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VASCULAR ENDOTHELIAL OXIDATIVE STRESS IN ALCOHOL-INDUCED HYPERTENSION

KAZIM HUSAIN

Department of Physiology, Pharmacology and Toxicology,
Ponce School of Medicine, Ponce, PR 00732, USA
Phone: 787-840-2575; Fax: 787-259-7085; Email: khusain@psm.edu

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Abstract- Epidemiological studies in humans and experimental studies in animals have shown the link between chronic alcohol consumption and the prevalence of hypertension. However, molecular mechanisms implicated with alcohol-induced increases in blood pressure (BP) remain elusive. The objective of this study was to investigate the relationship between BP and molecular as well as physiological changes in aortic endothelium in chronic ethanol treated rats. Male Fisher rats were given 20% ethanol (4 g/kg) orally and controls received 5% sucrose daily for 12 weeks. The BP was recorded weekly by tail-cuff method and after 12 weeks, rats were anesthetized with pentobarbital, thoracic aorta isolated and used for aortic reactivity using tissue bath and for biochemical analysis. The data show that ethanol ingestion significantly increased systolic, diastolic and mean BP after 12 weeks compared to control. The endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) expressions were down-regulated leading to depletion of aortic NO levels in ethanol treated rats compared to control. The aortic NADPH oxidase activity significantly enhanced with a concomitant increase in membrane lipid peroxidation and depressed ratio of reduced to oxidized glutathione in alcohol-treated rats compared to control. The aortic vasoconstriction was slightly enhanced in response to phenylephrine but vasorelaxation was significantly diminished in response to acetylcholine, adenosine and sodium nitroprusside in chronic ethanol treated rats. It is concluded that chronic ethanol ingestion induces aortic endothelial oxidative injury and the down regulation of nitric oxide generating system leading to impaired vasorelaxation and hypertension in rats.

Key Words: Alcohol; Aorta; Blood pressure; eNOS; Glutathione, Oxidative injury; Rat; VEGF

INTRODUCTION

Alcohol (ethanol) containing beverages are consumed by a large proportion of the population regularly or occasionally in the world. About two thirds of adult American populations consume ethanol (41). Light-to-moderate alcohol consumption (1-2 drinks/day) has been reported to improve the cardiovascular health by reducing the risks of diseases such as coronary artery diseases, ischemic stroke, and myocardial infarction including hypertension (22). However, chronic ethanol consumption (more than 3 drinks/day) increases the incidence and prevalence of cardiovascular complications including hypertension and stroke (9, 20, 38). Our recent studies have demonstrated that both dose and duration of ethanol ingestion is implicated in the induction of hypertension in a rat model (15, 16). Ethanol is extensively metabolized to cytotoxic acetaldehyde in the liver by the enzyme alcohol dehydrogenase (30) which is further oxidized to acetate by acetaldehyde dehydrogenase/oxidase, leading to the generation of reactive oxygen species (ROS) and free radicals (30, 39). Chronic ethanol ingestion more selectively induces microsomal cytochrome P450 II E1 in tissues leading to generation of 1-hydroxy ethyl radical (39). These reactive species oxidize cellular bio-molecules such as reduced glutathione, proteins and DNA and initiate membrane lipid peroxidation leading to cardiovascular dysfunction (32). Superoxide anion production in the cardiovascular endothelium and its interaction with nitric oxide (NO) to form peroxynitrite is also implicated in hypertension (2). Clinical studies in human suggested the role of superoxide anions in hypertension (6, 21). The principal sources of endothelial superoxide production are the activation NADPH oxidase and xanthine oxidase or uncoupling of NO synthases (10). The endothelial NO generating system such as vascular endothelial growth factor (VEGF) and endothelial NO synthase (eNOS) in the

Abbreviations: BP-blood pressure; **DNA**-deoxyribonucleic acid; **eNOS**-endothelial nitric oxide synthase; **ELISA**-enzyme linked immunosorbent assay; **GSH/GSSG**-glutathione reduced/glutathione oxidized; **H_2O_2**-hydrogen peroxide; **MDA**-malondialdehyde; **mRNA**-messenger ribonucleic acid; **NO**-nitric oxide; **O_2**-oxygen; **SDS-PAGE**-sodium dodecyl sulfate-poly acrylamide gel electrophoresis; **SOD**-superoxide dismutase; **RT-PCR**-reverse transcriptase polymerase chain reaction; **VEGF**- vascular endothelial growth factor

cardiovascular system plays an important role in maintaining the normal cardiovascular function including blood pressure. However, the molecular mechanisms and the role of possible endogenous mediators causing ethanol-induced rise in blood pressure are obscured. The endogenous vasoconstrictor/vasodilators as well as oxidant/antioxidant balance in the endothelium have pivotal role in protecting the vascular tissues thus allowing normal contractile function and blood pressure. We tested the hypothesis that chronic alcohol ingestion causes oxidative stress and the down-regulation of antioxidant/NO generating systems in the aortic endothelium leading to impaired vasorelaxation and hypertension in rats. Therefore, the objective of this study was to investigate the chronic ethanol-induced changes in BP (systolic, diastolic and mean), vascular reactivity responses in response to phenylephrine, acetylcholine, adenosine, and sodium nitroprusside and molecular alterations in the aorta of the rat.

MATERIALS AND METHODS

Chemicals

Chemicals such as acetylcholine, adenosine, phenylephrine, sodium nitroprusside, absolute alcohol, peroxidase conjugated secondary antibody and other chemicals were obtained either from Sigma Chemical Company or Fisher Scientific Company MO, USA. Nitric oxide assay kit was purchased from Cayman Chemical Co. MI, USA. Monoclonal antibodies for eNOS and VEGF were purchased from Upstate Biotechnology, NY, and R & D Systems, Minneapolis, MN, USA. RNA isolation kit, VEGF/β-actin primers and RT-PCR kit were obtained from Invitrogen Corporation, Carlsbad, CA, IDT, Coralville, IA, Qiagen Inc., Valencia CA, USA, respectively.

Animals

Male Fisher rats (8 weeks old, 200-250 g) were obtained from Charles River (Wilmington, MA, USA) and kept in the school's animal facility for one week for quarantine. Rats were provided food and water *ad libitum* and maintained on a 12:12 h light dark photoperiod at room temperature of 25^{o}C. They were randomly divided into two groups and treated as follows:

Group I [Control]: Rats were administered 5% sucrose [10 ml/kg, orally] daily for 12 weeks through orogastric tube in the morning to avoid circadian cycle effects and for equivalent calorific intake (n=7).

Group II [Ethanol]: Rats were given 20% ethanol (4 g/kg, orally) daily for 12 weeks through orogastric tube in the morning (n=7).

The animals were monitored for changes in systolic, diastolic and mean BP and recorded every week using tail-cuff method with NIBP-8 monitor (Columbus Instruments, OH, USA). The animals were sacrificed by decapitation under ether anesthesia 24 h after the last treatments. After 12 weeks post-treatment, the animals from each group were anesthetized in the morning with Phenobarbital (40 mg/kg, i. p.) to avoid the alteration in circadian rhythm, thorax was opened, aorta carefully dissected and rings were used for tissue bath experiments. The remaining parts of the aorta were immediately immersed in liquid nitrogen and stored at – 80 ^{o}C until analysis could be completed. 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 (IACUC) and as per the guidelines of the National Institute of Health (NIH).

Analysis of aortic reactivity using tissue bath

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 ^{o}C (Myobath-2, WPI, Sarasota, FL, USA) as described earlier (14). The steel wire was connected to a force displacement transducer for isometric recording of changes in force. The signals was recorded and analyzed by Biopack Systems Inc. (Santa Barbra, CA, USA). The composition of the Krebs solution was (mM): NaCl, 96.87; KCl, 5.16; $MgSO_4$, 1.22; $NaHCO_3$, 25.56; $CaCl_2$, 1.33; ethylenediaminetetraacetic acid (EDTA), 0.34; and dextrose, 1.01. The Krebs bicarbonate solution was equilibrated with 95% O_2 and 5% CO_2. The aortic segments were allowed to equilibrate for 1 hr with an initial tension of 1 g. After equilibration, aortic segments were pre-contracted with 5 X 10^{-7} M phenylephrine. In ring segments pre contracted with phenylephrine, concentration-response curves to acetylcholine, adenosine and sodium nitroprusside were generated.

Determination of vascular endothelial growth factor (VEGF) gene expression

Total RNA from frozen aortic tissues was isolated using guanidium thiocyanate-phenol-chloroform extraction with the TRIZOL reagent as per the manufacturer's instructions (Invitrogen).

Quantification of VEGF mRNA by RT- PCR

The measurement of VEGF-A transcripts was carried using RT- PCR technique. A 2 µg sample of total RNA was denatured by incubating at 70 ^{0}C for 5 min and then the tubes were placed on ice for 3 min. The denatured RNA (5 µl) was incubated for 60 min at 37 ^{0}C and for 5 min at 95 ^{0}C with 4 µl of 5 x reverse transcriptase buffer, 1 µl of oligo $(dT)_{15}$, 1 µl Rnasin (50 U/µl), 1 µl dNTPs (10 mmol/L), 1 µl reverse transcriptase (200 U/µl), and 7 µl of deionized water in a total volume of 20 µl. For polymerase chain reaction (PCR), 5 µl of the resulting cDNA, 31 µl of triple distilled water, 5 µl of 5 x PCR buffer, 3 µl $MgCl_2$ (25 mmol/L), 1 µl dNTPs, 1 µl each of sense and antisense primers (10 pmol/L) of VEGF and β-actin, and 1 µl Taq DNA polymerase (3U/µl) in a total volume of 50 µl was added. The samples were amplified through 35 cycles, each amplification consisting of denaturation at 90 ^{0}C for 40 s, primer annealing temperature from 60 ^{o}C to 50 ^{0}C decreased by 0.5 ^{0}C per cycle for 20 cycles, followed by an additional 15 cycles at an annealing temperature of 50 ^{0}C for 35 s. PCR products were analyzed on 2% agarose gel containing ethidium bromide and quantified by a complete gel documentation and analysis system. The mRNA expression levels were determined using ratio to β-actin as a standard. The sequences of primers for VEGF was sense

5'-TTTACTGCTGTACCTCCACCAT-3'

and antisense

5'-ATCTCTCCTATGTGCTGGCTTT-3'

(318 bp) and for β-actin, sense

5'-AAGTCCCTCACCCTCCCAAAAG-3' and antisense 5'-AAGCAATGCTGTCACCTTCCC-3' (100 bp) from IDT Inc. IA.

Vascular Endothelial Growth Factor (VEGF) Protein Expression Assay

The VEGF protein expression was analyzed using Western immunoblotting technique. Briefly, 10 µg of ventricular proteins was applied on electrophoresis using a 12% denaturing SDS-PAGE for 2 hrs. After electrophoresis, the protein on the gel was electrophoretically transferred to nitrocellulose membrane (Bio-Rad) overnight at 4^0C. The membrane was incubated in 5% nonfat dry milk in TBST buffer (10 mmol/L Tris-HCl [pH 7.2], 0.15 mol/L NaCl and 0.05% Tween-20) followed by incubation with specific monoclonal antibodies for VEGF-A (R & D Systems, MN) at 1:1000 dilutions. After rinsing with TBST buffer, the blot was incubated with HRP-conjugated secondary antibody (Sigma Chem. Co., MO) and developed with the use of enhanced chemiluminescence's system (ECL kit, Amersham). After washing with TBST buffer, the blot was exposed to Kodak films in x-ray cassettes with intensifying screens. The protein bands (45kDa for VEGF-A) were quantified using an image scanning densitometer (Epi Chemi II, VP Bioimaging Systems, Upland, CA, USA).

Endothelial (eNOS) Protein Expression Assay

The enzyme protein contents of eNOS were analyzed using ELISA technique as described earlier (12, 13).

Nitric Oxide Assay

Nitric oxide level in the tissue was determined by NO assay kit method (Cayman Chemical Co. MI, USA), as described in our previous publications (12-16).

NADPH Oxidase Assay

The enzyme activity was assayed based on superoxide-induced lucigenin photoemission as described by Cui and Douglas (4). Enzyme assay was carried out in a final volume of 1 ml containing 50 mM phosphate buffer pH 7.0, 1mM EGTA, 150 mM sucrose, 0.5 mM lucigenin, 0.1 mM NADPH and 0.5 mg protein of tissue homogenate. Enzyme reaction was initiated with the addition of lucigenin. Photoemission was measured for 3 minutes using luminometer.

Lipid peroxidation assay

This assay was used to determine malondialdehyde (MDA) levels as described by Ohkawa et al. (33).

Determination of glutathione (GSH) and its disulphide (GSSG)

Reduced Glutathione (GSH) and oxidized glutathione (GSSG) were determined as described earlier (12-16) using Glutathione Assay kit purchased from Cayman Chemical Company MI, USA.

Protein Assay

Protein concentration in the tissue was estimated according to the method of Read and Northcole (37) using Coomassie protein assay dye and bovine serum albumin as a standard.

Statistical Analysis

The data were expressed as mean ±S.E.M. The data for biochemical and physiological parameters were analyzed statistically using two-way analysis of variance (ANOVA) followed by Duncan's multiple range tests using the SAS statistical software package (SAS Institute, Cary, NC, USA) for comparison of treated groups with control group. The 0.05 level of probability was used as the criterion for statistical significance.

RESULTS

Effect of ethanol on blood pressure

Chronic ethanol administration significantly ($p<0.001$) increased systolic BP (172 ± 12.50 mm of Hg) compared to control (121 ± 10.45 mm of Hg) after 12 weeks. The diastolic BP was significantly ($p<0.001$) increased (114 ± 7.25 mm of Hg) in ethanol-treated rats compared to control (80 ± 9.30 mm of Hg) after 12 weeks. The mean BP was significantly ($p<0.001$) increased (145 ± 7.20 mm of Hg) in ethanol-treated rats compared to control (94 ± 6.10 mm of Hg) after 12 weeks.

Effect of ethanol on oxidative stress indices

The changes in lipid peroxidation end product (MDA concentration), reduced to oxidized glutathione ratio (GSH/GSSG) and NADPH oxidase activity in the aorta of control and alcohol treated rats are depicted in Table 1.

Table 1. Effect of chronic ethanol ingestion on aortic malondialdehyde (MDA) level, reduced to oxidized glutathione ratio (GSH/GSSG) and NADPH oxidase activity in rats.

Groups	MDA	GSH/GSSG	NADPH Oxidase
1. Control (n=7)	2.13± 0.28	3.98±0.32	0.44±0.12
2. Ethanol (n=7)	4.57±0.42*	2.61±0.19*	1.66±0.25**

Rats were given 20% ethanol at a dose of 4 g/kg, orally daily for 12 weeks. Control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks. All the values are expressed as mean ± S.E. MDA concentration is expressed as n moles/mg protein; NADPH oxidase activity as change in photoemission units/min/mg protein. *$p < 0.01$ compared to group 1; **$p < 0.001$ compared to group 1

The MDA concentration in the aorta significantly increased 215% of control ($p<0.01$) in response to alcohol after 12 weeks indicating oxidative injury to the aortic tissue. Reduced to oxidized glutathione ratio (GSH/GSSG), an indicator of oxidative stress, in the aorta showed a significant decrease 65% of control ($p<0.01$) in response to alcohol ingestion for 12 weeks. Aortic NDAPH oxidase activity significantly increased 377% of control ($p<0.001$) in response to alcohol after 12 weeks indicating enhanced superoxide anion generation in the aorta of rats.

Effect of ethanol on aortic nitric oxide system

The effect of chronic ethanol administration on aortic NO levels and eNOS protein expression is depicted in Table 2. Chronic ethanol treatment significantly depleted aortic NO levels (48% of control, $p<0.001$) after 12 weeks indicating the decreased NO bioavailability in the aortic endothelium of the rats. Chronic ethanol treatment significantly decreased aortic eNOS protein levels (67% of control, $p<0.01$) after 12 weeks indicating the NO generation through eNOS is impaired in the aortic endothelium of the rats.

Table 2. Effect of chronic ethanol ingestion on aortic nitric oxide (NO) and endothelial nitric oxide synthase (eNOS) protein levels in rats.

Groups	NO	eNOS
1. Control (n=7)	27.93± 1.74	3.88±0.32
2. Ethanol (n=7)	13.55±1.45**	2.61±0.29*

Rats were given 20% ethanol at a dose of 4 g/kg, orally daily for 12 weeks. Control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks. All the values are expressed as mean ± S.E. NO concentration is expressed as µmol/mg protein and eNOS protein level is expressed as µg/mg protein.
*$p < 0.01$ compared to group 1; **$p < 0.001$ compared to group 1.

The effect of chronic ethanol administration on aortic VEGF gene expression is depicted in Figure 1. Chronic ethanol treatment depleted aortic VEGF mRNA levels (50% of control) after 12 weeks indicating the down-regulation of aortic VEGF gene expression by chronic ethanol ingestion.

The effect of chronic ethanol administration on aortic VEGF protein expression is depicted in Figure 2. Chronic ethanol treatment significantly decreased aortic VEGF protein levels (60% of control, $p<0.01$) after 12 weeks indicating the down-regulation of protein expression in the aortic endothelium of rats.

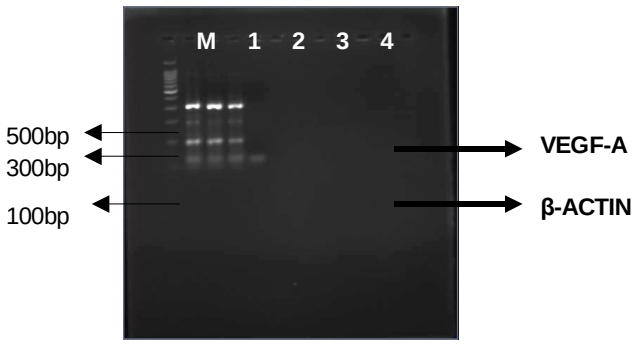

Figure 1. RT-PCR analysis demonstrated decreased VEGF-A mRNA expression in the aorta of rats after chronic ethanol ingestion as compared to control (5% sucrose).
The RT-PCR products were subjected to electrophoresis on 2% agarose gel. Lane 1: Control (5% sucrose); Lane 2: control (5% sucrose); Lane 3: Ethanol (4g/kg) for 12 weeks; Lane 4: Negative control (without cDNA template of the tissue samples). VEGF mRNA levels in the aorta of chronic alcohol treated rats depleted compared to control.

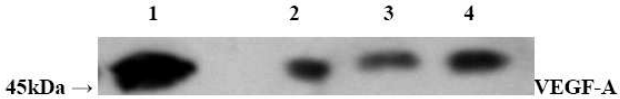

Figure 2. Effect of chronic ethanol ingestion (4 g/kg, orally) daily for 12 weeks on aortic vascular endothelial factor (VEGF) protein expression in rats. The control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks.
Western blot analysis demonstrated decreased VEGF protein expression after chronic ethanol ingestion as compared to control. Ten micrograms of aortic protein were loaded per lane and Electrophoresis was performed using 12% denaturing SDS-PAGE. Lane 1: Control (5% sucrose); Lane 2: Ethanol (4g/kg) for 12 weeks; Lane 3: Ethanol (4g/kg) for 12 weeks; Lane 4: Ethanol (4g/kg) for 12 weeks.

Effect of ethanol on aortic reactivity response

The changes in the phenylephrine-induced aortic contraction in control and alcohol treated rats for 12 weeks are depicted in Figure 3. Phenylephrine produced a slight but not a significant increase in aortic vasoconstriction in ethanol group compared to control.

The effects of chronic ethanol ingestion on the endothelium-dependent relaxation produced by acetylcholine in rat thoracic aortic rings are depicted in Figure 4. Chronic ethanol significantly decreased the endothelium-dependent relaxation of the aorta compared to control ($p<0.01$) indicating endothelial perturbation in ethanol-induced hypertensive rats.

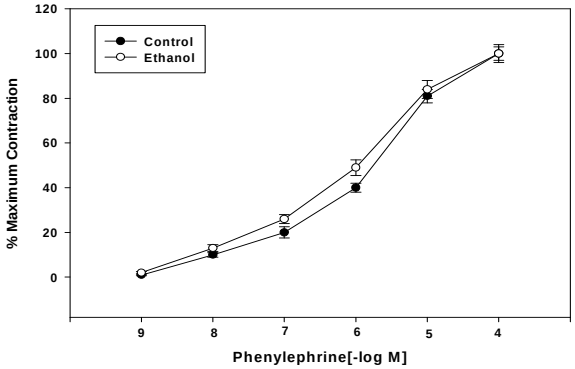

Figure 3. Effects of chronic alcohol ingestion for 12 weeks on the aortic contraction (g) produced by phenylephrine

in rats. The control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks.
Increase in tension (g) above an initial resting tension of 2 g in response to 5 x 10^{-7} M phenylephrine. Chronic ethanol ingestion slightly increased aortic contraction response elicited by phenylephrine in rats as compared to control.

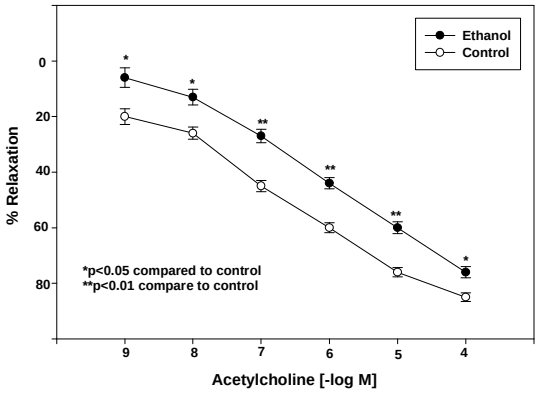

Figure 4. Effect of chronic ethanol ingestion (4 g/kg, orally) daily for 12 weeks on aortic endothelium-dependent relaxation by acetylcholine (ACh) in rats. The control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks.
Chronic ethanol ingestion significantly decreased aortic endothelium-dependent relaxation response elicited by acetylcholine in rats as compared to control (n = 7; *p<0.05 and **p<0.01).

The effects of chronic ethanol ingestion on the endothelium-independent relaxation produced by adenosine in rat thoracic aortic rings are depicted in Figure 5. Chronic ethanol significantly decreased the adenosine-induced relaxation of the aorta compared to control at higher concentrations of the adenosine (p<0.01).

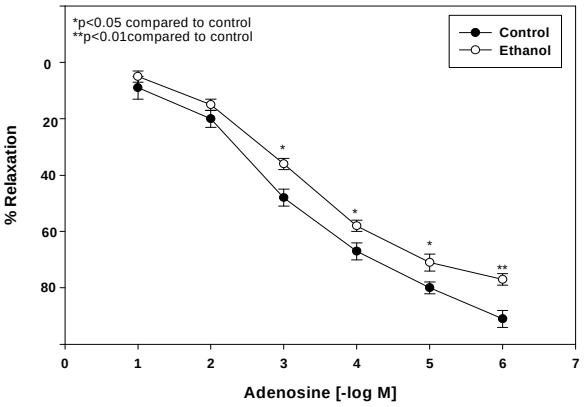

Figure 5. Effect of chronic ethanol ingestion (4 g/kg, orally) daily for 12 weeks on aortic endothelium-independent relaxation by adenosine in rats. The control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks.
Chronic ethanol ingestion significantly decreased aortic endothelium-independent relaxation response elicited by adenosine in rats as compared to control (n = 7; *p<0.05 and **p<0.01).

The effects of chronic ethanol ingestion on the endothelium-independent relaxation produced by sodium nitroprusside (SNP) in rat thoracic aortic rings are depicted in Figure 6. Chronic ethanol significantly decreased the SNP-induced relaxation of the aorta compared to control (p<0.05).

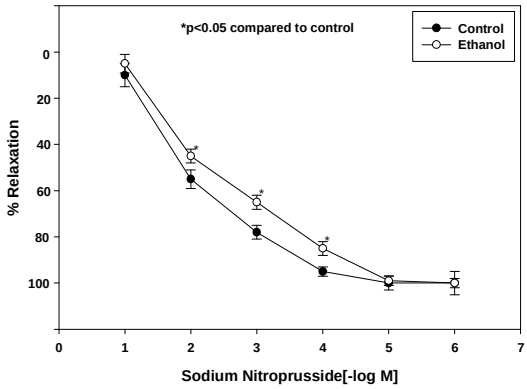

Figure 6. Effect of chronic ethanol ingestion (4 g/kg, orally) daily for 12 weeks on aortic endothelium-independent relaxation by sodium nitroprusside (SNP) in rats. The control animals received 5% sucrose (10 ml/kg, orally) daily for 12 weeks.
Chronic ethanol ingestion significantly decreased aortic endothelium-independent relaxation response elicited by sodium nitroprusside in rats as compared to control (n = 7; *p<0.05).

DISCUSSION

This study addresses the chronic ethanol-induced changes in blood pressure and its relationship with aortic endothelial oxidative stress, nitric oxide generating system and reactivity response in rats. The data indicate that administration of chronic dose of ethanol 4 g/kg for 12 weeks profoundly increased systolic, diastolic and mean BP in rats. The increase in BP is related to the depletion of aortic NO levels indicating the endothelial dysfunction in chronic ethanol-induced hypertension. NO is synthesized in the vascular endothelium by endothelial NO synthase (eNOS) enzyme (29). NO has been reported to activate guanylate cyclase and the consequent increase in cGMP levels induces a sequence of protein phosphorylation associated with smooth muscle relaxation (31) leading to low blood pressure. The production of NO by the endothelium is critically dependent on the

function of eNOS. The activity of eNOS is specifically targeted by various regulatory factors within the caveolae (lipid spheres in the plasma membrane)/caveolins (structural proteins of caveolae membrane) system that control NO levels (5, 29). Results of the present study show a significant depression of eNOS protein expression in the aorta of alcohol treated rats. It is likely that chronic heavy alcohol ingestion alters the endothelial membrane lipids and proteins as well as cofactor tetrahydrobiopterin causing the depression of eNOS expression causing reduced NO bioavailability leading to endothelial dysfunction and hypertension. Clinical as well as experimental studies have also shown that chronic ethanol consumption either interferes with NO production or release of NO from endothelial cells (36, 40, 42). The diminished NO bioavailability may be related to either reaction with superoxide anion to form peroxynitrite radicals or uncoupling/oxidative inactivation of endothelial nitric oxide synthase (eNOS) by ethanol-induced free radicals. It is most conceivable that ethanol-induced superoxide generation in the aortic endothelium as evidenced by NADPH oxidase activation (Table 1) and depressed eNOS protein expression (Table 2) is implicated in diminishing NO bioavailability leading to hypertension.

The production of NO by eNOS is also regulated by vascular endothelial growth factor (VEGF) in the endothelium (3, 43). Recent reports suggest a crucial role of VEGF and its receptors tyrosine kinases Flk1 (VEGFR-1) and Flt1 (VEGFR-2) in the regulation of endothelial NO production (3, 43), maintenance and repair of the luminal endothelium (19, 26) as well as endothelium-dependent vascular relaxation (25). In the present study chronic ethanol ingestion significantly decreased ventricular VEGF gene as well as protein expressions hence VEGF-induced NO production in the endothelium is also impaired in rats. Interestingly, low dose alcohol has been shown to increase VEGF expression in vitro as well as in vivo (8, 11) which may be attributed to the cardiovascular protection and lowering of the BP. However, other studies have shown the down regulation of NO generating system in the cardiovascular tissues and induction of hypertension in chronic alcohol treated animals and humans (15, 16, 36, 40). A recent study demonstrated the reciprocal regulation between NO level and VEGF expression in the in vitro tumor cell lines for the purpose of regulation of angiogenesis using NO donors exogenously (23) however we did not observe this relationship in our in vivo studies. Moreover, this regulation is very complex in vivo where various physiological factors play an important role in the modulation of the vascular VEGF and NO signaling pathways.

Chronic administration of ethanol to rats resulted in a significant decrease in the endogenous aortic GSH/GSSG ratio in rats. The down-regulation of GSH/GSSG ratio, an indicator of oxidative stress (46), can lead to impairment of the cellular defense against reactive oxygen species and may result in vascular injury and hypertension. Inhibition of glutathione synthesis by buthionine sulfoximine (BSO) has been demonstrated to increase blood pressure in rats (44). Therefore, it is suggested that maintenance of high GSH/GSSG ratio in the blood vessels is an important intracellular event for proper blood pressure regulation. Earlier studies have shown that chronic administration of ethanol to rats resulted in oxidative stress and down-regulation of the endogenous antioxidant enzymes in the cardiovascular system (17, 32, 45). The depression of GSG/GSSG ratio and activation of NADPH oxidase further enhances the influx of endogenous superoxide causing enhanced aortic membrane lipid peroxidation reaction as evidenced by enhanced MDA levels in ethanol treated hypertensive rats. Clinical studies in human suggested the role of superoxide anions in hypertension (6, 21). Additional studies demonstrated that enhanced ROS production perturbs the aortic Ca^{++} homeostasis leading to vascular contraction and hypertension (27, 48). We speculate that enhanced oxidative injury to the vascular endothelium of ethanol treated rats down regulate the NO generating system causes impaired vascular relaxation and enhanced vascular tone leading to increase in blood pressure.

The data of this study further show that chronic ethanol did not significantly alter the aortic contraction induced by phenylephrine compared to control 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 (1, 47). On the other hand, the endothelium-dependent relaxation elicited by acetylcholine is known to be mediated by nitric oxide (7) which plays a pivotal role in chronic alcohol-induced hypertension (15,

16, 35). Endothelial release of NO activates guanylate cyclase in smooth muscle cells, causing increased cyclic GMP leading to vasorelaxation (18). Other clinical and experimental studies have also shown that chronic ethanol consumption either interferes with NO production or release of NO from endothelial cells (35, 36, 40). Adenosine, an endogenous metabolite of ATP, is known to cause vasodilation through activation of adenosine receptors type A_2 (24) and provide endothelial protection by the activation of antioxidant enzymes through A_3 receptors (28). Our findings of reduced relaxation of the aorta elicited by adenosine by chronic ethanol treatment compared to 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 (34) and an enhanced oxidative stress (15-17, 45) by chronic ethanol ingestion. The data further revealed that the relaxant responses to nitrovasodilators such as SNP decreased in the aorta of chronic ethanol treated rats compared to control. Present findings are in agreement with other report (47). Taken together these data suggest that chronic ethanol ingestion suppresses the vascular relaxation response both endothelium-dependent and independent manner leading to hypertension in rats.

It is concluded that the chronic alcohol ingestion causes oxidative endothelial injury and down regulates the aortic endothelial nitric oxide generating system specifically VEGF and eNOS expression leading to diminished aortic relaxation response and hypertension in rats.

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