PHYSICAL TRAINING AMELIORATES CHRONIC ALCOHOL-INDUCED HYPERTENSION AND AORTIC REACTIVITY IN RATS

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(Received 4 October 2005; first review notified 3 January 2006; accepted in revised form 12 January 2006; advance access publication 8 February 2006)

Abstract — Aims: The aim of this study was to investigate the interactive effect of physical training and chronic ethanol ingestion on changes in blood pressure (BP) and aortic reactivity response in rats. Methods: Male Fisher rats were divided into four groups of seven animals each and treated as follows: (i) control (5% sucrose, orally) daily for 12 weeks; (ii) ethanol (4 g kg⁻¹, orally) daily for 12 weeks; (iii) exercise training on treadmill followed by sucrose daily for 12 weeks; (iv) exercise training on treadmill followed by ethanol daily for 12 weeks. The body weight and BP were recorded every week. The animals were anaesthetized with pentobarbital after 12 weeks; blood and thoracic aorta were isolated and analysed for ethanol and reactivity response using tissue bath technique, respectively. Results: The data show that exercise training significantly lowered the weight gain 6–12 weeks in ethanol-treated rats compared to ethanol alone or control rats. The systolic and mean BP significantly elevated 6–12 weeks, whereas diastolic BP elevated 8–12 weeks after ethanol ingestion. Exercise training lowered the BP close to the normal control values in ethanol fed rats. Blood ethanol level significantly elevated in ethanol group but decreased in exercise plus alcohol group. Aortic contractile response to phenylephrine in ethanol or control groups was attenuated by training with or without intact endothelium. Ethanol significantly reduced the aortic relaxation response to acetylcholine whereas training enhanced the relaxation response with intact endothelium. The relaxation responses to adenosine and sodium nitroprusside in the aortic ring segments of rats with or without endothelium were decreased in ethanol group which were attenuated by exercise training. Conclusions: Physical training attenuates the chronic ethanol-induced hypertension via reduction of body weight, clearance of ethanol, and augmentation of the aortic endothelial relaxation response in rats.

INTRODUCTION

Alcoholic beverages (ethanol) are consumed by most of the human societies in the world. About two-thirds of adult American populations consume ethanol (USDHHS, 1990). Many epidemiological evidences show that chronic high dose ethanol consumption is associated with hypertension (Kaplan, 1995; Estruch et al., 2005). We have recently shown that both the dose as well as the duration of ethanol consumption is important in elevating the blood pressure in a rat model (Husain et al., 2004; Husain et al., 2005). However, the mechanisms and the role of possible mediators causing ethanol-induced rise in blood pressure (BP) are obscure. The endogenous vasoconstrictor/vasodilators as well as oxidant/antioxidant balance in the endothelium have an important role in protecting the heart and aorta thus allowing normal contractile function and BP. There are contradictory reports about the influence of chronic alcohol consumption on vascular responsiveness in the presence and absence of endothelium to vasoactivators (Brown et al., 2002; Sahna et al., 2000; Williams et al., 1990). These conflicting reports are related to differences in dose, duration, and mode of ethanol exposure at different experimental conditions. Hence, this study is based on established exact and controlled oral hypertensive dose and duration of ethanol in a rat model.

Population throughout the world exercises daily to maintain good cardiovascular health. Many individuals participate in organized group exercise rehabilitation programmes or pursue individual exercise and also consume alcoholic beverages. This raises the question what would be the interactive effect of chronic ethanol ingestion and exercise training on BP and vascular reactivity response in rats? Exercise increases the utilization of oxygen in the body and up-regulates the antioxidant defence system in the cardiovascular system (Husain, 2004; Somani et al., 1995; Somani and Husain, 1996). Exercise training also generates nitric oxide (NO) in the cardiovascular system by induction of NO synthase and vascular endothelial growth factor (Husain, 2004; Sessa et al., 1993; Wang et al., 1993). Recent studies have shown the beneficial role of physical training in the control of BP in humans (McCarthy et al., 2003; Tsai et al., 2004) and experimental animals (Graham and Rush, 2004; Husain, 2002). However, the interactive effect of physical conditioning and chronic alcohol ingestion on alterations in BP and associated changes in vascular reactivity responses in rats has not been studied. This study tested the hypothesis that interaction of exercise training and chronic ethanol treatment would maintain/ regulate the BP through the balance of vascular reactivity with vasoconstrictor and vasodilators in rats. Therefore, this study investigated the interaction of exercise training and chronic ethanol treatment on alterations in blood ethanol concentration, body weight, and BP as well as aortic reactivity with and without endothelium in response to phenylephrine, acetylcholine, adenosine, and sodium nitroprusside (SNP) in rats.

MATERIALS AND METHODS

Chemicals

Chemicals such as phenylephrine, acetylcholine, SNP, adenosine, absolute alcohol and other chemicals were obtained from Sigma Chemical Company, and Fisher Scientific Company MO. Alcohol assay kit was purchased from OraSure Technologies Inc PA.
Animals

Male Fisher rats (2 months old, 200–250 g) were obtained from Charles River (Wilmington, MA) and kept in the school’s animal facility for 1 week for quarantine. The animals were divided into four groups of seven animals each and treated as follows: (i) control (5% sucrose 10 ml kg⁻¹, orally through orogastric tube daily in the morning for 12 weeks; (ii) ethanol (20%, v/v) at a dose of 4 g kg⁻¹, orally through orogastric tube daily in the morning for 12 weeks; (iii) exercise training on treadmill as indicated in the published protocol (Husain, 2004; Husain, 2003; Husain and Hazelrigg, 2002) shown in Table 1 followed by sucrose feeding daily in the morning for 12 weeks; (iv) exercise training on treadmill followed by ethanol feeding daily in the morning for 12 weeks. The control animals were given 5% sucrose for equivalent calorific intake as ethanol fed rats get extra calorific value. The animals were monitored for body weight changes throughout the study. The BP (systolic, diastolic, and mean) was measured through tail-cuff method weekly using non-invasive BP monitor model NIBP-8 (Columbus Instruments, OH). After 12 weeks post-treatment, the animals from each group were anaesthetized in the morning with phenobarbital (40 mg/kg, i.p.) to avoid the alteration in circadian rhythm, thorax was opened, and the blood was collected from the heart in heparinized capped vials for blood ethanol analysis. The care and use of the animals reported in this study were approved by Ponce School of Medicine’s Institutional Laboratory Animal Care and Use Committee and as per the guidelines of the National Institute of Health (NIH).

Determination of blood alcohol levels

In all groups of rats, blood alcohol was estimated using commercial kit QED A150 from OraSure Technologies, Inc. PA.

Analysis of aortic reactivity using tissue bath

Animals were anaesthetized with phenobarbital (40 mg/kg, i.p.). After anaesthesia, thorax was opened, and the descending thoracic aorta isolated carefully and cleaned of surrounding tissue. The aortic ring segments (2–3 mm) was mounted horizontally on stainless steel wire hooks in isolated organ bath containing 10 ml of Krebs buffer at 37°C (Myobath-2, WPI, Sarasota, FL). The steel wire was connected to a force displacement transducer for isometric recording of changes in force. The signals were recorded and analysed by Biopack Systems Inc. (Santa Barbara, CA). The composition of the Krebs solution is (mM): NaCl, 96.87; KCl, 5.16; MgSO₄, 1.22; NaHCO₃, 25.56; CaCl₂, 1.33; EDTA, 0.34; and dextrose, 1.01. The Krebs bicarbonate solution was equilibrated with 95% O₂ and 5% CO₂. In some of the segments the endothelium was mechanically removed by gently rubbing the intimal surface with teeth of the forceps. The successful removal of the endothelium was assessed by performing successive dose–response relaxation curves to acetylcholine. Acetylcholine failed to relax aortic rings pre-contracted by phenylephrine without endothelium, while acetylcholine relaxed the aortic rings with intact endothelium. The aortic segments (with and without endothelium) were allowed to equilibrate for 1 h with an initial tension of 1 g. After equilibration, aortic segments were precontracted with 5 x 10⁻⁷ M phenylephrine. In ring segments precontracted with phenylephrine, concentration–response curves to acetylcholine, adenosine and, SNP were generated.

Statistical analysis

The data were expressed as mean ± SEM. The data were analysed statistically using unpaired t-tests or one-way analysis of variance (ANOVA). ANOVA was followed by Duncan’s multiple range tests using SAS statistical software for comparison between different treatment groups. Statistical significance was set at $P < 0.05$.

RESULTS

There was no significant difference in body weight gain among control, ethanol, and exercise training plus ethanol groups 5 weeks after treatment. However, exercise training significantly reduced the weight gain in rats 6–12 weeks ($P < 0.01$) after treatment compared with control or ethanol group. The body weights of the rats in control, ethanol, exercise, and exercise plus ethanol after 12 weeks were 324 ± 12, 323 ± 10, 281 ± 11, and 295 ± 9 g, respectively.

Blood alcohol concentration significantly ($P < 0.001$) increased (20-fold) in rats treated with ethanol for 12 weeks (99.80 ± 6.10 mg %) compared with control (4.50 ± 1.50 mg %). In ethanol plus exercise training group and sucrose plus exercise training group the blood alcohol levels were 5.60 ± 1.90 and 3.90 ± 1.40 mg %, respectively. The changes in the systolic BP of rats in control, ethanol, and exercise training plus ethanol–treated groups for 12 weeks are depicted in Fig. 1. A significant increase in systolic BP was observed in rats treated with ethanol during 6–7 weeks ($P < 0.05$), during 8–9 weeks ($P < 0.01$) and during 10–12 weeks ($P < 0.001$) compared with control group. The combination of both exercise and ethanol resulted in normalization of BP close to control level indicating the beneficial role of exercise conditioning in mitigating the ethanol-induced hypertension in rats. Exercise training alone slightly decreased the systolic BP (105 ± 8 mm Hg) in rats compared with control.

The changes in the diastolic BP of rats in control, alcohol, and exercise training plus ethanol group for 12 weeks are depicted in Fig. 2. The diastolic BP was significantly increased in rats treated with ethanol for 8–9 weeks.

<table>
<thead>
<tr>
<th>Week</th>
<th>Belt speed (m/min)</th>
<th>Inclination (degrees)</th>
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Fig. 1. Effects of chronic ethanol ingestion, exercise training, and the combination of both for 12 weeks on systolic BP changes in rats. Ethanol increased systolic BP during 6–7 weeks (n = 7; *P < 0.01), during 8–9 weeks (n = 7; **P < 0.01) and during 10–12 weeks (***P < 0.001) compared with control group. Exercise training decreased the systolic BP value close to control level.

Fig. 2. Effects of chronic ethanol ingestion, exercise training, and the combination of both for 12 weeks on diastolic BP changes in rats. Ethanol significantly increased diastolic BP during 8–9 weeks (n = 7; *P < 0.01) and during 10–12 weeks (**P < 0.01) compared with control group. Exercise training brings down the diastolic BP value close to control level.

Table 2. Effects of chronic alcohol ingestion, exercise training, and the combination of both for 12 weeks on the aortic contraction (g) produced by phenylephrine in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control (n = 7)</th>
<th>Ethanol (n = 7)</th>
<th>Exercise training (n = 7)</th>
<th>Exercise + ethanol (n = 7)</th>
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<tr>
<td>Aorta with endothelium (+)</td>
<td>1.94 ± 0.12</td>
<td>1.89 ± 0.13</td>
<td>1.58 ± 0.13*</td>
<td>1.66 ± 0.11*</td>
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<tr>
<td>Aorta without endothelium (−)</td>
<td>1.92 ± 0.11</td>
<td>1.90 ± 0.12</td>
<td>1.53 ± 0.14*</td>
<td>1.61 ± 0.13*</td>
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Increase in tension (g) above an initial resting tension of 2 g in response to 5 × 10−7 M phenylephrine. Values are expressed as mean ± SEM. *Significantly different from control or ethanol group (P < 0.05).

Fig. 3. Effects of chronic ethanol ingestion, exercise training and the combination of both for 12 weeks on the endothelium-dependent relaxation produced by acetylcholine in rat aortic rings with (+) or without (−) intact endothelium. *Significantly different from aortic relaxation response with intact endothelium (+) compared with ethanol group (n = 7; P < 0.01). **Significantly different from control response (n = 7; P < 0.001) in aortic rings with intact endothelium (+). All aortic rings were sub-maximally pre-contracted with 5 × 10−7 mol l−1 phenylephrine.

The changes in the phenylephrine-induced rat aortic contraction in control, alcohol, and exercise training plus ethanol group for 12 weeks are depicted in Table 2. Phenylephrine produced almost similar aortic vasoconstriction in control and ethanol group with or without endothelium. Phenylephrine-induced vasoconstriction response was significantly (P < 0.05) decreased in exercise training plus ethanol group with and without endothelium compared with control or ethanol group. Exercise training significantly (P < 0.05) decreased phenylephrine-induced vasoconstriction of the aortic segments with or without endothelium compared with control.

The effects of chronic ethanol ingestion, exercise training, and the combination of the two on the endothelium-dependent relaxation produced by acetylcholine in rat thoracic aortic rings with and without endothelium are depicted in Fig. 3. Chronic ethanol significantly decreased the endothelium-dependent relaxation of the aorta compared with control (P < 0.001) indicating endothelial perturbation in ethanol-induced hypertensive rats. Exercise training significantly increased the relaxation of the aorta in ethanol-treated rats (P < 0.01) indicating the preservation of the endothelial...
functions by physical training. Removal of endothelium abolished the response to acetylcholine in the aorta of rats in all treatment groups.

The effects of chronic ethanol ingestion, exercise training, and the combination of the two on the endothelium-independent relaxation produced by adenosine in rat thoracic aortic rings with and without endothelium are depicted in Fig. 4. Chronic ethanol significantly decreased the adenosine-induced relaxation of the aorta compared with control at higher concentrations of the adenosine with endothelium (P < 0.05) or without endothelium (P < 0.01). Exercise training significantly increased the adenosine-induced relaxation of the aorta with or without endothelium in ethanol-treated rats (P < 0.001) indicating the beneficial role of exercise-induced adenosine generation.

The effects of chronic ethanol ingestion, exercise training, and the combination of the two on the endothelium-independent relaxation produced by SNP in rat thoracic aortic rings with and without endothelium are depicted in Fig. 5. Chronic ethanol significantly decreased the SNP-induced relaxation of the aorta compared with control with or without endothelium (P < 0.001). Exercise training significantly increased the SNP-induced relaxation of the aorta with or without endothelium in ethanol-treated rats (P < 0.01). The removal of endothelium shifted the relaxation curve for SNP to the left in control and ethanol-treated groups but to the right in exercise plus ethanol-treated group. SNP-induced aortic relaxation response was significantly more pronounced without endothelium in the control animals (P < 0.05) compared with aorta with intact endothelium. The decreasing differences among the different treatment groups at the highest concentrations of SNP on vascular relaxation are likely due to desensitization of the aorta at higher concentrations.

DISCUSSION

This study addresses the interaction of exercise training and chronic alcohol ingestion on alterations in body weight, blood ethanol level, BP and aortic reactivity response with or without endothelium in rats. The present data show that weight gain in rats after chronic ethanol dosing was similar to the control rats fed 5% sucrose orally for 12 weeks indicating an equivalent caloric intake by rats in both the groups. However, exercise training significantly reduced the weight gain in ethanol or sucrose fed rats. A significant increase in systolic, diastolic and mean BP observed in ethanol group which were normalized by physical conditioning. It is well known that physical inactivity and overweight trigger hypertension (Joshi et al., 2005; Ross et al., 2000) whereas; regular physical activity has been shown to decrease the BP and body-weight (McCarthy et al., 2003; Tsai et al., 2004; Husain, 2003; Husain, 2002). Hence, the beneficial effects of the training in the lowering of the BP are likely to be related to decrease in body weight of rats. Most of the earlier studies have shown the reduction of systolic BP by physical training, however, this study on rats and another study on human beings (Belardinelli et al., 1995) have demonstrated the reduction of diastolic BP also by exercise training. The data further show that the blood ethanol concentration increased after 12 weeks treatment compared with controls and significantly decreased by exercise training indicating enhanced metabolism and clearance of ethanol. Exercise training is known to enhance the metabolism of chronic ethanol via induction of microsomal cytochrome P450 II E1 along with cytosolic alcohol dehydrogenase (El-Sayed et al., 2005; Ardies et al., 1994; Suter et al., 1992) and also facilitates the elimination and clearance of ethanol from the body (El-Sayed et al., 2005; Husain and Somani, 1998; Ardies et al., 1989). The present
study demonstrates that physical conditioning attenuates chronic ethanol-induced hypertension through reduction of the body weight and enhanced metabolism and clearance of ethanol from the body.

The data of this study further show that chronic ethanol did not alter the aortic contraction induced by phenylephrine compared with control or without intact endothelium suggesting that chronic ethanol-induced hypertension is not related to the vascular constriction through alpha-adrenergic receptors. Our findings are also in agreement with the earlier studies demonstrating no alpha-adrenergic receptor mediated constriction of rat thoracic aorta after chronic ethanol ingestion in rats (Abdel-Rahman and Wooles, 1987; Williams et al., 1990). However, variability also exists pertaining to the effects of chronic ethanol ingestion on the vascular reactivity response to vasoconstrictors. Certain studies reported enhanced alpha-adrenergic-induced contraction (Altura and Altura, 1987; Pinardi et al., 1992) whereas others demonstrated diminished contraction after chronic ethanol ingestion (Sahna et al., 2000; Strickland and Wooles, 1988). On the other hand, exercise training significantly decreased the aortic contraction induced by phenylephrine compared with ethanol or control group with or without intact endothelium indicating the desensitization to contractile effects of phenylephrine. Physical training has been shown to decrease the adrenergic activity (Chandler and DiCarlo, 1997) and depletes the sarcoplasmic reticulum Ca$^{2+}$ levels (Stehno-Bittel et al., 1991) thus preventing the contraction of the aortic smooth muscle. These data suggest that the exercise training abrogated the hypertensive response of chronic ethanol ingestion by reducing the vascular contractility response in rats.

On the other hand, the endothelium-dependent relaxation elicited by acetylcholine is known to be mediated by NO (Furchgott and Zawadski, 1980) which plays a pivotal role in chronic alcohol-induced hypertension (Husain et al., 2005; Husain et al., 2004; Pinardi et al., 1992). This finding is supported by the fact that acetylcholine was unable to cause relaxation of the aorta without intact endothelium in control and ethanol-treated groups. Endothelial release of NO activates guanylate cyclase in smooth muscle cells, causing increased cyclic GMP leading to vasorelaxation (Ignarro and Kadowitz, 1985). Other clinical and experimental studies have also shown that chronic ethanol consumption either interferes with NO production or release of NO from endothelial cells (Pinardi et al., 1992; Slomiany et al., 1998; Puddey et al., 2001). The diminished endothelial NO availability may either be related to reaction with superoxide anion to form peroxynitrite radicals (Beckman et al., 1990) or oxidative inactivation/uncoupling of endothelial NO synthase by ethanol-induced free radicals (Gryglewaski et al., 1986; Peterson et al., 1999). The data further show that the endothelium-dependent relaxation of the aorta elicited by acetylcholine is significantly enhanced by exercise training which was reduced by chronic ethanol treatment. This effect of the training is related to increased acetylcholine levels (Dornay et al., 2000) through NO action in the vascular system (Graham and Rush, 2004; Husain, 2004; Husain, 2002; Wang et al., 1993). These data suggest that exercise training ameliorated alcohol-induced hypertension by enhancing the endothelium-dependent vascular relaxation response in rats.

Adenosine, an endogenous metabolite of ATP, is known to cause vasodilation through activation of adenosine receptors type A$_2$ (Leung et al., 1985) and provide endothelial protection by the activation of antioxidant enzymes through A$_3$ receptors (Maggirwar et al., 1994). Our findings of reduced relaxation of the aorta elicited by adenosine with and without intact endothelium by chronic ethanol treatment compared with control suggest that the endothelium-dependent as well as independent component of adenosine are down-regulated by the chronic ethanol treatment. Other possibilities for the reduced relaxation by adenosine could include either decreased number or sensitivity of the adenosine receptors due to perturbation of membranes (Peoples et al., 1996) and an enhanced oxidative stress (Husain et al., 2005; Husain et al., 2004; Husain and Somani, 1997; Vendimiale et al., 2001) by chronic ethanol ingestion. Exercise training enhanced relaxation of the aorta elicited by adenosine with and without intact endothelium compared with ethanol-treated group suggests that the endothelium-dependent as well as independent component of adenosine are up-regulated by the training. Other possibilities for the enhanced relaxation by adenosine could include either increased adenosine due to ATP breakdown (Watkinson et al., 1979), number or sensitivity of the adenosine receptors (Maggirwar et al., 1994) or the up-regulation of endothelial antioxidant system (Husain and Somani, 1997; Somani and Husain, 1998) by exercise training.

Results further revealed that the relaxant responses to nitrovasodilators such as SNP decreased in thoracic aorta of chronic ethanol-treated rats in the presence and absence of the endothelium compared with control. Present findings are in agreement with another report (Williams et al., 1990). These reports demonstrated that the removal of the endothelium shifted the relaxation curve to the left in both the ethanol-treated and control groups, suggesting that the endothelium can modulate the relaxant response of agents which were previously reported to act independent of endothelium (Rapoport and Murad, 1983). However, the relaxant responses to SNP increased in thoracic aorta of exercise trained plus chronic ethanol-treated rats in the presence of the endothelium compared with ethanol group but decreased in the absence of endothelium. These data suggested that the endothelium can modulate the relaxant response of nitrovasodilators in the aorta of exercise trained rats treated with chronic ethanol. These data are also supported by our earlier findings that exercise training modulates the chronic vascular effects of nitroglycerin in rats (Husain, 2003). Overall, these data suggest that chronic ethanol ingestion suppresses the vascular relaxation response with or without endothelium leading to hypertension whereas exercise training enhances the endothelium-dependent and independent vascular relaxation leading to lowering of BP in rats.

In summary, chronic ethanol ingestion caused hypertension in rats by reducing the endothelium-dependent vascular relaxation response and physical conditioning ameliorated the ethanol-induced hypertension by lowering the body weight and blood ethanol level and by enhancing the aortic relaxation response with or without endothelium.

Acknowledgement—This work was supported in part by The RCMI NIH grant no. 2G12RR03050-19.
REFERENCES


