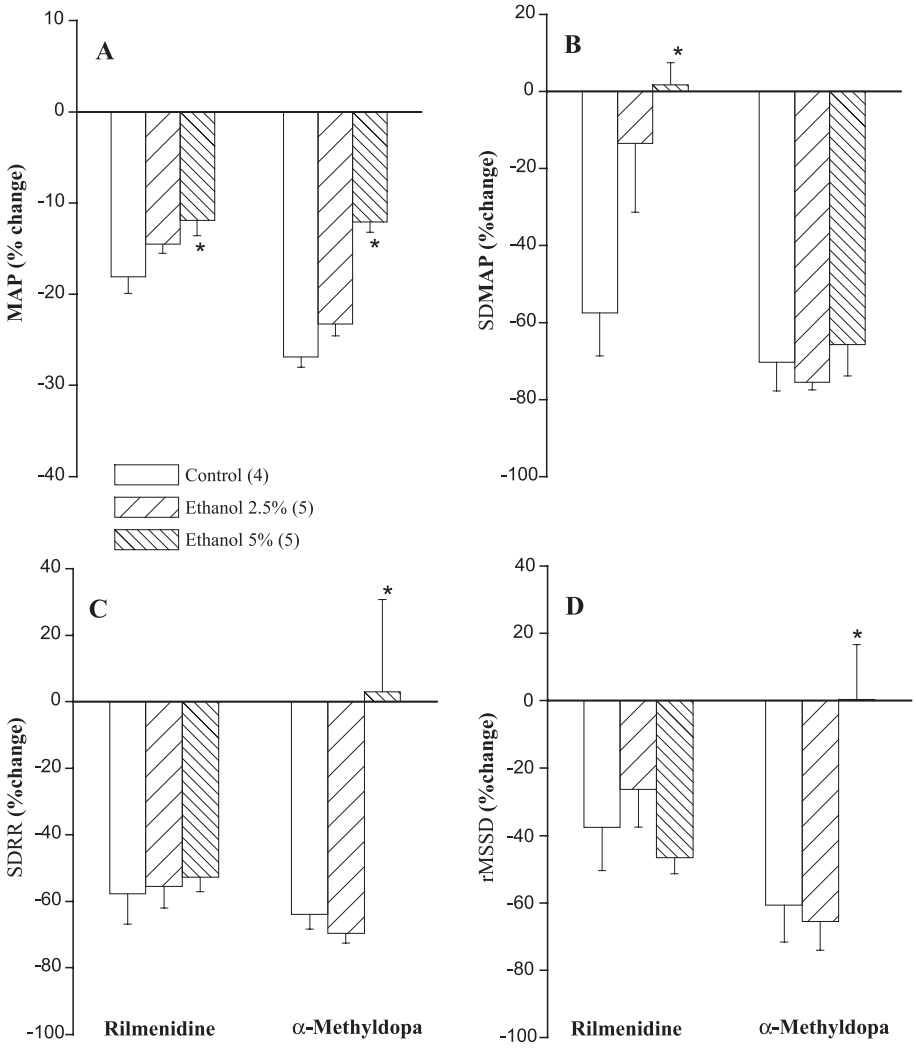

Fig. 6. Percentage changes in mean arterial pressure (MAP) and in the variability indices of mean arterial pressure (SDMAP) and heart rate (standard deviation of beat-to-beat intervals, SDRR, and the root mean square of successive beat-to-beat differences, rMSSD) evoked by rilmenidine (600 μg/kg) or α-methyldopa (100 mg/kg) in ethanol (2.5% and 5% w/v, 12 weeks) and pair-fed control SHR. Values are means±S.E.M. and number of rats in each group is shown in parentheses. *P<0.05 versus corresponding control values.

4. Discussion

In the present study, we investigated the effects of chronic ethanol on changes in locomotor activity, blood pressure and heart rate and their variability elicited by selective activation of imidazoline I_1 receptors (rilmenidine) or α_2-adrenoceptors (α-methyldopa) and the role of cardiovascular autonomic balance in this interaction. Hemodynamic and locomotor responses evoked by either hypotensive agent were evaluated in telemetered ethanol-fed SHR and their pair-fed controls. The time-domain measurement of blood pressure and heart rate variability was employed to determine changes in the cardiovascular autonomic control. The time-domain indices correlate with frequency-domain measurements and, therefore, provide a reliable evaluation of autonomic activity (Stein et al., 1994; Sgoifo et al., 1997). The main findings of the study include: (i) ethanol feeding significantly reduced blood pressure and blood pressure, but not heart rate, variability, (ii) rilmenidine or α-methyldopa significantly reduced blood pressure and hemodynamic variability, (iii) ethanol caused a concentration-dependent attenuation of α-methyldopa or rilmenidine hypotension, and (iv) ethanol attenuated the reductions in blood pressure and heart rate variability evoked by rilmenidine and α-methyldopa, respectively. These findings suggest that, unlike its differential effect on imidazoline I_1 receptor (counteraction) and α_2-adrenoceptor (no effect) hypotension when administered acutely (El-Mas and Abdel-Rahman, 1998, 1999), long-term ethanol feeding compromises the hypotension produced via activation of either type of receptors. Further, the alteration of the cardiovascular autonomic control may contribute, at least partly, to the deleterious effect of chronic ethanol on centrally evoked hypotension.

The present study showed that the hypotensive effect of rilmenidine or α-methyldopa in control SHR was associated with significant reductions in blood pressure and heart rate variability as measured by the time-domain parameters. These effects may be explained by at least three mechanisms. First, the reduced hemodynamic variability may relate to the inhibition of central sympathetic tone (Elghozi et al., 1991; Janssen et al., 1991; Tulen et al., 1993; Girard et al., 1995), the main mechanism mediating the hypotensive action of these drugs (Timmermans and Van Zwieten, 1982; El-Mas and Abdel-Rahman, 1999). This view is supported by the observation that the reduced blood pressure variability produced by rilmenidine in hypertensive humans coincides with a predominant reduction in blood pressure fluctuations in the mid-frequency range (0.1 Hz), which reflects sympathoinhibition (Girard et al., 1995). Further, time- or frequency-domain measurements highlight a role for sympathoinhibition in the reduction in hemodynamic variability that parallels centrally evoked hypotension (Elghozi et al., 1991; Janssen et al., 1991; Tulen et al., 1993). Second, the enhanced baroreflex sensitivity by imidazoline I_1 receptor/α_2-adrenoceptor agonists (Badoer et al., 1983; Head, 1995) might also contribute to their favorable effect on hemodynamic oscillations (Badoer et al., 1983; Elghozi et al., 1991; Head, 1995). It is believed that baroreflex activity is inversely related to blood pressure variability (Su et al., 1986). Previous studies including ours have shown that partial or complete arterial baroreceptor deafferentation elicits remarkable increases in blood pressure oscillations (Krieger, 1964; El-Mas et al., 1994a). Baroreflex facilitation was not evident in the present study because the variability in the adjacent interbeat intervals, which largely reflects cardiac vagal activity (Stein et al., 1994; Sgoifo et al., 1997), was reduced after rilmenidine or α-methyldopa administration. It is possible that the reduction in the variability in the adjacent interbeat intervals might be a counter-regulatory mechanism to the decline in sympathetic activity that may have functioned to maintain the sympathovagal balance. Third, α_2-adrenoceptor agonists produce sedation (van Zwieten, 1997), which may favor hemodynamic stability. Clonidine, a mixed imidazoline I_1 receptor/α_2-adrenoceptor agonist, reduces blood pressure variability only during the dark period when the rats are active (Janssen et al., 1991). In the present study, α-methyldopa-evoked reductions in blood pressure variability coincided with reduced locomotor activity. Compared with α-methyldopa, rilmenidine had no effect on locomotor activity and caused a short-lasting reduction in blood pressure variability. The relatively lower affinity of rilmenidine, compared with α-methyldopa or clonidine, to α_2-adrenoceptors may explain its lesser sedative side effect (van Zwieten, 1997).

Reported findings from our laboratory have shown that acute ethanol administration counteracts the hypotensive and sympathoinhibitory responses to imidazoline I_1 receptor but not α_2-adrenoceptor activation (El-Mas and Abdel-Rahman, 1998, 1999). A main objective of the present study was to investigate whether chronic ethanol feeding elicits a similar selective adverse effect on imidazoline I_1 receptor-mediated hypotension and to determine the role of sympathovagal balance in this interaction. Interestingly, findings of the present study, in contrast to previous acute ethanol studies (El-Mas and Abdel-Rahman, 1998, 1999), showed that chronic ethanol feeding elicited a concentration-related attenuation of the hypotensive response to α-methyldopa or rilmenidine. The apparently greater attenuation by ethanol of α-methyldopa compared to rilmenidine hypotension (65% vs. 35%) might relate to the greater and longer-lasting hypotension produced by α-methyldopa. This may constitute a possible limitation of the present study since the achievement of similar falls in blood pressure is necessary to precisely determine whether ethanol interact differently with central pathways involved in imidazoline I_1 receptor and α_2-adrenoceptor-mediated hypotension. Notably, the delayed and more sustained hypotensive effect of α-methyldopa are expected since the drug effects depend on its metabolic degradation into α-methylnorepinephrine, which is the pharmacologically active form (Sweet, 1984). The reason

for the paradoxical effects of acute (no effect; El-Mas and Abdel-Rahman, 1998, 1999) and chronic (attenuation; this study) ethanol on α_2-adrenoceptor mediated hypotension is not clear. One possible explanation may relate to the ability of chronically administered ethanol to reduce α_2-adrenoceptor density in the nucleus tractus solitarius (El-Mas and Abdel-Rahman, 2001a), a possible brainstem target for α-methyldopa hypotension (Kubo and Misu, 1981). Further, biochemical evidence is available that chronic ethanol reduces α_2-adrenoceptor sensitivity in the SHR brain (Szmigielski et al., 1989). There is also the possibility that chronic accumulation of acetaldehyde, the metabolic product of ethanol, may interfere with the degradation pathway of α-methyldopa (Collins et al., 1990), which mediates its hypotensive effect. Together, our findings may suggest that ethanol feeding to SHR alters the function of neuronal pathways involved in the elicitation of the hypotensive responses evoked via activation of imidazoline I_1 receptors and α_2-adrenoceptors. Nonetheless, it remains to be determined whether the deleterious interaction between chronic ethanol and imidazoline I_1 receptor/α_2-adrenoceptor mediated hypotension is specific to the hypertensive status or it can be similarly demonstrated in normotensive animals. The notion should be considered, however, that centrally acting antihypertensive agents elicit little, if any, drop in blood pressure in normotensive rats and ethanol, administered acutely, fails to alter such response (El-Mas et al., 1994b; El-Mas and Abdel-Rahman, 1997a,b).

The role of cardiovascular sympathovagal balance in ethanol attenuation of centrally evoked hypotension was evaluated in the current investigation. The results showed that the effect of ethanol on hemodynamic variability depended on the type of receptors (imidazoline I_1 receptor or α_2-adrenoceptor) involved. Ethanol abolished rilmenidine (but not α-methyldopa)-induced reductions in blood pressure variability. In contrast, ethanol attenuated reductions in heart rate variability evoked by α-methyldopa but not by rilmenidine. Although evidence is available that changes in blood pressure and heart rate variability might not be related (Janssen et al., 1991), the reason for the discrepancy in ethanol effects on hemodynamic variability responses to rilmenidine and α-methyldopa is not clear. Such selectivity in the effect of ethanol may suggest a differential interaction with neuronal pathways mediating these responses. Notably, the simultaneous attenuation of centrally evoked reductions in blood pressure and hemodynamic variability by ethanol may suggest a role for sympathetic activity and, therefore, the overall sympathovagal balance in this interaction. This view may be supported by the observations that: (i) the reductions in hemodynamic variability and sympathoinhibitory effects of imidazoline I_1 receptor/α_2-adrenoceptor agonists are positively correlated (Elghozi et al., 1991; Janssen et al., 1991; Tulen et al., 1993; Girard et al., 1995), and (ii) sympathoexcitation is implicated in the ethanol counteraction of centrally evoked hypotension (Mao and Abdel-Rahman, 1998; El-Mas and Abdel-Rahman, 1999). It is notable, however, that whereas the hypotensive effect of rilmenidine was attenuated by ethanol (2.5% and 5%) in a concentration-dependent manner (Fig. 4A), the attenuation by ethanol of rilmenidine-induced reductions in blood pressure variability was not dependent on the concentration of ethanol (Fig. 5A). These findings may suggest that factors other than alterations in the sympathovagal balance may have also contributed to the antagonistic ethanol–rilmenidine interaction on blood pressure. Remarkably, the present findings that ethanol adversely affected the beneficial effects of antihypertensive agents on the variability of blood pressure and heart rate is clinically important. The reduction in blood pressure variability by imidazoline I_1 receptor/α_2-adrenoceptor agonists contributes to the regression of ventricular and vascular hypertrophy (Timio et al., 1987; Strauer, 1988; Kohno et al., 1990). Moreover, hemodynamic oscillation abnormalities associate with life-threatening cardiovascular events including sudden stroke, ventricular arrhythmias and myocardial infarction (Stein et al., 1994).

In agreement with our previous studies (El-Mas and Abdel-Rahman, 2000; Rekik et al., 2002), the present study showed that baseline blood pressure and its variability index were reduced in ethanol-fed compared with control SHR. The finding that chronic ethanol lowers blood pressure has also been reported by other investigators (Beilin et al., 1992) and may be attributed to the ethanol-induced myocardial depression (Kelback et al., 1985), facilitation of vascular endothelial activity (Rekik et al., 2002), vasodilation (Turlapaty et al., 1979), or α-adrenoceptor blockade (Abdel-Rahman et al., 1985). The argument may be raised that the reduced effects of rilmenidine or α-methyldopa on blood pressure and hemodynamic variability in ethanol-fed rats might be due to the lower baseline values of these hemodynamic parameters. To rule out such possibility, responses to either hypotensive agent were expressed as percentages of baseline values (i.e. pre-rilmenidine or pre-α-methyldopa) in the ethanol and control groups. The results showed that the attenuating effect of ethanol was still apparent even when the responses were expressed as percentages of baseline values, which suggests that differences in baseline hemodynamic values may not explain ethanol effects. This notion is further supported by the observations that (i) ethanol attenuated centrally evoked hypotension in normotensive rats (El-Mas et al., 1994b), whose baseline blood pressure was less than that of ethanol-fed rats in the present study, and (ii) clonidine hypotension was attenuated in ethanol-fed SHR whose baseline blood pressure was similar to that of control SHR (Abdel-Rahman, 1994).

Finally, it is imperative to comment on the biphasic heart rate response elicited by α-methyldopa in the current study. Whereas the late bradycardic effect of α-methyldopa is consistent with sympathoinhibition (Head, 1995; El-Mas and Abdel-Rahman, 1999), the early tachycardia is peripherally mediated and involves activation of cardiac â-adrenoceptors (van der Maas et al., 1986). The tachycardic effect of α-methyldopa is unlikely to be a compensatory barore-

flex response to the evoked hypotension because the maximum increase in heart rate occurred shortly after α-methyldopa administration and preceded the fall in blood pressure as showed in this study and by others (van der Maas et al., 1986). Further, this response does not seem to be caused by the animal handling during the i.p injection because: (i) no increase in heart rate was evident in the rilmenidine or saline group, and (ii) the tachycardia was abolished in ethanol-fed rats, possibly due to ethanol-evoked myocardial depression (Kelback et al., 1985).

In conclusion, the present study provides, for the first time, a detailed analysis of the effects of chronic ethanol on the hypotensive and hemodynamic variability responses to selective activation of imidazoline I_1 receptor or α_2-adrenoceptor in telemetered SHR. Unlike a preferential adverse effect of acutely administered ethanol on imidazoline I_1 receptor but not α_2-adrenoceptor-mediated hypotension (El-Mas and Abdel-Rahman, 1998, 1999), chronic ethanol significantly attenuated the hypotension elicited by activation of either receptor site. Ethanol also compromises the beneficial effects of rilmenidine or α-methyldopa on hemodynamic stability, which may implicate, at least in part, the cardiovascular sympathovagal balance in the antagonistic effect of ethanol on centrally evoked hypotension.

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References

Abdel-Rahman, A.-R.A., 1989. Reversal by ethanol of the hypotensive effect of clonidine in conscious spontaneously hypertensive rats. Hypertension 14, 531–541.

Abdel-Rahman, A.A., 1994. Alcohol abolishes the hypotensive effect of clonidine in spontaneously hypertensive rats. Hypertension 24, 802–807.

Abdel-Rahman, A.-R.A., Dar, M.S., Wooles, W.R., 1985. Effects of chronic ethanol administration on arterial baroreceptor function and pressor and depressor responsiveness in rats. J. Pharmacol. Exp. Ther. 232, 194–201.

Abdel-Rahman, A.-R.A., Merrill, R.H., Wooles, W.R., 1987. Effect of acute ethanol administration on the baroreceptor reflex control of heart rate in normotensive human volunteers. Clin. Sci. 72, 113–122.

Abdel-Rahman, A.A., Carroll, R.G., El-Mas, M.M., 1992. Role of the sympathetic nervous system in alcohol–guanabenz hemodynamic interaction. Can. J. Physiol. Pharm. 70, 1217–1224.

Badoer, E., Head, G., Korner, P.I., 1983. Effects of intracisternal and intravenous alpha-methyldopa and clonidine on haemodynamics and baroreceptor-heart rate reflex properties in conscious rabbits. J. Cardiovasc. Pharmacol. 5, 760–767.

Beilin, L.J., Hoffmann, P., Nilsson, H., Skarphedinsson, J., Folkow, B., 1992. Effect of chronic ethanol consumption upon cardiovascular reactivity, heart rate and blood pressure in spontaneously hypertensive and Wistar-Kyoto rats. J. Hypertens. 10, 645–650.

Bousquet, P., Feldman, J., Tibirica, E., Bricca, G., Greney, H., Dontenwill, M., Stutzmann, J., Belcourt, A., 1992. Imidazoline receptors: a new concept in central regulation of the arterial blood pressure. Am. J. Hypertens. 5, 47S–50S.

Brockway, B.P., Millis, P.A., Azar, S.H., 1991. A new method for continuous chronic measurement and recording of blood pressure, heart rate and activity in the rat via radiotelemetry. Clin. Exp. Hypertens., Part A Theory Pract. A13, 885–895.

Collins, M.A., Ung-Chhun, N., Cheng, B.Y., Pronger, D., 1990. Brain and plasma tetrahydroisoquinolines in rats: effects of chronic ethanol intake and diet. J. Neurochem. 55, 1507–1514.

Elghozi, J.L., Laude, D., Janvier, F., 1991. Clonidine reduces blood pressure and heart rate oscillations in hypertensive patients. J. Cardiovasc. Pharmacol. 17, 935–940.

El-Mas, M.M., Abdel-Rahman, A.A., 1997a. Contrasting effects of urethane, ketamine, and thiopental anesthesia on ethanol–clonidine hemodynamic interaction. Alcohol., Clin. Exp. Res. 21, 19–27.

El-Mas, M.M., Abdel-Rahman, A.A., 1997b. Role of cardiac output in ethanol-evoked attenuation of centrally-mediated hypotension in conscious rats. Hypertension 30, 288–294.

El-Mas, M.M., Abdel-Rahman, A.A., 1998. Ethanol selectively counteracts hypotension evoked via central II-imidazoline but not α_2-adrenergic receptor activation in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 32, 382–389.

El-Mas, M.M., Abdel-Rahman, A.A., 1999. Ethanol counteraction of I_I-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, M.M., Abdel-Rahman, A.A., 2000. Radiotelemetric evaluation of the hemodynamic effects of long-term ethanol in spontaneously hypertensive and Wistar-Kyoto rats. J. Pharmacol. Exp. Ther. 292, 944–951.

El-Mas, M.M., Abdel-Rahman, A.A., 2001a. Effect of long-term ethanol feeding on brainstem α_2-receptor binding in Wistar-Kyoto and spontaneously hypertensive rats. Brain Res. 900, 324–328.

El-Mas, M.M., Abdel-Rahman, A.A., 2001b. Evidence for the involvement of central I_1-imidazoline receptor in ethanol counteraction of clonidine hypotension in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 38, 417–426.

El-Mas, M.M., Carroll, R.G., Abdel-Rahman, A.A., 1994a. Centrally mediated reduction in cardiac output elicits the enhanced hypotensive effect of clonidine in conscious aortic barodenervated rats. J. Cardiovasc. Pharmacol. 24, 184–193.

El-Mas, M.M., Tao, S., Carroll, R.G., Abdel-Rahman, A.A., 1994b. Ethanol–clonidine hemodynamic interaction in normotensive rats is modified by anesthesia. Alcohol 11, 307–314.

Girard, A., Fevrier, B., Elghozi, J.L., 1995. Cardiovascular variability after rilmenidine challenge: assessment of acute dosing effects by means of spectral analysis. Fundam. Clin. Pharmacol. 9, 366–371.

Head, G.A., 1995. Importance of imidazoline receptors in the cardiovascular actions of centrally acting antihypertensive agents. Ann. N.Y. Acad. Sci. 763, 531–540.

Janssen, B.J., Tyssen, C.M., Struyker-Boudier, H.A., 1991. Modification of circadian blood pressure and heart rate variability by five different antihypertensive agents in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 17, 494–503.

Kelback, H., Gjorup, T., Brynjolf, I., Christensen, N.J., Godtfredsen, J., 1985. Acute effects of alcohol on left ventricular function in healthy subjects at rest and during upright exercise. Am. J. Cardiol. 55, 164–167.

Kohno, M., Yasunari, K., Murakawa, K., Yokokawa, K., Horio, T., Kurihara, N., Takeda, T., 1990. Effect on atrial natriuretic peptides of chronic treatment with alpha-methyldopa and hydralazine in spontaneously hypertensive rats. Am. J. Hypertens. 3, 111–116.

Krieger, E.M., 1964. Neurogenic hypertension in the rat. Circ. Res. 15, 511–521.

Kubo, T., Misu, Y., 1981. Pharmacological characterization of the α-adrenoceptors responsible for a decrease of blood pressure in the nucleus tractus solitarii of the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 317, 120–125.

Lieber, C.S., DeCarli, L.M., 1982. The feeding of alcohol in liquid diets: two decades of applications and 1982 update. Alcohol., Clin. Exp. Res. 6, 523–531.

Mao, L., Abdel-Rahman, A.A., 1998. Ethanol counteraction of clonidine-evoked inhibition of norepinephrine release in rostral ventrolateral medulla of rats. Alcohol., Clin. Exp. Res. 22, 1285–1291.

Potter, J.F., Beevers, F., 1984. Pressor effect of alcohol in hypertension. Lancet 1, 119–122.

Puddey, I.B., Beilin, L.J., Vandongen, R., 1987. Regular alcohol use raises blood pressure in treated hypertensive subjects. A randomized controlled trial. Lancet 2, 647–651.

Rekik, M., El-Mas, M.M., Mustafa, S.J., Abdel-Rahman, A.A., 2002. Role of endothelial adenosine receptor-mediated vasorelaxation in ethanol-induced hypotension in hypertensive rats. Eur. J. Pharmacol. 452, 205–214.

Sgoifo, A., de Boer, S.F., Westenbroek, C., Maes, F.W., Beldhuis, H., Suzuki, T., Koolhaas, J.M., 1997. Incidence of arrhythmias and heart rate variability in wild-type rats exposed to social stress. Am. J. Physiol. 273, H1754–H1760.

Stein, P.K., Bosner, M.S., Kleiger, R.E., Conger, B.M., 1994. Heart rate variability: a measure of cardiac autonomic tone. Am. Heart J. 127, 1376–1381.

Strauer, B.E., 1988. Regression of myocardial and coronary vascular hypertrophy in hypertensive heart disease. J. Cardiovasc. Pharmacol. 12 (Suppl. 4), S45–S54.

Su, D.F., Cerutti, C., Barres, C., Vincent, M., Sassard, J., 1986. Blood pressure and baroreflex sensitivity in conscious hypertensive rats of Lyon strain. Am. J. Physiol. 251, H1111–H1117.

Sweet, C.S., 1984. New centrally acting antihypertensive drugs related to methyldopa and clonidine. Hypertension 6, II51–II56.

Szmigielski, A., Szmigielska, H., Wejman, I., 1989. The effect of prolonged ethanol administration on central alpha2-adrenoceptors sensitivity. Pol. J. Pharmacol. Pharm. 41, 263–272.

Timio, M., Venanzi, S., Gentili, S., Ronconi, M., Del Re, G., Del Vita, M., 1987. Reversal of left ventricular hypertrophy after one-year treatment with clonidine: relationship between echocardiographic findings, blood pressure, and urinary catecholamines. J. Cardiovasc. Pharmacol. 10 (Suppl. 12), S142–S146.

Timmermans, P.B.M.W.M., Van Zwieten, P.A., 1982. α_2-Adrenoceptors: classification, localization, mechanisms and targets for drugs. J. Med. Chem. 25, 1389–1401.

Tulen, J.H., Smeets, F.M., Man in 't Veld, A.J., van Steenis, H.G., van de Wetering, B.J., Moleman, P., 1993. Cardiovascular variability after clonidine challenge: assessment of dose-dependent temporal effects by means of spectral analysis. J. Cardiovasc. Pharmacol. 22, 112–119.

Turlapaty, P.D.M.V., Altura, B.T., Altura, B.M., 1979. Ethanol reduces Ca^{2+} concentration in arterial and venous smooth muscle. Experientia 35, 639–640.

van der Maas, M.L., Head, G.A., de Jong, W., 1986. Mechanism of the tachycardia following systemic alpha-methyldopa administration in conscious rats. J. Cardiovasc. Pharmacol. 8, 1014–1019.

van Zwieten, P.A., 1997. Central imidazoline (I_I) receptors as targets of centrally acting antihypertensives: moxonidine and rilmenidine. J. Hypertens. 15, 117–125.

Visser, E.K., van Reenen, C.G., van der Werf, J.T., Schilder, M.B., Knaap, J.H., Barneveld, A., Blokhuis, H.J., 2002. Heart rate and heart rate variability during a novel object test and a handling test in young horses. Physiol. Behav. 76, 289–296.

Volicer, L., Volicer, B.J., Gravras, H., 1978. The effect of alcohol intake on compliance with antihypertensive treatment. In: Messiha, F.S., Tyner, G.S. (Eds.), Alcoholism: A Perspective. PJD Publishing, New York, pp. 88–99.