ORIGINAL ARTICLE

Blood pressure and vascular reactivity to endothelin-1, phenylephrine, serotonin, KCl and acetylcholine following chronic alcohol consumption in vitro

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ABSTRACT

Ethanol has been reported to cause hypertension, the mechanism of which is unknown. Therefore, the effect of chronic ethanol consumption on vascular responsiveness and blood pressure was investigated. Systolic blood pressure was recorded weekly by tail-cuff method. Aortic rings from rats fed chow ad libitum or pair-fed liquid diets containing either ethanol (7.2% v/v) or isocaloric carbohydrate for 4 weeks were placed in organ chambers for isometric tension measurement. There was a mild but significant elevation of the systolic blood pressure in the alcohol-fed rats by week 1 compared to baseline measurements and this remained higher. No significant changes in reactivity of rat isolated aortas to phenylephrine, serotonin, endothelin-1 (ET-1) and KCl were seen in chronic ethanol consumption. In addition, the sensitivity (i.e. pD_2) of alcohol-fed aortic rings to the vasoconstrictors was also unchanged compared to controls. Chronic ethanol consumption, however, increased relaxation to acetylcholine with increased pD_2 values, but did not alter relaxation to sodium nitroprusside, a cyclic guanosine monophosphate (cGMP)-dependent direct smooth muscle dilator. The results indicate that chronic ethanol consumption significantly potentiates endothelium-dependent relaxations in aortic rings, probably through interference with the production and/or the release of nitric oxide (NO) or adaptive alterations in muscarinic receptors on the endothelial cells, and that increased vascular responsiveness to several vasoconstrictors is not a mechanism responsible for the blood pressure elevation in the chronic alcohol consumption in rats.

INTRODUCTION

It is well established that endothelial cells make important contributions to cardiovascular regulation through the release of vasoconstrictor and vasodilator factors. At present, the most potent endothelium-derived vasoconstrictor identified is the peptide, ET-1, whereas the major vasodilator produced by endothelial cells is NO [1,2]. The production of ET-1 and NO by endothelial cells and the interactions between these factors is likely to be abnormal in conditions in which endothelial cell function is disordered; one such condition is hypertension [3,4].

Patients with chronic alcoholism are known to develop hypertension [5,6,7]. Although the etiology of hypertension in chronic alcohol consumption is not understood, it has been suggested that this complication is associated with an alteration in the reactivity of blood vessels to neurotransmitters and hormones. There are many conflicting reports in the rat after chronic ethanol ingestion. Reports in the literature describe increases [8,9], decreases [10,11] and no change [12,13] in

vascular reactivity to vasoactive agents as well as NO [12,14,15,16] in chronic alcohol consumption. The discrepancy between the above-mentioned results might be attributable to differences in treatment protocols, sex or the duration of alcohol consumption of the animals at the time of the various studies.

Although chronic alcohol consumption is known to produce altered reactivity of vascular smooth muscle to neurotransmitters and hormones, there have been no reports concerning changes in ET-1-mediated responses. Recently, ethanol has been reported to stimulate ET-1 and 2 release from cultured human umbilical vein endothelial cells and this may be related to ethanol-induced cardiovascular diseases such as hypertension [17]. In the present study therefore we investigated the contractile response to ET-1, serotonin, phenylephrine and KCl and the relaxant response to acetylcholine and sodium nitroprusside as well as systolic blood pressure and compared the responses of aortic rings from control and chronic alcohol-fed rats at 4 week.

METHODS

Animal procedures

Thirty-two adult male Wistar rats weighing 200–250 g were placed in a quiet, temperature- and humidity-controlled room (22 ± 3 °C and 62 ± 7%, respectively) in which a 12–12 h light-dark cycle was maintained (08:00–20:00 h light).

Rats were individually housed in metal cages and divided into three groups: standard diet-fed rats ($n = 12$), sucrose-fed rats ($n = 8$) and ethanol-fed rats ($n = 12$). Ethanol (7.2% v/v) was given to rats in a modified liquid diet for 4 weeks as previously described [18]. The liquid diet was prepared daily. The weight of the rats was recorded every day, and daily ethanol intake was measured and expressed as grams per kilogram per day. Sucrose-fed animals were given an identical volume of diet that was made isocaloric with sucrose instead of ethanol for the same period. Standard diet-fed rats were given the standard rat chow that was made isocaloric with the other two groups for the same period.

The experiments reported in this study have been carried out in accordance with the Declaration of Helsinki. Ethical approval was granted by the Kocaeli University Ethics Committee (Kocaeli, Turkey).

Blood ethanol determination

Blood ethanol concentration was determined by the headspace gas chromatography method [19]. Blood samples were taken by intracardiac puncture from the rats under light ether anaesthesia.

Blood pressure determination

Indirect systolic blood pressure and heart rate were recorded once a week by the tail-cuff plethysmography (MAY-COM BPHR 200; COMMAT Iletisim Co., Ankara, Turkey). In this procedure, conscious rats were placed in a restraining holder from which the tail protruded. Vasodilatation was achieved by local warming of the tail with an infrared bulb. A cuff and pulse sensor was placed around the tail, and the cuff was inflated until the pulse disappeared. When the cuff was deflated, the point of reappearance of the pulse indicated the value of systolic blood pressure. The average value of blood pressure and heart rate in each rat were obtained from four sequential cuff inflation–deflation cycles. Tail-cuff pressure was then regularly measured for the 4 weeks following ethanol treatment.

Organ bath studies

At the end of the 4-week period of ethanol consumption, rats were sacrificed by an overdose of ether. Blood was collected for glucose assay and the thoracic aorta – from the aortic arch to the diaphragm – was excised. After excision, vessels were immediately placed in Kreb's solution (for composition see below) and were dissected carefully and then rings were prepared. The rings were transferred to 20 mL organ baths containing Kreb's solution maintained at 37 °C by a thermoregulated water circuit and continuously aerated with 95% O_2 and 5% CO_2. The pH of the solution was 7.4. All tissues were allowed to equilibrate for 90 min prior to beginning the experiments. During this period, the bath fluid was routinely changed every 15 min. Resting tension was set at 1.5 g by repeat adjustments and remained unchanged throughout the experiment. At the completion of each experiment, tissues were lightly blotted and weighed.

Each ring was connected to a force-displacement transducer (MAY-COM FDT 10-A; COMMAT Iletisim Co.) for the measurement of isometric force, which was continuously displaced and recorded on-line on a computer via a four channel transducer data acquisition system (TDA 94; COMMAT Iletisim Co.), using software (Polywin 95 1.0; COMMAT Iletisim, Co.) that also had the capacity to analyse the data.

Agonist-induced contractions

Tissues were exposed to 80 mM KCl for 5 min to test the viability of the preparation. Tissues were then washed,

and the contractile responses to phenylephrine (10^{-9}–10^{-4} M), serotonin (10^{-9}–10^{-4} M) and ET-1 (10^{-10}–10^{-8} M) were obtained cumulatively. The concentration of the agonist in the bath was increased approximately threefold at each step after the response to the previous dose reached a plateau. ET-1 was added at intervals of between 5 and 20 min depending on the time taken for the previous response to each a plateau.

Between successive dose–response curves, tissues were rinsed with fresh buffer and allowed to recover for 30 min, during which time tension returned to basal levels.

Agonist-induced relaxations

Each aortic ring was contracted by treatment with 3×10^{-7}–10^{-6} M phenylephrine. These concentrations produced 85–87% of the maximal response to phenylephrine. After the phenylephrine-induced contraction had reached a plateau, the concentration-response relationships for acetylcholine (10^{-9}–10^{-5} M) or sodium nitroprusside (10^{-11}–10^{-5} M) were obtained by adding one of these agents to the bath in a cumulative manner.

Solution and drugs

The ionic composition of the Krebs solution was as follows (mM): NaCl 118, KCl 4.71, $MgCl_2$ 1.05, NaH_2PO_4 1.33, $NaHCO_3$ 25, $CaCl_2$ 2.7 and glucose 5.6. In the high K^+ solution, NaCl was exchanged for equimolar amounts of KCl. Fresh solutions were prepared on the day of the experiments.

Drugs were prepared daily in distilled water and kept in ice during the course of experiments. The following drugs were used: phenylephrine hydrochloride (Sigma Chemical Co., St Louis, MO, USA), acetylcholine chloride (Sigma Chemical Co.), endothelin-1 (Sigma Chemical Co.), serotonin creatinine sulfate (Sigma Chemical Co.) and sodium nitroprusside (Adeka Drug and Chemical Co., Samsun, Turkey).

Statistics

All data were expressed as a mean value ± standard error of the mean.

The contractile force was expressed as milligrams of developed tension per milligram of tissue wet weight. The relaxant effects of agonists were expressed as a percentage of the precontraction to phenylephrine. Concentration–response curves were fitted by nonlinear regression with simplex algorithm and E_{max} and pD_2 ($-\log EC_{50}$) were calculated using the software of the transducer data acquisition system. Briefly, cumulative concentration–response curve data were computer fit as previously described to a four parameter logistic equation of the following form:

$$E = E_m/(1 + (EC_{50}/[D]^n))$$

where E is the observed effect in grams of tension, [D] is the concentration of agonist, E_m is the calculated maximal effect, EC_{50} is the [D] at 0.5 E_m, and n is the slope factor parameter.

Significance was tested by one-way analyses of variance (ANOVA) with a posthoc Tukey's–Kramer test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

In blood pressure experiments, effects of time (weeks) were statistically evaluated with repeated measures ANOVA. When the relevant *F*-values were significant at the 5% level, pairwise comparisons were made for the effect of time in alcohol-fed group, using Dunnett's test with baseline measurements (week 0) as a control.

RESULTS

As shown in *Table I*, the body weight of the rats and dry weights of aortic rings of all groups were not statistically different.

Ethanol consumption and blood ethanol levels

Daily ethanol consumption of the rats was in a range of 12.3–18.5 g/kg. The mean blood ethanol level was 293.6 ± 5.2 mg/dL ($n = 5$) in the ethanol-fed rats, whereas scarcely any blood ethanol level could be detected in control rats.

Blood pressure measurements

The tail-cuff blood pressure prior to ethanol treatment and over a 4-week ethanol feeding is shown in *Figure 1*. Blood pressure increased in rats receiving ethanol ($P < 0.05$), whereas there was no change in heart rate (week 0, 302 ± 13; week 1, 279 ± 12; week 2, 283 ± 10; week 3, 314 ± 11; week 4, 307 ± 11). Blood pressure did not change in standard diet- and sucrose-fed

Table I Body weight and aortic dry weigh of standard diet-fed, sucrose-fed and alcohol-fed rats.

	Body weight (g)	Aortic dry weight (mg)
Standard diet-fed	249.3 ± 19.6	0.91 ± 0.040
Sucrose-fed	245.6 ± 12.1	0.91 ± 0.053
Alcohol-fed	258.5 ± 16.5	0.90 ± 0.045

Values are mean ± SEM of 8–12 observations in each group.

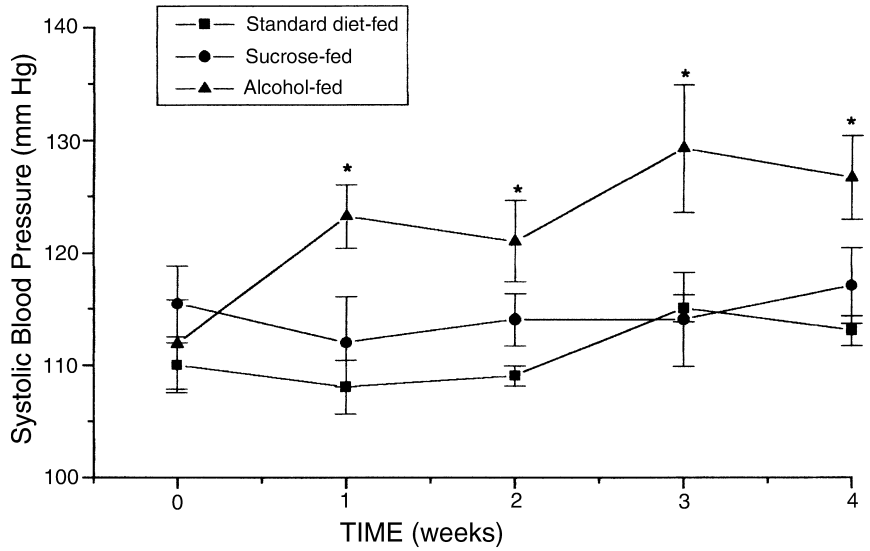


Figure 1 Changes in systolic blood pressure over a 4-week ethanol feeding period in Wistar rats. Systolic blood pressure was measured by the tail-cuff method. Values are mean ± SEM for 4–5 rats. Blood pressure was significantly higher at week 1–4 (*$P < 0.05$) as compared with baseline (week 0).

rats at various times (*Figure 1*). In addition, there was no change in heart rate in standard diet- and sucrose-fed rats groups (week 0, 300 ± 9; week 4, 298 ± 5; week 0, 313 ± 7; week 4, 300 ± 9, respectively).

Contractile responses

Cumulative concentration–response curves were obtained for phenylephrine, serotonin, and ET-1 in aortic rings from alcohol-fed, sucrose-fed and normal diet-fed rats. ET-1 caused concentration-related slowly developing contractions. The contractile responses to phenylephrine, serotonin or ET-1 did not change in aortas from ethanol-fed rats when compared to the vessels from sucrose- or standard diet-fed rats (*Figures 2, 3 and 4*). The contractile responses to 80 mM KCl also did not change in these animals (*Table II*). As shown in

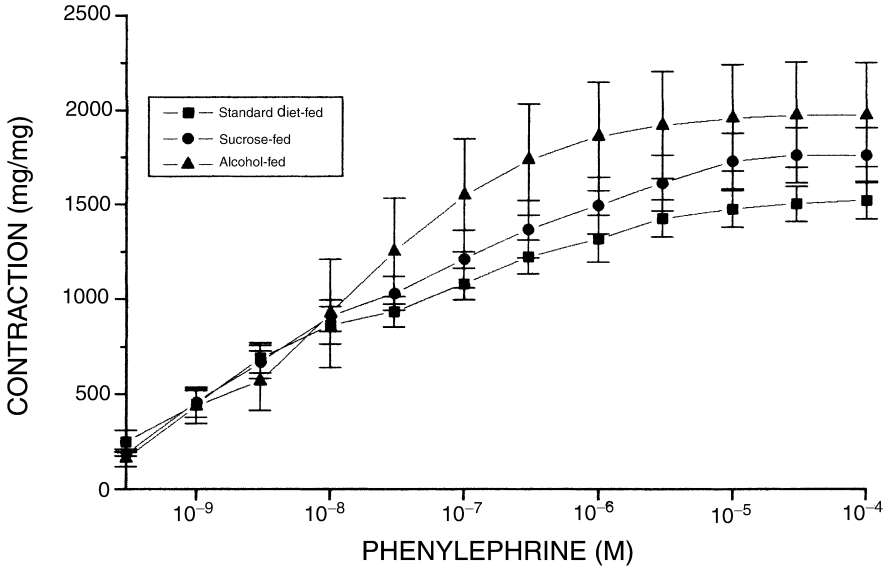


Figure 2 Phenylephrine concentration–response curves in thoracic aortic rings. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM ($n = 8$–12 rings of each group).

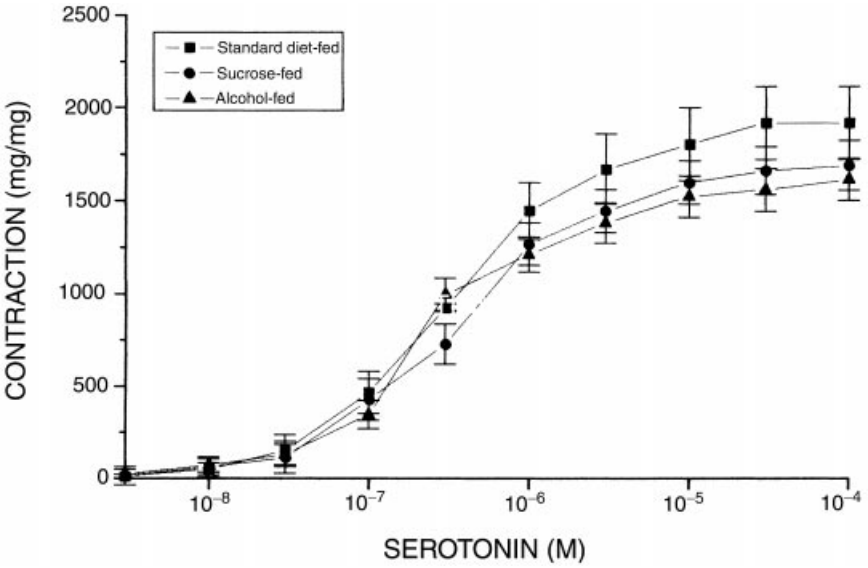


Figure 3 Serotonin concentration–response curves in thoracic aortic rings. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM ($n = 8$–12 rings of each group).

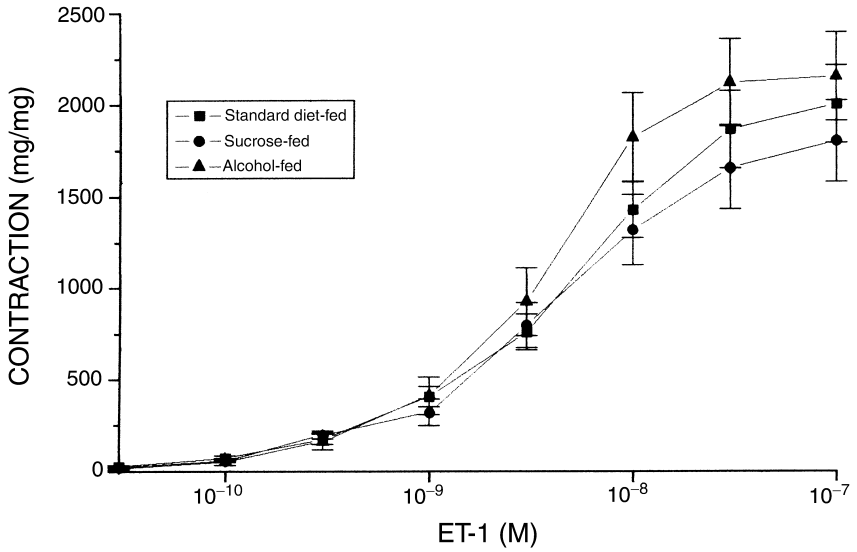


Figure 4 ET-1 concentration–response curves in thoracic aortic rings. Each point is expressed as mg tension/mg tissue and is given as the mean ± SEM ($n = 8$–12 rings of each group).

Table II, there was no significant difference between maximum responses or pD_2 values of rings from alcohol-fed and sucrose- or standard diet-fed rats.

Relaxation responses

The ability of acetylcholine or sodium nitroprusside to relax the arteries precontracted with a submaximal dose of phenylephrine was determined. The precontractile tone was similar in the all groups (1561 ± 296, 1357 ± 150 and 1197 ± 128 mg tension, mean ± SE; $n = 8$–12, in the alcohol-, sucrose-, and the standard diet-fed groups, respectively). The maximum response to acetylcholine was increased in alcohol-fed rats (*Figure* 5). In addition, the sensitivity (pD_2 value) of alcohol-fed aortas to acetylcholine was significantly increased compared to sucrose-fed rats (*Table II*). However, the relaxation response to sodium nitroprusside did not show any difference among the three groups (*Figure 6*, *Table II*).

DISCUSSION

A mild elevation in the systolic pressure was detected in the alcohol-fed group. This is consistent with the results of previous reports on rats with chronic alcohol-treatment [5–7,13,20]. In aortic rings from chronically alcohol-fed rats, contractile responses to serotonin,

Table II Maximum contraction response values (E_m, mg/mg) for phenylephrine, serotonin, ET-1 and 80 mM KCl and maximum relaxation response values (% of 3.10^{-7}–10^{-6} M phenylephrine) for acetylcholine and sodium nitroprusside and pD_2 values on exposure to agonist in rings of thoracic aortae obtained from the three groups of rats.

	Standard diet-fed	Sucrose-fed	Alcohol-fed
KCl			
E_m	1354.2 ± 113.11	1331.1 ± 173.10	1306.5 ± 72.34
Phenylephrine			
E_m	1507.8 ± 88.7	1750.1 ± 179.1	1990 ± 276.1
pD_2	8.22 ± 0.15	8.05 ± 0.35	7.83 ± 0.17
Serotonin			
E_m	1903 ± 167.9	1711.2 ± 142.4	1661.8 ± 112
pD_2	6.49 ± 0.16	6.46 ± 0.11	6.15 ± 0.18
ET-1			
E_m	2301.4 ± 215.2	1903.6 ± 304.1	2354.7 ± 105
pD_2	8.30 ± 0.065	7.98 ± 0.19	8.42 ± 0.07
Acetylcholine			
E_m	84.4 ± 1.29	81.1 ± 1.44	97.4 ± 1.39*
pD_2	7.67 ± 0.14	7.85 ± 0.12	8.92 ± 0.13*
SNP			
E_m	97.3 ± 2.32	97.2 ± 2.07	97.6 ± 3.55
pD_2	10.08 ± 1.93	9.93 ± 2.26	10.14 ± 1.99

Values are arithmetic means ± SEM of 8–12 observations in each group.
*$P < 0.05$ statistically different from all other groups.

ET-1, phenylephrine and KCl were not altered in comparison to the controls. However, chronic alcohol consumption increased endothelium-dependent relaxation induced by acetylcholine, thus confirming previous reports [14–16].

In recent years, hypertension due to chronic alcohol consumption has been recognised as a serious problem. Later studies emphasized the importance of increased vascular responsiveness to neurotransmitters or hormones in blood pressure elevation [8]. Although, to our knowledge, there is no study demonstrating the effects of chronic alcohol consumption on vascular responsiveness to ET-1, a powerful vasoconstrictor – vascular responsiveness to serotonin, phenylephrine or KCl – has been reported [12]. Altura *et al.* [21] showed hypersensitivity to K^+, catecholamines and angiotensin II after ethanol treatment. In contrast, other authors showed no change in the responsiveness to noradrenaline or serotonin after ethanol treatment [12,22]. In our study, there were no differences in the vasoconstrictor response of aortic rings between the control and the ethanol-fed rats. These findings imply that increased vascular responsiveness is not a mechanism responsible for the blood pressure elevation in the alcohol-fed rats. It is possible that the blood pressure elevation in chronic alcohol consumption may be, at least in part, associated with an expansion in plasma volume [13].

Although the endothelium–dependent relaxation of rat aorta to acetylcholine is reported to be depressed by the acute administration of alcohol [23,24], in previous studies [14–16] and in this study, using aorta preparations from rats, acetylcholine-induced endothelium–dependent relaxation was increased by chronic alcohol consumption. The mechanism by which alcohol increa-

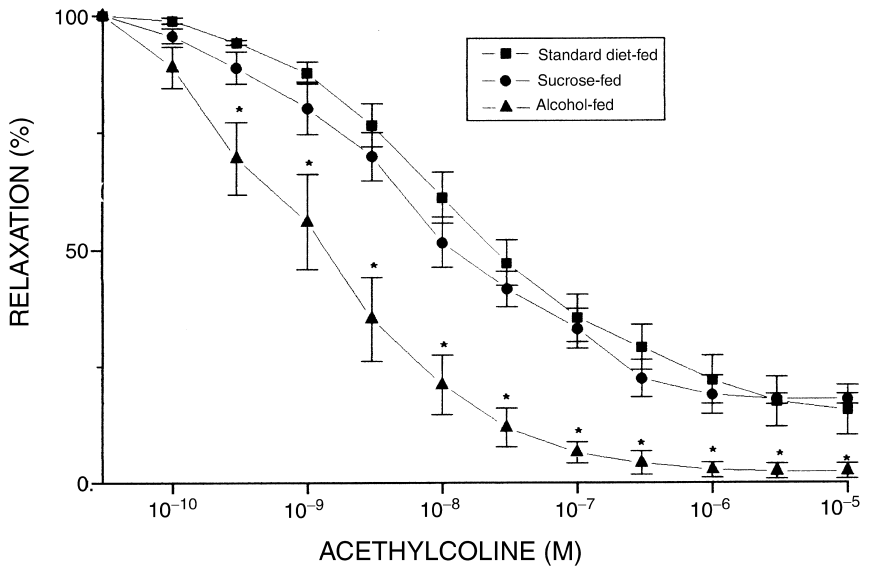


Figure 5 Acetylcholine concentration–response curves in rat isolated thoracic aortic rings precontracted with phenylephrine. Contraction elicited by phenylephrine alone was set at 100% and each point of relaxation induced by acetylcholine is expressed as a percentage of the contraction to phenylephrine. Data are the mean ± SEM. *$P < 0.05$, statistically different from the response of rings from standard diet- and sucrose-fed rats ($n = 8$–12 rings of each group).

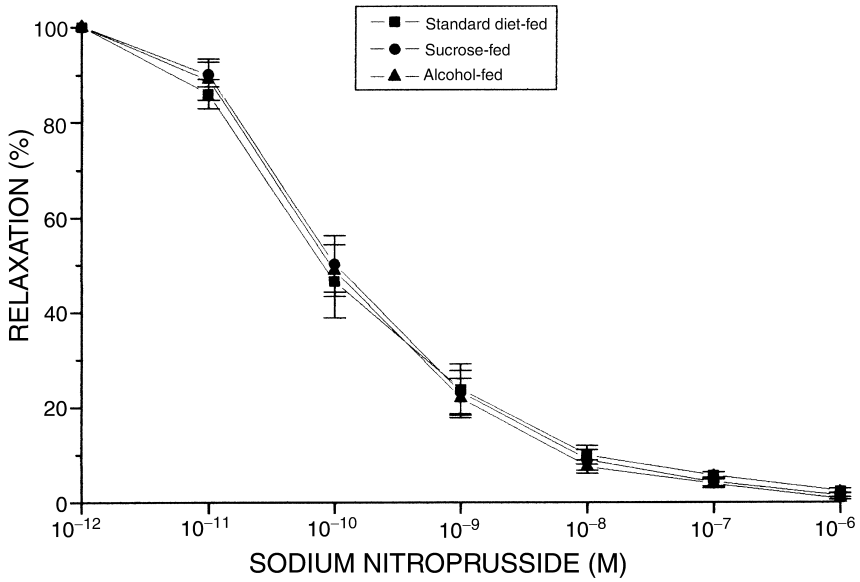


Figure 6 Sodium nitroprusside concentration–response curves in rat isolated thoracic aortic rings precontracted with phenylephrine. Contraction elicited by phenylephrine alone was set at 100% and each point of relaxation induced by sodium nitroprusside is expressed as a percentage of the contraction to phenylephrine. Data are the mean ± SEM ($n = 8$–12 rings of each group).

ses endothelium–dependent relaxation is not clear but it seems to be associated with possible alterations in either properties and functions of the membrane receptor system or sensitivity of the vascular NO production in the vascular tissue. As ethanol is a membrane-fluidizing agent [25], its action may alter the properties and functions of the membrane receptor system, such as muscarinic cholinergic receptors [26]. Therefore, membrane changes in endothelial cells may increase the sensitivity of receptors leading to an increase in the relaxation.

Furthermore, the magnitude of the precontraction was similar in preparations from alcohol-, sucrose- and standard-fed rats for phenylephrine, thus ensuring that any difference in relaxation among the three groups was not due to differences in the degree of precontraction. Therefore, increased sensitivity of vascular smooth muscle cells to NO or increased relaxation mechanism in the smooth muscle cells may occur.

The relaxations induced by NO, like those to sodium nitroprusside, are mediated through an increase in cGMP in vascular smooth muscle [27]. The mechanism of increased endothelium-dependent vasodilatation appears to be related to the increased production of cGMP. Because the response to the sodium nitroprusside was the same in vascular tissue from control and alcohol-fed rats, it may be assumed that the responsiveness of aorta to the effects that are mediated by NO was unchanged. These findings also suggest that the increase in the endothelium–dependent relaxation response to acetylcholine in alcohol-fed rats probably occurs at the level of the endothelium and not the smooth muscle cells, and is most likely to be due to endothelial cell response to acetylcholine receptor-mediated activation.

The results of the present study may also be explained by increased synthesis and/or release of NO, mainly of endothelial origin, and it is conceivable that this increment can lead to mild elevation in systolic pressure instead of significant elevation. It is suggested that increased sensitivity to vasoconstrictor agents and decreased vascular responses to vasodilator agents is observed in hypertension [28].

It should be noted that the observations of different studies are highly variable. Konishi and Su [3] reported that the endothelial lining of the blood vessel may provide a compensatuary response that will mask altered sensitivity to vasoactive agents. Recently it was demonstrated that ethanol augments both basal and stimulated NO production and this effect is associated with increased eNOS protein and mRNA expression levels [29]. This data also shows that an adaptive mechanism masking increased blood pressure may exist in ethanol consumption.

As is well known, chronic ethanol consumption enhances production of prostacyclin that may contribute to the vasodilator actions of ethanol [30]. The mechanism of increased acetylcholine-induced endothelium–dependent relaxation appears to be, at least in part, related to the production of prostacyclin. Because in this study we did not perform experiments with a

cyclooxygenase inhibitor, such as indomethacin, we can not draw any conclusion regarding the involvement of prostacyclin at the present time. In addition, a candidate to account for increased vasorelaxation is endothelium-derived hyperpolarizing factor (EDHF) [31]. Previous work has shown that EDHF plays a role in endothelium–dependent vasorelaxation of arterial vessels in the rat [32]. Further experiments are required to establish whether EDHF or prostacyclin contributes to endothelium–dependent vasorelaxation in chronic alcohol consumption.

In conclusion, the results of the study indicate that in male rats, chronic alcohol consumption is associated with no significant change in vascular contractile response to ET-1, phenylephrine and serotonin but enhanced acetylcholine-induced, endothelium–dependent vasorelaxation and mild elevation of systolic blood pressure. The mechanism of the enhanced relaxation is not known but does not appear to involve alterations in the cGMP–dependent relaxations of vascular smooth muscle. It is possible that the effect might be due to adaptive alterations in muscarinic receptors on the endothelial cells or increased NO production in the vascular tissue.

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