JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 985–993

Received January 17, 2007
Accepted March 12, 2007
DOI 10.1211/jpp.59.7.0011
ISSN 0022-3573

Effect of ethanol consumption on blood pressure and rat mesenteric arterial bed, aorta and carotid responsiveness

Carlos R. Tirapelli, Andreia F. C. Leone, Eduardo B. Coelho, Leonardo B. M. Resstel, Fernando M. A. Corrêa, Vera L. Lanchote, Sergio A. Uyemura, Cláudia M. Padovan, Ana M. de Oliveira

Department of Psychiatry Nursing and Human Sciences, College of Nursing of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, São Paulo, Brazil

Carlos R. Tirapelli

Department of Internal Medicine, Nephrology Division, Faculty of Medicine of Ribeirão Preto, USP, Ribeirão Preto, São Paulo, Brazil

Andreia F.C. Leone, Eduardo B. Coelho

Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, USP, São Paulo, Brazil

Leonardo B.M. Resstel, Fernando M.A. Corrêa

Department of Clinical, Toxicological and Food Science Analysis, Faculty of Pharmaceutical Sciences, USP, Ribeirão Preto, São Paulo, Brazil

Vera L. Lanchote, Sergio A. Uyemura

Department of Psychology and Education, Faculty of Philosophy and Sciences, USP, Ribeirão Preto, São Paulo, Brazil

Cláudia M. Padovan

Department of Physics and Chemistry, Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, USP, Ribeirão Preto, São Paulo, Brazil

Ana M. de Oliveira

Correspondence: Carlos Renato Tirapelli, Universidade de São Paulo, Departamento de Enfermagem Psiquiátrica e Ciências Humanas, Escola de Enfermagem de Ribeirão Preto-USP, Av. Bandeirantes 3900, CEP 14040-902, Brazil. E-mail: crtirapelli@eerp.usp.br

Acknowledgements: We thank Juliana A. Vercesi, Mirian C.C. Melo, Eduardo Tozatto, Sonia A. C. Dreossi and Antonio Zanardo Filho for technical support.

Abstract

This study investigates whether chronic ethanol consumption increases blood pressure and alters vascular reactivity in different tissues. Changes in reactivity to phenylephrine and acetylcholine were investigated in the aorta, carotid artery and mesenteric arterial bed (MAB) isolated from rats pretreated with ethanol for 2 or 6 weeks. Mild hypertension was observed in chronically ethanol-treated rats, which was due to rises in both systolic and diastolic pressures. Chronic ethanol consumption increased the contractile response to phenylephrine of endothelium-intact and denuded rat aortic rings from rats pretreated with ethanol for 2 or 6 weeks. Conversely, no differences were found in acetylcholine-induced relaxation. Neither phenylephrine-induced contraction nor acetylcholine-induced relaxation were altered in the rat carotid. Six weeks' ethanol consumption enhanced the contractile response to phenylephrine of endothelium-intact, but not denuded rat MAB. On the other hand, 2 weeks' ethanol consumption did not affect phenylephrine-induced increase in perfusion pressure. Moreover, acetylcholine-induced endothelium-dependent relaxation in the MAB was reduced after treatment with ethanol for 6 weeks but not after 2 weeks. In conclusion, ethanol affects both blood pressure and vessel reactivity, but the effect on vascular reactivity may take longer to become apparent in MAB than in the aorta, and was not evident in the carotid. Moreover, we provide evidence that the effect of ethanol depends on the agonist and blood vessel studied.

Introduction

Excessive ethanol intake is a known cardiovascular risk factor in the general population (Kauhanen et al 1999; Malyutina et al 2002). Some studies have reported that a more-than-moderate ethanol intake elevates blood pressure in humans (Moore et al 1990) and animals (Resstel et al 2006). The precise mechanism(s) responsible for the increased blood pressure associated with ethanol consumption is/are uncertain. Several mechanisms have been postulated, such as enhanced secretion of hormones and neurotransmitters, stimulation of the sympathetic nervous system (Chan et al 1985) and a myogenic mechanism, which involves alteration in the contractile properties of vascular smooth muscle (Chan and Sutter 1983). Previous reports have suggested that enhanced vascular reactivity to vasoconstrictor agents (Pinardi et al 1992) or impairment of vascular relaxation (Kahonen et al 1999) contribute to the cardiovascular complications associated with ethanol-induced increases in blood pressure. In fact, increased vascular reactivity to α_1-adrenoceptor agonists has been demonstrated in the aorta (Pinardi et al 1992; Stewart and Kennedy 1999; Tirapelli et al 2006a) and superior mesenteric artery (Hatton et al 1992) from ethanol-treated rats. Although chronic ethanol consumption is associated with increased vascular responsiveness to α_1-adrenoceptor agonists, contradictory results have also been published, including unchanged (Chan and Sutter 1983; Utkan et al 2001) and attenuated (Strickland and Wooles 1988) responses. The reasons for these differences are not entirely clear, but contributing factors may be different experimental designs, different protocols of ethanol administration, duration of chronic ethanol treatment and the blood vessel studied (Sahna et al 2000; Utkan et al 2001).

Some studies have shown a positive correlation between the duration and extent of ethanol intake and the development of hypertension (Abdel-Rahman & Wooles 1987; Strickland & Wooles 1988). Thus, it appears likely that the period of exposure to ethanol is a major factor in the development of hypertension. Recently, by using the same experimental model for ethanol feeding, we showed increased blood pressure in conscious rats after 2 weeks' treatment (Resstel et al 2006), which coincides with the enhancement of phenylephrine-induced contraction in the rat aorta (Tirapelli et al 2006a).

Based on the aforementioned observations, we investigated whether ethanol consumption induces an increase in mean arterial pressure (MAP) and changes in the reactivity of the mesenteric arterial bed (MAB), aorta and carotid artery. We have also verified whether there is a relationship between the period of exposure to ethanol and alterations in blood pressure and vascular reactivity. Using the same protocol for ethanol feeding, we compared the effect of ethanol consumption for 2 and 6 weeks on blood pressure and changes in reactivity of three different blood vessels to phenylephrine and acetylcholine.

Material and Methods

Drugs

The following drugs were used: phenylephrine hydrochloride, acetylcholine hydrochloride (Sigma, St Louis, MO, USA). The drugs were dissolved in distilled water.

Experimental design

Male Wistar rats (obtained from the animal facility from the Campus of Ribeirão Preto, University of São Paulo, Ribeirão Preto-SP) were housed under standard laboratory conditions. The housing conditions and experimental protocols are in accordance with the Ethical Animal Committee from the Campus of Ribeirão Preto (University of São Paulo).

The rats, initially weighing 300–350 g (80–100 days old), were randomly divided into three groups: control, isocaloric and ethanol. Control rats received tap water ad libitum. Rats in the ethanol group received ethanol 20% (v/v) in their drinking water (Tirapelli et al 2006a, b). Rats in the isocaloric-treated group received a solution containing an isocaloric amount of sucrose (290.50 g L^{-1}) instead of ethanol. In order to avoid considerable loss of animals, the rats in the ethanol-treated group were submitted to a brief and gradual adaptation period: they received 5% ethanol in their drinking water in the first week, 10% in the second week and 20% in the third week. The experimental stage began at the end of the third week. The same procedure was adopted for the isocaloric-treated group, where the sucrose was used to compensate isocalorically for the calorie content of the ethanol solution. In these groups, the calorie content of the liquid diet was adjusted to match that of the ethanol-exposed groups. The isocaloric-treated groups were included in the study protocol to evaluate whether alterations in calorie intake associated with ethanol consumption might explain the possible influences of ethanol on arterial responses. The rats were treated for 2 or 6 weeks and were weighed weekly. Animals had free access to food (Purina rat chow). Consumption of solid and liquid intake was measured weekly.

Blood ethanol and serum glucose measurements

Animals were not fasted prior to blood collection. Blood (5 mL per rat) was collected from the aorta of anaesthetized rats using heparinized syringes as described previously (Tirapelli et al 2006a). Samples (1 mL) were placed in 10 mL headspace vials, to which were added 1.0 g sodium chloride, 1.0 mL water, 100 μL internal standard (acetonitrile, 1 mL L^{-1}). Ethanol analysis was carried out using a CG-17A gas chromatography system (Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and a HSS-4A headspace sampler (Shimadzu). Calibration standards (0.10–3.16 mg mL^{-1}) were prepared in headspace vials. Ethanol concentrations are expressed in mg mL^{-1} blood.

Glucose concentration was measured because elevated glucose levels have been reported to alter vascular responsiveness (Tesfamariam et al 1991). Blood samples (5 mL per rat) for measurement of glucose concentration were collected using non-heparinized syringes. Care was taken to avoid haemolysis. Samples were centrifuged at 8000–10000 *g* for 10 min at room temperature. The serum (100 μL) was analysed (in triplicate) for glucose content using commercially available kits (Labtest Diagnóstica, São Paulo, Brazil) and an auto-analyzer (ABAA model, Abbott, VP, USA). Glucose concentrations are expressed in mg dL^{-1} blood.

Measurement of blood pressure and heart rate in conscious rats

In-vivo procedures were performed as described previously (Resstel et al 2006). One day before the measurement of blood pressure and heart rate (HR), rats were anaesthetized with tribromoethanol (250 mg kg^{-1} i.p.) and a catheter (4 cm segment of PE-10 heat-bound to a 13 cm segment of PE-50, Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta via the femoral artery for recording of blood pressure and HR. The catheter was tunnelled under the skin and exteriorized at the animal's dorsum. During the experiment, freely moving rats were kept in individual cages and MAP and HR were recorded using an HP-7754A amplifier (Hewlett Packard, Waltham, MA, USA) connected to a signal acquisition board (Windaq di 190, DATAQ, Akron, OH, USA) and computer processed. Basal MAP, diastolic arterial pressure (DAP), systolic arterial pressure (SAP) and HR were recorded.

Vessel ring preparation

Rats were not fasted prior to tissue collection. Animals were anaesthetized with tribromoethanol (1 mL per 100 g body weight i.p.) and killed approximately 5 min later by aortic exsanguination. The thoracic aorta and carotid artery were quickly removed, cleaned of adherent connective tissue and cut into four and two rings, respectively (5–6 mm in length).

The endothelium was removed mechanically from some rings by gently rolling the lumen of the vessel on a thin wire. Two stainless-steel stirrups were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Letica Scientific Instruments, Barcelona, Spain) to measure tension in the vessels. The rings were placed in a 5 mL organ chamber containing Krebs solution gassed with 95% oxygen / 5% carbon dioxide and maintained at 37°C. The composition of the Krebs solution was as follows (mmol L^{-1}): NaCl, 118.0; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 15.0; glucose, 5.5; $CaCl_2$, 2.5. The rings were stretched to an optimal basal tension of 1.5 g (aorta) and 1.0 g (carotid artery), determined by length–tension relationship experiments, and were allowed to equilibrate for 60 min. The bathing solution was changed every 15–20 min.

Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (1 μmol L^{-1}) in the presence of contractile tone induced by phenylephrine (0.1 μmol L^{-1}). For studies in endothelium-intact vessels, rings were discarded if relaxation with acetylcholine was not 80% or greater. For studies in endothelium-denuded vessels, rings were discarded if there was any degree of relaxation.

Concentration–response curves for phenylephrine and acetylcholine

After 60 min equilibration, each aortic or carotid ring was exposed three times to phenylephrine (0.1 μmol L^{-1}) to attain its maximum contractility. Each ring was sequentially washed and re-equilibrated and was allowed to relax to baseline. After 30 min, cumulative concentration–response curves for phenylephrine (10^{-10}–10^{-5} mol L^{-1}) were obtained. In another set of experiments, steady tension was evoked by phenylephrine (0.1 μmol L^{-1}) and then acetylcholine was added cumulatively (10^{-10}–10^{-5} mol L^{-1}). Relaxation was expressed as percentage change from phenylephrine-contracted levels. The vascular responsiveness to the agonists was studied in aortic and carotid rings from control, isocaloric- and ethanol-treated rats after treatment for 2 or 6 weeks.

Mesenteric arterial bed perfusion

The rat isolated MAB perfused in-vitro was used as a model of vascular resistance, as described previously by Leone and Coelho (2004) modified from the Macgregor method (Macgregor 1965). In brief, rats were anaesthetized with 2.5% tribromoethanol (1 mL per 100 g body weight i.p.), the abdominal cavity was opened and the intestinal loops were exposed. The superior mesenteric artery was dissected close to its origin in the abdominal aorta and cannulated with a PE-50 polyethylene catheter. The MAB was perfused with 1 mL Krebs solution (composition (mmol L^{-1}): NaCl 120.0, KCl 4.7, $NaHCO_3$ 25.0, $CaCl_2{\cdot}2H_2O$ 2.4, $MgCl_2{\cdot}6H_2O$ 1.4, KH_2PO_4 1.17, glucose 11.0) containing 500 IU heparin. The intestinal loops were removed en bloc, the MAB was separated by cutting close to the intestinal loops, and the preparation was placed in a cuvette warmed to 37°C. The cannulated superior mesenteric artery was coupled to a perfusion pump (LKB 2215 Multiperpex pump, LKB, Broma, Sweden) and the MAB was perfused with Krebs solution bubbled with 95% oxygen / 5% carbon dioxide, pH 7.4, at a constant flow of 4 mL min^{-1}. A pressure transducer (R 511A, Beckman Instruments, Schiller Park, IL, USA) was coupled in a 'y' arrangement to the system for perfusion pressure recording. The pre-amplified and filtered outlet signal was coupled to the data acquisition system (DATAQ DI-150, Akron, OH, USA) connected to the RS 232 parallel port of a Pentium II personal computer, and stored for later analysis using the Windaq software, version 2.5 (DATAQ).

Vascular reactivity to vasoactive agents

The MAB was perfused with Krebs solution and left to rest for 15 min to allow stabilization of the basal perfusion pressure, which was recorded continuously. Increasing bolus doses of phenylephrine (0.5–80 μg) were then injected with a 50 μL Hamilton syringe. The interval between injections was 5 min or the time needed for perfusion pressure to return to initial values. In some preparations, the endothelium was removed with a solution of sodium deoxycholate (2 mL bolus of a 1 mg mL^{-1} solution). After 10 min for equilibration, a dose–response curve was constructed as described above. At the end of the experiment, the MAB was pre-contracted with a dose of phenylephrine capable of increasing the basal perfusion pressure by 60 mmHg, and a bolus injection of acetylcholine (10 nmol L^{-1}) was applied; absence of a dilating response indicated effective removal of the vascular endothelium. Dose–response curves for acetylcholine were also obtained. The MAB was pre-contracted with a dose of phenylephrine capable of increasing the basal perfusion pressure by 60 mmHg. Increasing bolus doses of acetylcholine (0.5–50 μg) were then injected using a 50 μL Hamilton syringe. Relaxation was expressed as percentage change from the phenylephrine-contracted levels.

Statistical analysis

Results are expressed as mean ± s.e.m. Statistical analyses were performed using one- or two-way (treatment vs time) analysis of variance (ANOVA), as indicated in the text. Post hoc comparisons were performed using Dunnett's test. Student's *t*-test was used to compare the blood ethanol levels between 2- and 6-week-treated rats. A P value below 0.05 was considered significant. Statistical analysis was performed using commercially available software (SPSS version 9.0, Chicago, IL, USA).

Results

Body weight, food consumption, liquid intake, blood ethanol and serum glucose measurements

Before beginning treatment, control rats had a mean body weight of 295.8 ± 8.0 g, isocaloric-treated rats weighed 300.8 ± 13 g and ethanol-treated rats weighed 301.4 ± 7.8 g. After 2 weeks' ethanol treatment, rats' body weight (419.2 ± 7.6 g) was lower than that of control rats (464.1 ± 7.1 g) and isocaloric-treated rats (473.3 ± 9.2 g)

($P<0.05$; ANOVA). Similarly, 6 weeks' treatment reduced the body weight of rats in the ethanol group (429.4 ± 15.2 g) and increased the body weight of rats in the isocaloric-treatment group (585.9 ± 12.0 g) compared with the control group (520.1 ± 10.3 g) ($P<0.05$; ANOVA).

Solid food consumption (g per week) was reduced in ethanol-treated rats (131.7 ± 3.9 g) and isocaloric-treated rats (132.2 ± 7.4 g) compared with control animals (274.7 ± 4.5 g) ($P<0.05$; ANOVA) in the 2-week treatment programme. Similarly, treatment for 6 weeks reduced the solid consumption of rats in the ethanol-treated (127.8 ± 2.55 g) and isocaloric-treated rats (100.2 ± 1.4 g) compared with control rats (205.7 ± 8.1 g) ($P<0.05$; ANOVA). Liquid intake (mL per week) at week 2 was higher in control rats (460 ± 9.6) and isocaloric-treated rats (490.12 ± 39.0) than in ethanol-treated rats (273.4 ± 8.5 mL) ($P<0.05$; ANOVA). Similarly, liquid intake at week 6 was reduced in the ethanol group (246.4 ± 5.8) compared with control (456 ± 6.9) and isocaloric-treated rats (488.2 ± 32.6) ($P<0.05$; ANOVA).

Blood ethanol levels (in mg mL^{-1}) in the ethanol-treated rats averaged 1.85 ± 0.18 in the second week (n = 9) and 1.97 ± 0.17 in the sixth week (n = 8). No ethanol was detectable in the blood of control and isocaloric-treated animals. Blood ethanol concentrations were not significantly different between the two different periods of treatment (Student's *t*-test).

In the 2-week treated rats, serum glucose concentrations (in mg dL^{-1}) in the control (n = 9), isocaloric-treated (n = 8) and ethanol (n = 10) groups averaged 107.15 ± 5.40, 104.15 ± 5.90, and 109.10 ± 5.88, respectively. No significant differences were found in serum glucose levels after 6 weeks' treatment (control: 100.06 ± 6.30, n = 9; isocaloric: 110.22 ± 11.11, n = 11; ethanol: 115.25 ± 10.75, n = 8) ($P<0.05$; ANOVA). These data indicate that the experimental protocol employed did not induce an enhancement of glucose levels.

Effect of chronic ethanol consumption on blood pressure and heart rate

Figure 1 shows representative traces of basal pulsatile arterial pressure, MAP and HR from control, isocaloric- and ethanol-treated rats at 2 and 6 weeks. Baseline values for MAP and DAP were increased by chronic ethanol treatment by approximately 25% after the two different periods of treatment when compared with control and isocaloric-treated rats. Similarly, treatment with ethanol for 2 or 6 weeks increased the SAP by approximately 20% compared with control and isocaloric-treated rats (Table 1). No

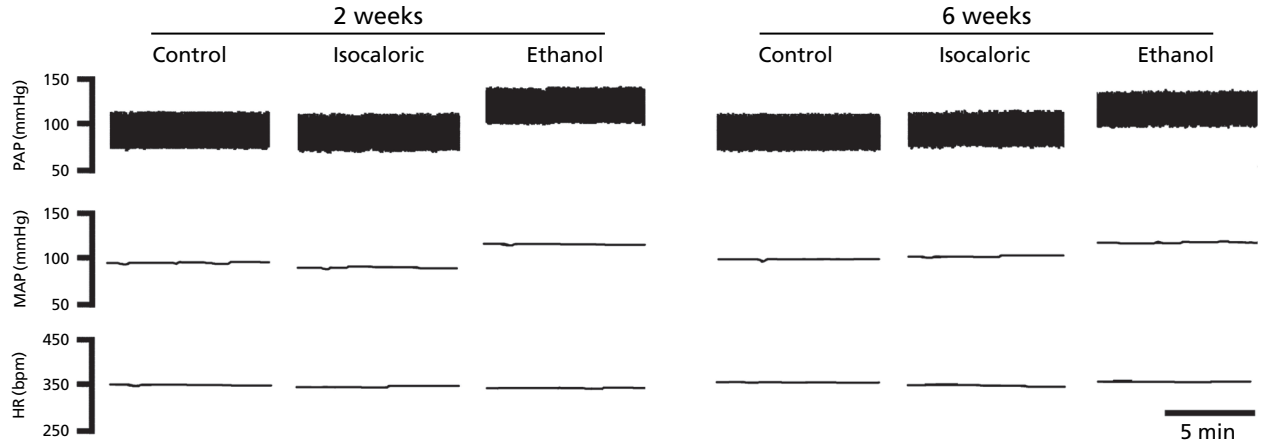

Figure 1 Original traces of pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) from 2- and 6-week control, isocaloric- and ethanol-treated rats. bpm, beats per minute.

Table 1 Systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) in rats treated with ethanol or isocaloric sucrose, and controls

	Control		Isocaloric		Ethanol	
	2 weeks	6 weeks	2 weeks	6 weeks	2 weeks	6 weeks
SAP	122.5 ± 2.87 (5)	120.5 ± 1.44 (4)	122.7 ± 1.93 (4)	123.0 ± 2.48 (4)	144.50 ± 3.57* (4)	148.7 ± 4.11* (4)
DAP	79.5 ± 3.75 (5)	81.0 ± 1.63 (4)	83.3 ± 2.68 (4)	81.5 ± 1.44 (4)	101.5 ± 2.25* (4)	103.5 ± 2.10* (4)
MAP	96.5 ± 3.86 (5)	97.0 ± 0.70 (4)	101.7 ± 1.93 (4)	100.2 ± 1.65 (4)	121.8 ± 3.03* (4)	122.3 ± 1.88* (4)

All values are in mmHg. Numbers in parentheses indicate the number of animals. Values are means ± s.e.m. *$P<0.05$ vs control and isocaloric groups (two-way analysis of variance followed by Dunnett's test).

changes in HR (given in beats per min) were observed after 2 weeks (control: 336.25 ± 12.35; isocaloric: 331.25 ± 17.76; ethanol: 316 ± 13.20) or 6 weeks (control: 332.25 ± 18.81; isocaloric: 335.75 ± 10.35; ethanol: 310.50 ± 10.10) (two-way ANOVA). MAP, DAP, SAP and HR values in the isocaloric-treated group were similar to those in the control group.

Effect of chronic ethanol consumption on aorta and carotid responsiveness

The maximum effect elicited by an agonist (E_{max}, given in g) of the concentration–response curve was significantly higher in aortic rings from ethanol-treated rats than in those from control or isocaloric-treated animals but there were no significant differences in pD_2 (–log EC50, where EC50 is the concentration of the agonist that elicits half the maximal response) values. The E_{max} and pD_2 values for phenylephrine-induced contraction did not differ significantly between control and isocaloric-treated animals (Figure 2, Table 2). Mechanical removal of the endothelium significantly increased both E_{max} and pD_2 values for phenylephrine (Table 2) in arteries from control, isocaloric- or ethanol-treated rats. The concentration–response to phenylephrine obtained in denuded aortic rings from ethanol-treated rats showed a significant difference in E_{max} values compared with values obtained for denuded arteries from control and isocaloric-treated rats (Figure 2, Table 2). The magnitude of the enhancement observed after ethanol treatment on phenylephrine-induced contraction did not differ between the two different periods of treatment in either endothelium-intact or denuded rings.

Acetylcholine-induced relaxation in the rat aorta did not significantly differ between the three groups of rats after 2 weeks' treatment (control: E_{max} 99.50 ± 0.84%, pD_2 7.67 ± 0.08, n = 12; isocaloric: E_{max} 98.19 ± 1.89%, pD_2 7.64 ± 0.09, n = 12; ethanol-treated E_{max} 95.97 ± 1.62%, pD_2 7.82 ± 0.23, n = 6) or 6 weeks' treatment (control:

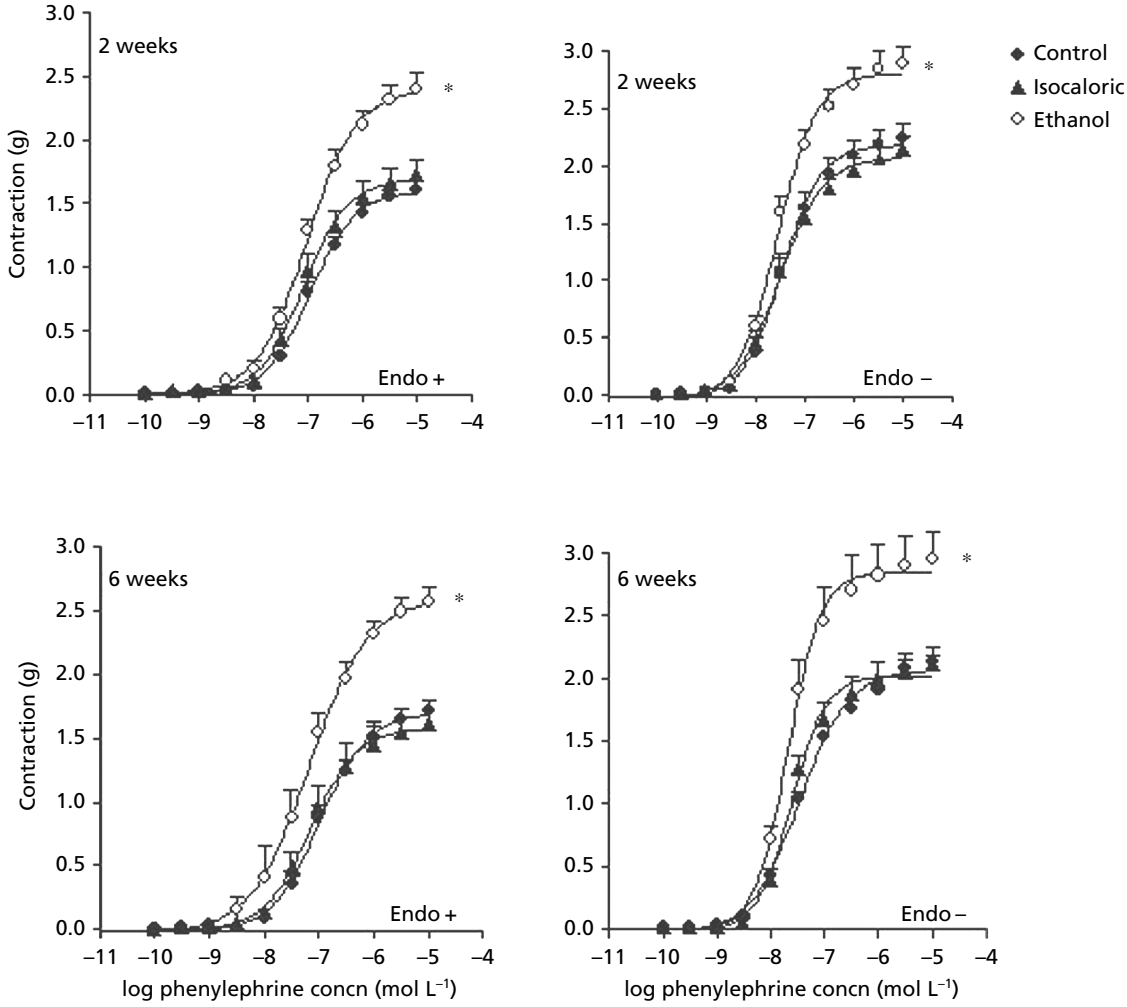

Figure 2 Effect of chronic ethanol consumption on phenylephrine-induced contractile response in rat aortic rings. Concentration–response curves for phenylephrine were determined in endothelium-intact (Endo+, left) or endothelium-denuded (Endo–, right) aortic rings from control, isocaloric- and ethanol-treated rats. *$P < 0.05$ vs control and isocaloric groups (two-way analysis of variance followed by Dunnett's test).

Table 2 Maximum response (E_{max} (g)) and pD_2 values for phenylephrine in endothelium-intact (Endo+) or denuded (Endo–) aortic rings from rats treated with ethanol or isocaloric sucrose, and controls

	Control		Isocaloric		Ethanol	
	Endo+	Endo–	Endo+	Endo–	Endo+	Endo–
E_{max}						
2 weeks	1.61 ± 0.05 (10)	2.23 ± 0.13† (8)	1.71 ± 0.12 (7)	2.14 ± 0.10† (8)	2.40 ± 0.12* (6)	2.89 ± 0.14*,† (6)
6 weeks	1.71 ± 0.09 (8)	2.12 ± 0.07† (4)	1.60 ± 0.18 (6)	2.11 ± 0.14† (6)	2.57 ± 0.11* (5)	2.95 ± 0.22*,† (6)
pD_2						
2 weeks	6.93 ± 0.07 (10)	7.48 ± 0.06† (8)	7.05 ± 0.08 (7)	7.50 ± 0.08† (8)	7.02 ± 0.10 (6)	7.52 ± 0.10† (6)
6 weeks	6.97 ± 0.07 (8)	7.45 ± 0.05† (4)	7.10 ± 0.09 (6)	7.61 ± 0.05† (6)	7.36 ± 0.15 (5)	7.65 ± 0.07† (6)

Numbers in parentheses indicate the number of preparations. Values are mean ± s.e.m. *$P < 0.05$ ethanol-treatment vs control and isocaloric-treatment groups, †$P < 0.05$ Endo+ vs Endo– groups (two-way analysis of variance followed by Dunnett's test).

E_{max} 96.75 ± 3.31%, pD_2 7.73 ± 0.08, n = 12; isocaloric: E_{max} 102.57 ± 1.50%, pD_2 7.60 ± 0.11, n = 6; ethanol-treated: E_{max} 100.45 ± 0.50%, pD_2 7.64 ± 0.08, n = 7) (two-way ANOVA).

Phenylephrine-induced contraction in rat carotid rings was not altered by treatment with ethanol in either endothelium-intact or denuded rings (Table 3). Acetylcholine-induced relaxation in the carotid artery did not differ significantly between controls (E_{max} 111.05 ± 4.07%, pD_2 7.00 ± 0.18, n = 8), isocaloric (E_{max} 103.55 ± 4.09%, pD_2 7.29 ± 0.18, n = 6) or ethanol-treated rats (E_{max} 103.45 ± 2.69%, pD_2 7.18 ± 0.13, n = 9) after treatment for 2 weeks. Similarly, treatment for 6 weeks did not alter acetylcholine-induced relaxation in arteries from ethanol-treated rats (E_{max} 112.50 ± 3.49%, pD_2 7.19 ± 0.09, n = 6) compared with arteries from control (E_{max} 101.54 ± 8.30%, pD_2 7.28 ± 0.10, n = 8) or isocaloric-treated rats (E_{max} 108.46 ± 4.27%, pD_2 7.24 ± 0.10, n = 6) (two-way ANOVA).

Concentration–response curves for phenylephrine and acetylcholine in the MAB

Baseline perfusion pressure was similar in MAB from 2-week ethanol-treated (20.25 ± 1.80 mmHg), control (20.54 ± 0.50 mmHg) and isocaloric-treated rats (21.12 ± 0.40 mmHg) ($P < 0.05$; two-way ANOVA). Similarly, baseline perfusion pressure did not differ between the groups after 6 weeks' treatment (control 19.40 ± 1.44 mmHg; isocaloric 21.34 ± 1.50 mmHg; ethanol 19.71 ± 1.11 mmHg; $P < 0.05$; two-way ANOVA). The maximum increase in perfusion pressure (E_{max}, given in mmHg) induced by phenylephrine was significantly higher in the MAB from 6-week ethanol-treated rats than in tissues from control or isocaloric-treated animals but no differences in E_{max} values with phenylephrine were found after 2 weeks' treatment. E_{max} values for phenylephrine-induced increases in perfusion pressure did not differ significantly between control and isocaloric groups after 2 or 6 weeks' treatment (Table 4).

E_{max} values from the concentration–response curves obtained with phenylephrine were similar in endothelium-denuded rings from ethanol-treated rats, control and isocaloric-treated rats, in both 2- and 6-week-treated animals (Table 4). Acetylcholine-induced endothelium-dependent relaxation in 2-week-treated rats did not differ between the groups. On the other hand, the relaxation induced by acetylcholine (50 μg) in endothelium-intact MAB from 6-week ethanol-treated rats was significantly reduced compared with MABs from control or isocaloric-treated rats (Figure 3).

Table 3 Maximum response (E_{max} (g)) and pD_2 values for phenylephrine in endothelium-intact (Endo+) or denuded (Endo–) carotid rings from rats treated with ethanol or isocaloric sucrose, and controls

	Control		Isocaloric		Ethanol	
	Endo+	Endo–	Endo+	Endo–	Endo+	Endo–
E_{max}						
2 weeks	0.32 ± 0.03 (10)	0.46 ± 0.03* (9)	0.30 ± 0.03 (10)	0.44 ± 0.03* (6)	0.33 ± 0.03 (8)	0.47 ± 0.03* (8)
6 weeks	0.33 ± 0.03 (8)	0.44 ± 0.02* (11)	0.32 ± 0.03 (6)	0.43 ± 0.04* (8)	0.34 ± 0.03 (8)	0.45 ± 0.04* (12)
pD_2						
2 weeks	6.95 ± 0.05 (10)	7.42 ± 0.07* (9)	7.10 ± 0.11 (10)	7.55 ± 0.14* (6)	6.96 ± 0.06 (8)	7.44 ± 0.12* (8)
6 weeks	7.05 ± 0.08 (8)	7.41 ± 0.09* (11)	7.11 ± 0.15 (6)	7.54 ± 0.12* (8)	7.14 ± 0.12 (8)	7.50 ± 0.11* (12)

Numbers in parentheses indicate the number of preparations. Values are mean ± s.e.m. *$P < 0.05$ Endo+ vs Endo– groups (two-way analysis of variance followed by Dunnett's test).

Table 4 Maximum response (E_{max} (mmHg)) with phenylephrine in endothelium-intact (Endo+) or denuded (Endo−) mesenteric arterial beds from rats treated with ethanol or isocaloric sucrose, and controls

	Control		Isocaloric		Ethanol	
	Endo+	Endo−	Endo+	Endo−	Endo+	Endo−
2 weeks						
0.5 μg	7.6 ± 1.9	13.0 ± 1.2	8.4 ± 2.4	15.0 ± 3.8	6.9 ± 1.2	17.5 ± 3.6
2.5 μg	48.0 ± 7.9	74.1 ± 11.6	34.9 ± 3.5	58.4 ± 12.8	36.5 ± 3.6	61.5 ± 6.5
20 μg	104.3 ± 10.4	135.5 ± 11.0	85.2 ± 6.6	112.8 ± 11.9	100.5 ± 4.7	136.2 ± 12.0
40 μg	118.4 ± 7.7	146.0 ± 11.1	105.5 ± 3.2	132.6 ± 10.3	116.7 ± 4.5	142.5 ± 9.5
80 μg	112.0 ± 7.1	149.0 ± 12.4	110.8 ± 3.9	140.9 ± 11.5	116.1 ± 5.2	147.5 ± 10.1
6 weeks						
0.5 μg	6.1 ± 1.2	14.1 ± 0.7	6.5 ± 1.89	11.8 ± 0.7	8.8 ± 2.8	13.0 ± 1.2
2.5 μg	38.4 ± 5.6	73.5 ± 8.2	40.9 ± 6.9	62.8 ± 5.0	67.3 ± 8.7[a]	70.0 ± 9.1
20 μg	103.0 ± 4.2	135.8 ± 9.9	106.3 ± 6.5	127.7 ± 6.3	129.8 ± 2.1[a]	130.0 ± 12.1
40 μg	112.0 ± 4.5	140.0 ± 9.1	107.2 ± 3.4	141.1 ± 6.2	142.0 ± 8.1[a]	137.0 ± 10.4
80 μg	111.9 ± 3.5	146.3 ± 6.8	107.0 ± 3.7	140.9 ± 3.5	148.6 ± 11.0[a]	147.5 ± 17.5

Values are mean ± s.e.m. of n=5 or 6 preparations *$P < 0.05$ vs control and isocaloric groups (two-way analysis of variance followed by Dunnett's test).

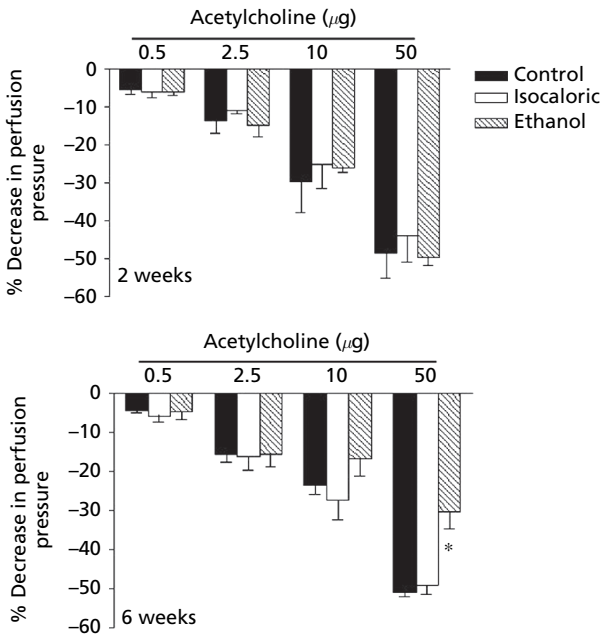

Figure 3 Effect of chronic ethanol consumption on acetylcholine-induced endothelium-dependent relaxation in rat mesenteric arterial bed (MAB). Relaxation induced by acetylcholine was determined in endothelium-intact MAB from 2-and 6-week ethanol-treated rats and age-matched controls. *$P < 0.05$ vs control and isocaloric groups (two-way analysis of variance followed by Dunnett's test).

Discussion

Mild hypertension following chronic ethanol ingestion was observed in this study, consistent with previous reports (Chan et al 1985; Resstel et al 2006). Increase in blood pressure after ethanol feeding, which is the consequence of rises in both SAP and DAP, was seen within 2 weeks of treatment and continued over the subsequent 4 weeks. Our data show that ethanol consumption for 2 or 6 weeks did not affect resting HR. Therefore, it appears that HR enhancement does not play an appreciable role in mediating elevation of blood pressure in ethanol-treated rats. Similarly, Beilin et al (1992) and Resstel et al (2006) reported that resting HR was not altered by long-term ethanol consumption.

The increment in blood pressure coincided with the enhanced responsiveness to phenylephrine in endothelium-intact and -denuded rat aortas. We found no relationship between the period of treatment with ethanol (2 or 6 weeks) and the magnitude of enhancement of blood pressure and aorta responsiveness to phenylephrine. Interestingly, the increase in blood pressure had a similar profile to that of vascular reactivity, where an increase of similar magnitude was found in 2- and 6-week-treated animals. As vascular contractility of the aorta does not contribute largely to increases in blood pressure, changes to vascular reactivity in this vessel at week 2 may be coincident with blood pressure increases. However, from our data it is not possible to establish a cause and effect relationship where the increased reactivity of the aorta to phenylephrine would be the mechanism underlying the increased blood pressure induced by ethanol consumption. It has been suggested that ethanol-induced increases in blood pressure are responsible for changes in vascular reactivity in this vessel, or that ethanol works by different mechanisms to affect both blood pressure and vascular reactivity.

The enhanced response to phenylephrine is not the result of a non-specific increase in the reactivity of the rat aorta induced by chronic ethanol consumption. Using the same protocol for ethanol feeding, we previously observed that the contractile response to endothelin-1 or KCl was not affected by ethanol consumption (Tirapelli et al 2006a), further indicating that the effect of ethanol depends on the agonist

studied. The current observation that acetylcholine-induced relaxation is not altered by chronic ethanol consumption corroborates previous results from our laboratory, namely that the effect elicited by ethanol on the responsiveness of rat aorta is endothelium independent (Tirapelli et al 2006a). In the same study, we verified that endothelium-independent enhancement of the vascular response to phenylephrine observed in the aorta of ethanol-treated rats is maintained by two mechanisms: an increased release of vascular smooth muscle-derived vasoconstrictor prostanoids (probably thromboxane A_2) and an enhanced extracellular calcium ion influx (Tirapelli et al 2006a).

Interestingly, ethanol intake did not seem to induce a general alteration in the vascular reactivity, since there were no differences in phenylephrine-induced contraction or acetylcholine-induced relaxation in the carotid artery. However, using the same experimental protocol, we have previously observed that ethanol consumption for 2 and 6 weeks enhanced endothelin-1-induced contraction and reduced the relaxation induced by IRL1620, a selective agonist for endothelin ET_B receptors in the rat carotid (Tirapelli et al 2006b). The effect of ethanol on the endothelinergic system is probably caused by reduced expression of relaxing endothelial ET_B receptors (Tirapelli et al 2006b). Reasons for these differences in the effect of ethanol consumption on blood vessels reactivity to different vasoactive agents are not clear; however, our findings strengthen the initial observation in the aorta that the effect of ethanol depends on the agonist studied. The lack of effect of ethanol treatment on phenylephrine-induced contraction in the rat carotid was an unexpected finding. Previously, we observed that vasoconstrictor prostanoids mediate the contractile response induced by phenylephrine in rat aorta (Tirapelli et al 2006a). A possible explanation for the disparity between the findings in the aorta and carotid could be attributed to the role of the prostanoids in mediating the response to phenylephrine in these arteries. However, further studies are needed to clarify this point. Thus, although the present observations did not provide evidence for alterations in the reactivity of the rat carotid to phenylephrine, we cannot conclude that ethanol consumption does not alter the reactivity of the rat carotid, since we previously observed that ethanol alters other pathways, such as the endothelinergic system. It is important to note that the altered vascular reactivity of the aorta described here, as well as the increased response of carotid arteries to endothelin-1 described previously (Tirapelli et al 2006b), were already apparent after 2 weeks' treatment. Whether the altered reactivity is a causative factor or a consequence of the increased blood pressure associated with ethanol consumption remains to be clarified.

The mesenteric circulation of the rat receives approximately one-fifth of the cardiac output (Nichols et al 1985); regulation of this bed therefore makes a significant contribution to the regulation of systemic blood pressure. The observation that there is an enhanced responsiveness to phenylephrine in perfused MAB from 6-week, but not 2-week, ethanol-treated rats suggests that the altered responsiveness to phenylephrine in the MAB is a consequence of the increased blood pressure associated with ethanol intake. The enhanced responsiveness to phenylephrine in MAB from ethanol-treated rats was not observed after endothelium denudation, further indicating a role for the endothelium in the increased sensitivity of α-adrenoceptors after ethanol treatment. Moreover, the finding that acetylcholine-induced vasodilatation of the MAB, which is mainly mediated by nitric oxide (NO) in this tissue, was reduced in vessels from ethanol-treated rats suggests that endothelial cell receptor-stimulated NO production/release and/or NO action are influenced by chronic ethanol consumption.

Some reports suggest that the period of exposure to ethanol is a major factor in the development of cardiovascular abnormalities (Abdel-Rahman & Wooles, 1987; Strickland & Wooles, 1988). Results presented here demonstrate that chronic ethanol consumption produced an enhanced responsiveness to phenylephrine and a decreased responsiveness to acetylcholine in the MAB, providing evidence of a relationship between the period of treatment and the enhancement of phenylephrine-induced contraction. It is important to note that blood pressure was already increased after 2 weeks' ethanol treatment, whereas increased responsiveness to phenylephrine was observed only in 6-week-treated rats. As mentioned above, increased response to phenylephrine was apparent in isolated aortas from 2-week ethanol-treated rats. Reasons for these differences are not clear, although they could be explained by the different role of conduit and peripheral arteries in the control of blood flow. This observation supports the notion that ethanol consumption differentially affects the reactivity of blood vessels to the same agonist, a fact that seems to be dependent on the period of exposure to ethanol.

The isocaloric-treatment group was included in the present study to evaluate whether alteration in calorie intake associated with ethanol consumption might explain the possible influences of ethanol on cardiovascular responses. Also, vascular responsiveness to vasorelaxant agents has been reported to be caused by elevated glucose (Tesfamariam et al 1991). Sucrose feeding did not affect arterial blood pressure and vascular reactivity, suggesting that the calorie content of the ethanol diet did not play a significant role in the present findings. Furthermore, blood glucose concentrations did not differ between the groups. Sucrose was used to compensate for calories provided by ethanol and not for total calorie intake. However, it is clear from our data that the animals in the ethanol group were of lower body weight, a fact that is possibly associated with the decreased consumption of food. Together, those observations indicate that total calorie intake may have been lower in the animals in the ethanol group. Thus, a decreased intake of essential nutrients from solid food may account for observed changes in the vascular reactivity.

Contradictory data regarding the effects of ethanol consumption on vascular reactivity and blood pressure have been published. The most important contributing factors indicated in the literature for these discrepancies are: different experimental designs for ethanol feeding, different protocols of ethanol administration, duration of chronic ethanol treatment and the type of blood vessel studied (Sahna et al 2000; Utkan et al 2001). The main contribution of the present investigation is that it uses the same protocol for ethanol feeding to evaluate the blood pressure and compare the responsiveness of three blood vessel types.

Conclusion

The major conclusion to be drawn from this study is that ethanol affects both blood pressure and vessel reactivity, but the effect on vascular reactivity may take longer to become apparent in the MAB than in the aorta, and was not evident in the carotid. Moreover, considering previous observations from our group (Tirapelli et al 2006a, b), we can conclude that the effect of ethanol depends on the agonist studied. Furthermore, ethanol consumption affects different vascular tissues in different ways. The reasons for this observation remain unclear, but it could be related to differences in function, localization or the type of vasomodulatory substances produced by these vessels. Finally, the period of exposure to ethanol is another factor that determines the altered responsiveness of blood vessels, as observed for the MAB, where the increased vascular reactivity seems to be a consequence of the increased blood pressure.

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