Dietary Mg^{2+} supplementation restores impaired vasoactive responses in isolated rat aorta induced by chronic ethanol consumption[☆]

Ricardo A. Brown [a], Karl J. Ilg [b], Alex F. Chen [c], Jun Ren [d,*]

[a]*Department of Biology, Morgan State University, Baltimore, MD 21251, USA*
[b]*School of Medicine, Wayne State University, Detroit, MI 48201, USA*
[c]*Department of Pharmacology and Toxicology, College of Human Medicine, Michigan State University, East Lansing, MI 48824-1317, USA*
[d]*Department of Pharmacology, Physiology, and Therapeutics, School of Medicine, University of North Dakota,*
501 N. Columbia Road, Grand Forks, ND 58203, USA

Abstract

Chronic ethanol consumption contributes to cardiovascular dysfunction possibly related to loss of Mg^{2+}. This study was designed to examine the role of dietary Mg^{2+} supplementation on chronic ethanol ingestion-induced vascular alteration. Rats were fed an ethanol liquid diet supplemented with or without Mg^{2+} for 12 weeks. The force-generating capacity was examined in thoracic aortic rings. Ethanol-consuming animals exhibited significantly elevated blood pressure. In aorta with intact endothelium, the contractile responses to norepinephrine and KCl were greatly attenuated and potentiated, respectively. Interestingly, the ethanol-induced alterations in blood pressure and vasoconstrictive response were restored by Mg^{2+} supplementation. Pretreatment with the β_1-adrenoceptor antagonist atenolol in intact aortic rings abolished the difference in response to norepinephrine between the control and ethanol groups, which implies the involvement of a weakened β_1-adrenoceptor component in vessels from the ethanol-fed rats. The norepinephrine-induced vasoconstriction in intact aorta rings was completely abolished by the α_1-adrenoceptor antagonist prazosin. In endothelium-denuded aorta, the contractile response to norepinephrine or KCl was not significantly different between the ethanol and Mg^{2+} groups. Endothelium-dependent vasorelaxation to carbamylcholine chloride was not altered by either ethanol or Mg^{2+} supplementation. Sodium nitroprusside-induced vasorelaxation was depressed by ethanol, and restored by Mg^{2+}, in aorta with or without endothelium. These data suggest that chronic ethanol consumption contributes to alterations of endothelium-dependent and -independent vascular response. These alterations can be compensated by dietary Mg^{2+} supplementation. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Mg^{2+}; Ethanol; Vascular response; Endothelium

1. Introduction

Chronic ethanol consumption is associated with cardiovascular dysfunctions independent of other known risk factors (Altura and Altura, 1982, 1987a; Patel et al., 1997). Although light to moderate ethanol consumption may lower blood pressure and benefit cardiovascular function, excessive ethanol consumption is believed to be associated with a number of cardiovascular diseases such as hypertension (Moore et al, 1990; Patel et al., 1997). Several mechanisms have been postulated for the hypertensive response to chronic ethanol consumption such as secretion of hormones and neurotransmitters, stimulation of the sympathetic nervous system, and alteration of baroreceptor activity (Altura and Altura, 1982, 1987a). Evidence also suggests the existence of a myogenic mechanism(s) involving alteration in the contractile properties of vascular smooth muscle (Altura and Altura, 1982). Chronic ethanol exposure has been suggested to facilitate Ca^{2+} influx in smooth muscle cells, thus increasing the intracellular Ca^{2+} concentration and vascular tone (Altura and Altura, 1982). However, the exact mechanism(s) underlying ultrastructural changes, myocardial depression, and alterations in vascular reactivity following chronic exposure to ethanol remain only partially understood.

While the etiology of alcoholic cardiomyopathy may be multifactorial, the association of chronic ethanol consumption and profound hypomagnesemia has been recognized (Flink et al, 1953; Flink, 1986; Brautbar and Altura, 1987). Indeed, although a number of therapeutic agents such as the cardiac glycosides, anticancer drugs, and diuretic agents are positively correlated with Mg^{2+} deficiency in humans, ethanol has often been described as the most notorious cause of Mg^{2+} wasting (Kalbfleish et al, 1963). Mg^{2+} is a fundamental constituent of soft tissue and bone. It catalyzes the activity of at least 350 enzymes (such as hexokinase, pyruvate dehydrogenase, enolase, and creatine phosphokinase) by causing a conformational change during catalytic processes and promoting aggregation of multienzyme complexes (Altura and Altura, 1984; Murphy, 2000; Romani and Scarpa, 2000). Mg^{2+} also plays a role in mitochondrial membrane permeability and maintenance of low resting levels of intracellular free Ca^{2+} (Altura and Altura, 1995; Resnick, 1995). Compelling evidence has suggested that Mg^{2+} may flux across the cell membrane in either direction in response to hormonal and non-hormonal stimuli, resulting in major changes in total and, to a lesser extent, free Mg^{2+} content within tissues (Altura and Altura, 1995; Resnick, 1995). Mg^{2+} is considered as an intracellular messenger and has been shown to modulate a number of physiologic properties in vascular smooth muscle cells including ion (Ca^{2+}, K^+) channel conductance, membrane oxidation, and lipid composition (Morrill et al., 1997, 1998; Yang et al., 1999; Romani and Scarpa, 2000; Shi and Cui, 2001). In addition, dietary Mg^{2+} deficiency induces hypertension while dietary Mg^{2+} supplementation prevents the development of hypertension in animals genetically predisposed to higher systolic blood pressure (Altura et al., 1984, 1992). Since both Na^+ and Ca^{2+} membrane transport and vascular smooth muscle contraction are regulated by Mg^{2+}-dependent ATPase activity, it is possible that increases in intracellular Ca^{2+} and Na^+ concentration can be attributed to hypomagnesemia and are possibly related to hypertension (Altura and Altura, 1984; Altura et al., 1992).

The primary mechanisms underlying alcohol-induced hypomagnesemia are reduced dietary intake and enhanced renal excretion (Flink, 1986). Altura and Altura (1986, 1987a) demonstrated that dietary Mg^{2+} supplementation in experimental animals prevented the development of hypertension following long-term ethanol exposure, suggesting that Mg^{2+} depletion may play an important role in the pathogenesis of chronic ethanol-induced hypertension. We have recently shown that chronic ethanol-induced myocardial contractile dysfunction can be partially prevented by dietary Mg^{2+} supplementation (Brown et al., 1998). Despite these important findings, the role of dietary Mg^{2+} supplementation in chronic ethanol-related vascular dysfunction has not been reported. The purpose of this study was to examine the effects of dietary Mg^{2+} supplementation on vascular contractile function under the influence of chronic ethanol consumption.

2. Materials and methods

2.1. Experimental animals

Experimental protocols described in this study were approved by the animal care committee of Wayne State University and have been previously described (Brown and Savage, 1996). Adult Sprague–Dawley rats (both genders) were obtained as pairs of littermates weighing approximately 50 g. All animals were housed in a temperature-controlled room under a 12-h light/dark illumination cycle and allowed tap water ad libitum. Animals were initially maintained on standard rat chow for a 1-week quarantine period.

2.2. Chronic ethanol exposure

Following the quarantine period, all animals were introduced to a nutritionally complete liquid diet (Shake and Pour Bioserv, Frenchtown, NJ, USA) for a 1-week acclimation period (De Carli and Leiber, 1967; Brown et al., 1998). The employment of a liquid diet is based on the observations made by Keane and Leonard (1989) that ethanol self-administration resulted in less nutritional deficiencies and less stress to the animals in comparison to forced-feeding regi-

Table 1
General characteristics of animals upon sacrifice

	Control ($N=11$)	ETOH ($N=10$)	Mg^{2+}/control ($N=12$)	Mg^{2+}/ETOH ($N=12$)
Body weight (g)	489.1 ± 16.7	451.6 ± 27.1	478.8 ± 19.7	487.3 ± 21.0
Heart weight (g)	1.49 ± 0.10	1.40 ± 0.05	1.45 ± 0.07	1.44 ± 0.05
Heart weight/body weight (mg/g)	3.15 ± 0.33	3.22 ± 0.23	3.11 ± 0.21	3.05 ± 0.22
Liver weight (g)	13.8 ± 0.7	13.8 ± 0.9	13.9 ± 0.7	16.4 ± 0.7[a,b]
Liver weight/body weight (mg/g)	28.5 ± 1.5	31.5 ± 2.7	29.3 ± 1.3	33.3 ± 0.6[a]
Kidney weight (g)	3.05 ± 0.20	3.17 ± 0.09	2.94 ± 0.15	3.13 ± 0.08
Kidney weight/body weight (mg/g)	6.27 ± 0.39	7.24 ± 0.44	6.22 ± 0.33	6.51 ± 0.21
Serum Mg^{2+} (mg/dl)	3.72 ± 0.09	3.12 ± 0.15[a]	3.65 ± 0.26	3.70 ± 0.19[b]
ETOH (mg/dl)	0	63.8 ± 2.5[a]	0	40.3 ± 4.9[a,b]

ETOH: ethanol.

[a] $P<0.05$ vs. respective control group.
[b] $P<0.05$ vs. respective ETOH group.

mens, intravenous administration, or aerosolized inhalation. Upon completion of the acclimation period, one littermate was maintained on the liquid diet without ethanol, and the remaining littermates began a 7-day period of ethanol introduction. On days 1–4, ethanol-consuming animals were given a diet in which 12% of the total calories were isocalorically replaced by ethanol. Subsequently, on days 5–7, the caloric content of the diet provided by ethanol was increased to 24%. On day 8, ethanol-consuming animals were introduced to a diet in which 36% of the total calories were derived from ethanol. Supplementation with Mg^{2+} also began on day 8, which marked the beginning of the 12-week experimental period of chronic ethanol exposure. Mg^{2+}-supplemented animals received a diet containing 0.65 g/l of Mg^{2+} although nonsupplemented animals were given a diet that consisted of 0.13 g/l of Mg^{2+}. This value of Mg^{2+} content for the nonsupplemented diet corresponds to approximately 0.056% Mg^{2+} by weight of the liquid diet. An isocaloric (250 cal/l) pair-feeding regimen was employed to eliminate the possibility of nutritional deficits. Non-ethanol-consuming animals were offered the same quantity of diet ethanol-consuming animals drank the previous day. Consumption of liquid diet throughout the experimental period was measured daily. Blood pressure was determined by the tail-cuff method. Body weight recordings were obtained on a weekly basis. The ethanol concentration in blood samples was determined using a biochemistry analyzer (YSI 2700 Biochemistry Analyzer, Yellow Springs, OH, USA). Serum electrolyte levels were determined at weeks 4, 8, and 12 by University Laboratories, Wayne State University Health Center utilizing the methylthymol blue method for the determination of serum Mg^{2+} levels.
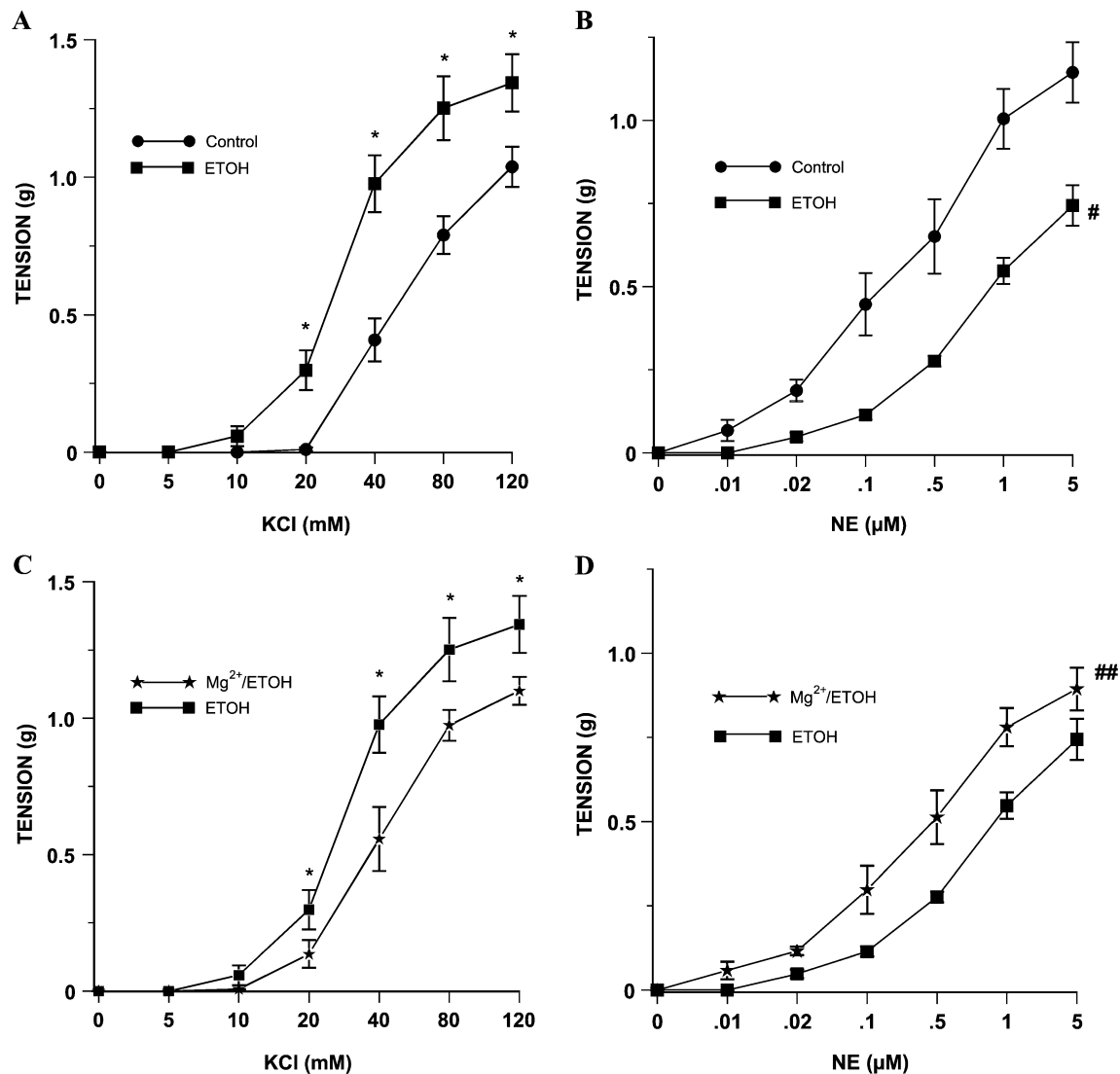

Fig. 1. Effects of chronic ethanol (ETOH) exposure and dietary Mg^{2+} supplementation on KCl- (panels A and C) or norepinephrine-induced (panels B and D) vasoconstriction in isolated rat aortic ring segments with intact endothelium. Data are mean ± S.E.M., $n = 6$–12 animals. * $P < 0.05$ compared to baseline; # $P < 0.05$ compared to control group; ## $P < 0.05$ compared to ETOH group.

2.3. Aortic ring isolation procedure and tension recording

At the end of the 12-week experimental period, animals were anesthetized with a ketamine/xylazine solution (3:1, 1.32 mg/kg, i.p.). The thoracic aorta was quickly removed and placed in an oxygenated physiologic salt solution (PSS) of the following composition (in mM): KCl 5.4, NaCl 136.9, $NaHCO_3$ 11.9, NaH_2PO_4 0.45, $MgCl_2$ 0.50, $CaCl_2$ 2.7, and glucose 5.6. Vessels were trimmed free of connective tissue and fat. The transverse segments (3 mm in length) were cut with special care to avoid damage to the endothelial layer. One aortic segment from each animal was left intact, while the other segment was mechanically denuded of endothelium by gently rubbing the intimal surface of the vessel. Muscle rings were then mounted vertically using fine wires inserted through the lumen for isometric tension recording in 20-ml organ baths containing PSS gassed with 95% O_2 and 5% CO_2 at 37 °C and pH ~ 7.4. All rings were stretched to ~ 0.7 g of resting tension and allowed to equilibrate for 90 min during which the bath solution was changed every 30 min. Isometric contractile tension was measured by using Grass FT03 force–displacement transducers coupled to a Grass Model 79 polygraph. Presence of functional endothelium was determined by greater than 50% relaxation by addition of carbamylcholine (2 μM) to segments precontracted with norepinephrine (1 μM). Aortic segments relaxing less than 50% were considered to be without functional endothelium (Brown and Savage, 1996).

2.4. Experimental protocols for vascular tension study

Aortic ring segments, once mounted, were allowed to equilibrate in PSS for 1 h prior to the start of each experiment. Dose–response curves were then constructed to norepinephrine, potassium chloride (KCl), carbamylcholine chloride, and sodium nitroprusside (Aldrich Chemical, Milwaukee, WI, USA). Each agonist was added cumulatively, and concentrations were allowed to elicit maximal response before subsequent doses were applied. To define the adrenergic contribution in norepinephrine-induced vasoconstriction, a selective β_1-adrenoceptor antagonist, atenolol (Sigma, St. Louis, MO, USA), and an α_1-adrenoceptor antagonist, prazosin (Sigma), were tested by pre-incubating with the vessels for 30 min prior to initiation of the norepinephrine protocol.

2.5. Statistical analysis

Two aortic segments were collected per animal (one endothelium-intact; one endothelium-denuded). Experimental groups include results from 10 to 12 rats. For each experimental series, data are presented as mean ± S.E.M. Statistical significance ($P < 0.05$) was estimated by a two-way analysis of variance (ANOVA) or t-test, where appropriate. A Dunnett's test was used for post hoc analysis when required.

3. Results

3.1. General characteristics of animals

The pattern of weekly diet consumption and body weight gain were similar in all groups (data not shown). The pair-feeding regimen ensured that consumption did not differ between groups at any time throughout the study. However, diet consumption increased linearly through the entire 12-week experimental period. During week 1, the control and ethanol-consuming animals drank approximately 52.8 ml/day while the Mg^{2+}-supplemented control and ethanol-consuming animals drank approximately 49.9 ml/day. By week 12, their consumption increased to 82.9 and 85.7 ml/day, respectively. The average weight gain in all animals was approximately 30 g/week.
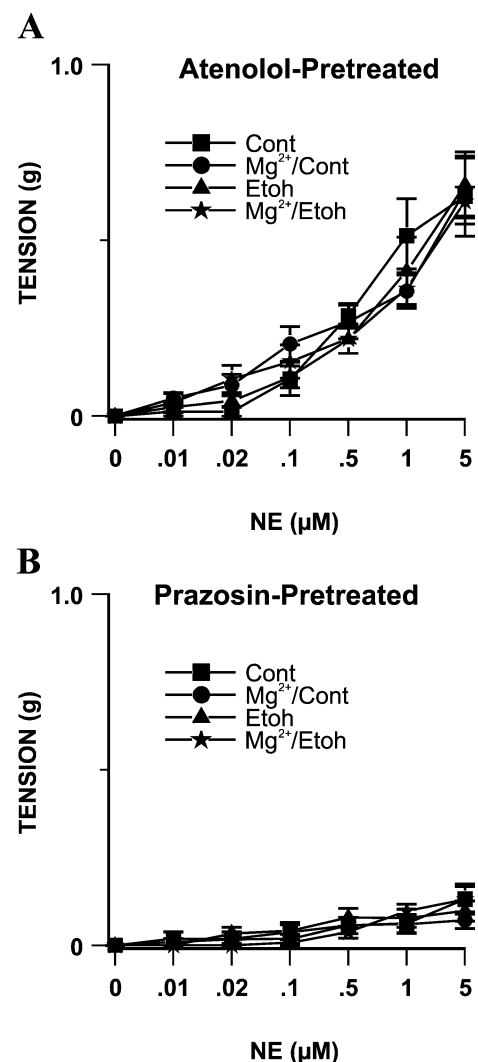

Fig. 2. Effects of pretreatment of the β_1-adrenoceptor antagonist atenolol (1 μM, panel A) and the α_1-adrenoceptor antagonist prazosin (1 μM, panel B) on norepinephrine-induced vasoconstriction in aortic ring segments with intact endothelium. Data are mean ± S.E.M., $n = 8$ animals.
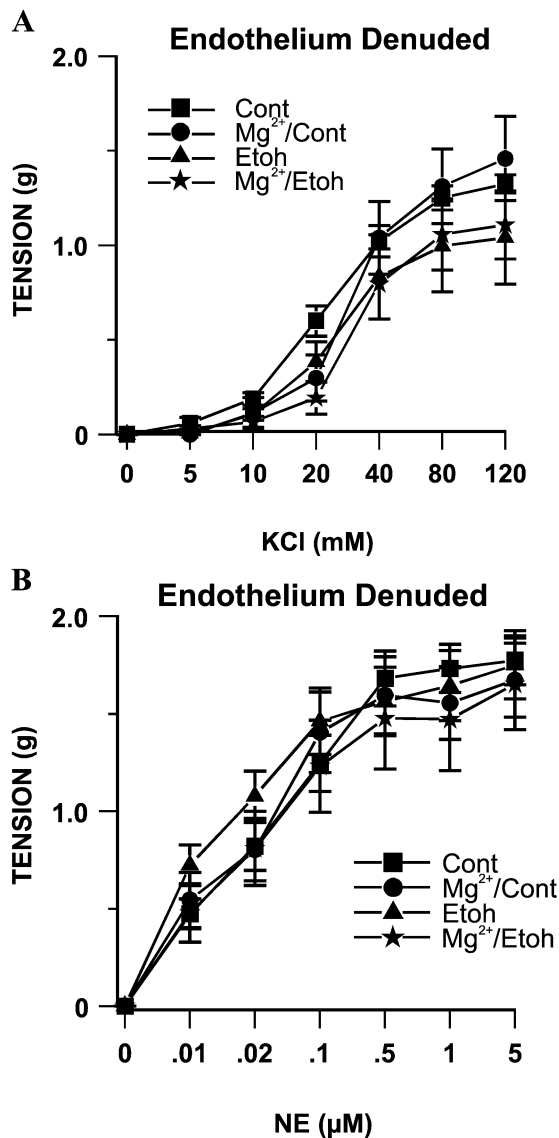
A

Endothelium Denuded

B

Endothelium Denuded

Fig. 3. Effects of chronic ethanol (ETOH) exposure and dietary Mg^{2+} supplementation on KCl- (panel A) or norepinephrine-induced (panel B) vasoconstriction in isolated rat aortic ring segments with denuded endothelium. Data are mean $\pm$ S.E.M., $n=6-9$ animals.

The general characteristics of experimental animals are shown in Table 1. Animals from all groups exhibited similar body weight gain over the experimental period. Neither chronic ethanol consumption nor dietary Mg^{2+} supplementation affected the heart and kidney weight/size. The liver weight and size were significantly larger in those animals that consumed ethanol while being supplemented with Mg^{2+}. Serum analysis revealed that ethanol-consuming animals had significantly lower Mg^{2+} levels than the control group. It is interesting that the Mg^{2+}-supplemented/ethanol-consuming group had lower blood ethanol concentrations than the non-Mg^{2+}-supplemented ethanol-consuming animals.

Ethanol-consuming animals exhibited elevated systolic blood pressures compared to those of the control group

(151.6 ± 0.6 vs. 132.9 ± 2.7 mm Hg, $P < 0.05$). Mg^{2+} supplementation significantly attenuated ethanol-induced elevation of blood pressure (136.4 ± 1.4 mm Hg in Mg^{2+}-supplemented ethanol-consuming animals, $P < 0.05$ vs. the ethanol group). Furthermore, Mg^{2+} supplementation also significantly attenuated blood pressure in non-ethanol-consuming control animals (125.0 ± 3.1 mm Hg for Mg^{2+}-supplementing control animals, $P < 0.05$ vs. the control group).

3.2. Vasoconstricting response to KCl and norepinephrine (endothelium-intact)

3.2.1. Effect of chronic ethanol consumption

As expected, bath application of KCl (5–120 mM) produced dose-dependent vasoconstriction of aortic ring segments with intact endothelium from both control and ethanol-consuming animals (Fig. 1A). Chronic ethanol consumption shifted the dose–response curve of KCl to the left when compared to the control animals. Maximal tension developed at 120 mM KCl was increased by 31% in the ethanol group compared to control (1.34 and 1.02 g for ethanol and control, respectively). Submaximal response at 40 mM KCl was increased by 113% in the ethanol group (0.98 g) compared to control (0.46 g). Similar to KCl, bath application of norepinephrine (0.01–5 μM) produced dose-dependent contractions in aortic ring segments with intact endothelium from control and ethanol groups (Fig. 1B). However, chronic ethanol consumption shifted the dose-dependent response curve to the right when compared to control animals.
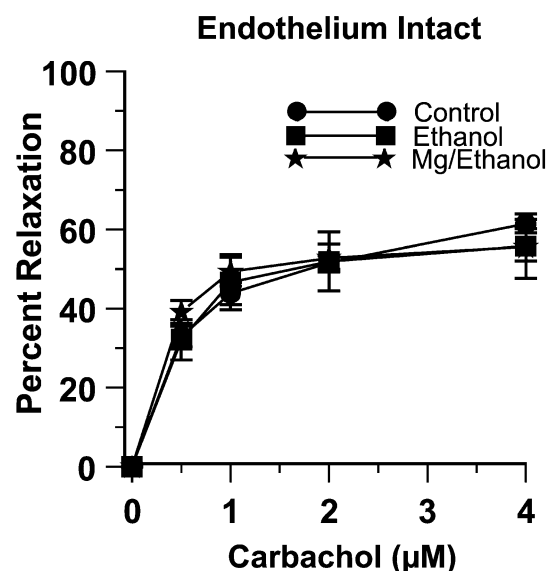
Endothelium Intact

Fig. 4. Effects of chronic ethanol (ETOH) exposure and dietary Mg^{2+} supplementation on carbamylcholine chloride-induced endothelium-dependent vasorelaxation. Data are mean $\pm$ S.E.M., $n=6-9$ animals.

3.2.2. Effect of dietary Mg^{2+} supplementation

Bath application of KCl produced dose-dependent contraction in ring segments with intact endothelium from Mg^{2+}-supplemented ethanol-consuming animals. Interestingly, dietary Mg^{2+} supplementation prevented ethanol-induced alteration in KCl-induced vasoconstriction by shifting the dose-dependent response curve to the right when compared to the ethanol-consuming group (Fig. 1C). Maximal tension developed at 120 mM KCl was decreased by 18% with dietary Mg^{2+} supplementation. Submaximal response at 40 mM KCl was decreased by 43% with Mg^{2+} supplementation. Bath application of norepinephrine

produced dose-dependent vasoconstriction in aortic ring segments with intact endothelium from the Mg^{2+}-supplemented, ethanol-consuming group (Fig. 1D). However, dietary Mg^{2+} supplementation shifted the dose–response curve leftwards when compared to the ethanol group. Dietary Mg^{2+} supplementation did not cause any significant effect to KCl- or norepinephrine-induced vasoconstriction in the non-ethanol-consuming control group (data not shown). Collectively, these data suggest that chronic ethanol consumption-induced alterations in vasoconstriction in response to KCl and norepinephrine are reversed by dietary Mg^{2+} supplementation.
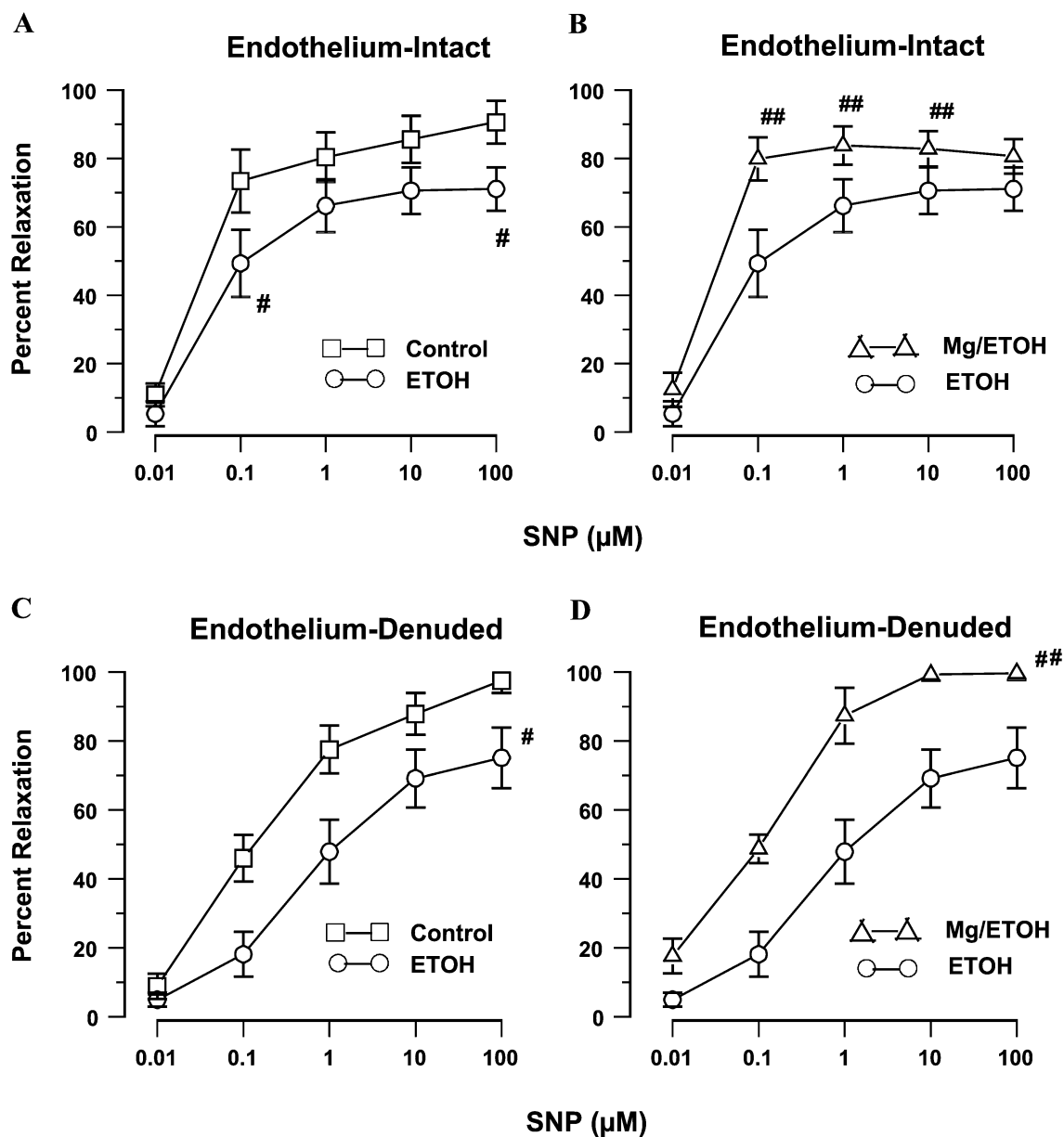

Fig. 5. Effects of chronic ethanol (ETOH) exposure and dietary Mg^{2+} supplementation on nitric oxide donor sodium nitroprusside-induced endothelium-independent vasorelaxation in endothelium-intact (panels A and B) and endothelium-denuded (panels C and D) aortic ring segments. Data are mean ± S.E.M., $n = 6-9$ animals. #$P < 0.05$ compared to control group; ##$P < 0.05$ compared to ETOH group.

3.3. Vasoconstriction of norepinephrine in the presence of atenolol and prazosin (with endothelium)

To examine the potential contribution of the adrenergic pathways in the altered norepinephrine-induced vasoconstrictive response following chronic ethanol consumption, the endothelium-intact aortic ring segments from all groups were pre-incubated with a selective α_1-adrenoceptor antagonist, prazosin (1 μM), or a selective β_1-adrenoceptor antagonist, atenolol (1 μM), for 30 min prior to the initiation of the norepinephrine dose–response. None of the antagonists affected resting tension development (data not shown). Results in Fig. 2 indicate that pretreatment by atenolol shifted the norepinephrine concentration–response (0.01–5 μM) curve to the right in vessels from the control group but did not significantly affect the responsiveness in vessels from the ethanol group. However, pretreatment with atenolol in aortic rings abolished the altered response to norepinephrine between the two groups, implicating the involvement of a weakened β_1-adrenoceptor component in vessels from the ethanol-fed rats. The norepinephrine-elicited vasoconstrictive response was almost completely abolished by prazosin, compared to the tension development in the absence of the antagonists (shown in Fig. 1). These results suggest a predominant role of the α-adrenergic pathway in norepinephrine-induced vasoconstriction.

3.4. Vasoconstricting response to KCl and norepinephrine (endothelium-denuded)

As shown in Fig. 3, neither chronic ethanol consumption nor dietary Mg^{2+} elicited any effect on the vasoconstriction in response to KCl (5–120 mM) or norepinephrine (0.01–5 μM) in aortic ring segments lacking intact endothelium.

3.5. Vasorelaxant response to carbamylcholine chloride (endothelium-intact)

The endothelium-dependent vasorelaxation to the acetylcholine analog, carbamylcholine chloride, was not different in ring segments with intact endothelium among the four animal groups tested (control, ethanol-consuming, Mg^{2+}-supplementing control, and Mg^{2+}-supplementing ethanol). Carbamylcholine chloride (0.5–4 μM) exhibited dose-dependent vasorelaxation in endothelium-intact aortic rings among all groups (Fig. 4). The vasorelaxant response to carbamylcholine chloride was not significantly different, as sensitivity and maximal response were the same among control, ethanol-consuming, and Mg^{2+}-supplemented ethanol-consuming groups.

3.6. Vasorelaxant response to sodium nitroprusside (endothelium-intact and endothelium-denuded)

Sodium nitroprusside is a nonspecific nitric oxide (NO) donor-mediating vascular smooth muscle relaxation independent of the endothelium. Results shown in Fig. 5 exhibit the dose-dependent vasorelaxant response of sodium nitroprusside (0.01–100 μM) in aortic ring segments with or without endothelium. Aortic ring segments obtained from chronic ethanol-consuming animals (both endothelium-intact and endothelium-denuded) showed an attenuated vasorelaxant response to sodium nitroprusside when compared to the control group. Dietary Mg^{2+} supplementation restored the vasorelaxant response which was diminished in the ethanol group, most notably at the lower dose range (0.1 and 1 μM). The maximal vasorelaxation elicited by sodium nitroprusside appeared to be larger in the endothelium-denuded rings compared to the rings with intact endothelium, suggesting an enhanced NO sensitivity. Lastly, dietary Mg^{2+} supplementation did not affect the sodium nitroprusside-induced vasorelaxant response in the non-ethanol-consuming control group (data not shown). These data suggest that chronic ethanol consumption-induced depression in endothelium-independent vasorelaxant response was reversed with dietary Mg^{2+} supplementation.

4. Discussion

Chronic ethanol ingestion leads to both Mg^{2+} deficiency and vascular dysfunction, contributing to elevated risk of cardiovascular disease (Flink, 1986; Altura and Altura, 1987a, 1994; Brown and Savage, 1996). Mg^{2+} is necessary for the normal vascular function while its deficiency may contribute to the development of cardiovascular disease (Altura et al., 1984, 1992; Altura and Altura, 1986). Our current study confirmed previous reports (Altura and Altura, 1978; Nishio et al., 1988) that the loss of serum Mg^{2+} leads to vascular dysfunction following chronic ethanol consumption. More importantly, our results indicate that alcoholism-related vascular dysfunction may be directly associated with hypomagnesemia and may be restored by dietary Mg^{2+} supplementation. This is consistent with previous findings that ethanol-induced cerebral vascular dysfunction, especially severe vasospasm, ischemia, and stroke, may be ameliorated by Mg^{2+} supplementation (Altura and Altura, 1994; Altura et al., 1995; Ema et al., 1998).

Mild hypertension following chronic ethanol ingestion was observed in this study, consistent with previous reports (Altura and Altura, 1982, 1987a; Chan and Sutter, 1983). As stated earlier, the rise in blood pressure may be attributed to several mechanisms including secretion of hormones and neurotransmitters, stimulation of the sympathetic nervous system, alteration of baroreceptor activity (Altura and Altura, 1982, 1987a), and volume overload (Chan and Sutter, 1983). The chronic ethanol ingestion-induced reduction in serum Mg^{2+} levels observed in this study is consistent with previous reports and may contribute to the elevation in blood pressure (Flink et al, 1953; Flink, 1986; Brautbar and Altura, 1987). However, a rise in blood pressure following chronic ethanol ingestion may occur in

the absence of hypomagnesemia (Chan and Sutter, 1983). In our study, Mg^{2+} supplementation prevented ethanol-induced hypertension. This is supported by the fact that Mg^{2+} supplementation reduced the plasma ethanol level, suggesting that Mg^{2+} may facilitate ethanol metabolism as reported (Walker, 1994). It is not known whether the elevated liver size (liver-to-body weight ratio) in the Mg^{2+} supplementation group reflects an Mg^{2+}-induced alteration in hepatic enzymatic activity. It is worthy pointing out that the nonsignificant decrease in body weight may have also contributed to the enhanced ratio. In addition, other factors such as ethanol- and/or Mg^{2+}-induced myogenic alteration in vascular smooth muscle cells may exist and contribute to the blood pressure alteration.

Mg^{2+} plays an important role in the control of vascular smooth muscle function by modulating myogenic tone and the contractile response to various physiological stimuli (Altura et al., 1984, 1992). A reduction of Mg^{2+} potentiates (Altura and Altura, 1978; Altura et al., 1984; Nishio et al., 1988), whereas an elevation of Mg^{2+} attenuates the vasoconstrictive response (Yang et al., 2000). The chronic ethanol ingestion-induced increase in intracellular Ca^{2+} in vascular smooth muscle (Zhang et al., 1997) has been shown to be a result of the hypomagnesemic defect in the Ca^{2+} transport mechanism, leading to vascular hyperactivity, vasoconstriction, and increased peripheral resistance (Altura and Altura, 1978, 1982, 1987a, 1994; Altura et al., 1992). The present study revealed that Mg^{2+} therapy abolished the ethanol-induced hypertension. This result is consistent with previous reports (Altura and Altura, 1987a, 1994; Brown et al., 1998) and further supports the important role of Mg^{2+} metabolism in alcoholism-related vascular dysfunction.

One important finding from our study is that the altered vasoconstrictive response to KCl and norepinephrine following chronic ethanol consumption was prevented by dietary Mg^{2+} supplementation. Although the mechanism(s) behind the disparate vasoconstrictive responsiveness between KCl and norepinephrine is not fully understood, several potential explanations may be considered. Vasoconstriction has been shown to increase when the ratio of K^+/Mg^{2+} concentration rises, depicting an interaction between the two ions in the control of vascular tone (Murakawa et al., 1988). High extracellular Mg^{2+} interferes with Ca^{2+} entry through voltage-dependent Ca^{2+} channels (Delpiano and Altura, 1996). Both of these mechanisms infer an augmented vasoconstrictive response to KCl (which depolarizes the plasma membrane) following chronic ethanol ingestion-induced Mg^{2+} deficiency, corresponding with the increased blood pressure. On the other hand, the observation that ethanol ingestion attenuated the norepinephrine-induced vasoconstriction may indicate a reduced level or function of adrenoceptor or post-receptor signaling mechanisms. Reduced extracellular Mg^{2+} levels may directly lead to a rise in nuclear and perinuclear free Ca^{2+} via an extracellular Ca^{2+}-dependent mechanism (Altura et al., 2001). It is

possible that the overloaded intracellular compartment(s) may therefore lose sensitivity to norepinephrine stimuli. This may be supported by the notion that subtle changes in extracellular Mg^{2+} significantly affect the contractile response to norepinephrine, likely through modification of the Ca^{2+} availability rather than binding of norepinephrine to its receptor (Altura and Altura, 1978, 1982; Chan and Sutter, 1983). It is worthy pointing out that other ethanol-related mechanisms may have also contributed to the altered vascular response to norepinephrine. Reduced extracellular Mg^{2+} levels potentiate (instead of attenuate) norepinephrine-induced vasoconstriction (Turlapaty and Altura, 1980). The prazosin-induced blockade of the norepinephrine response favors a predominant role of α_1-adrenoceptor in the response discussed here. The similarity in the vasoconstrictive response to norepinephrine or KCl among all groups with denuded endothelium suggests that the altered vasoconstrictive response following ethanol ingestion may be endothelium-dependent. Furthermore, the fact that dietary Mg^{2+} supplementation was capable of restoring the ethanol-induced alteration in vasoconstrictive response confirms that Mg^{2+} ion is crucial in the regulation of intracellular Ca^{2+} homeostasis in the vascular endothelium (Altura et al., 1992; Yang et al., 1999).

The attenuated endothelium-independent vasorelaxant response to sodium nitroprusside observed in our study indicates impaired vasodilatation following chronic ethanol consumption. Interestingly, the endothelium-dependent vasorelaxation to carbamylcholine chloride in the chronic ethanol ingestion group was similar to that of the control group, consistent with Mg^{2+} deficiency (Nishio et al., 1988). These data favor chronic ethanol consumption interfering with the sensitivity of NO rather than the production of endogenous NO. Several mechanisms may be postulated for the reduced NO responsiveness including altered guanylate cyclase activity or other down-stream NO pathways such as decreased PI-3 kinase and G-kinase efficacy. Ethanol has been shown to elicit a biphasic effect on microcirculation, blood flow, and blood pressure. The vasodilatory effect of ethanol may be, in large part, a consequence of its direct actions on vascular smooth muscle cells (Altura and Altura, 1982). Paradoxically, certain doses of ethanol over a long-term may also induce vasoconstriction on peripheral blood vessels, the effects of which are dependent on intracellular free Ca^{2+} (Altura and Altura, 1982; Yang et al., 2001). It is believed that NO plays a critical role in the ethanol-induced microcirculatory and vascular contractile response (Yang et al., 2000, 2001). Ethanol (e.g., red wine) induces endothelial generation and release of NO, a potent vasodilator through cGMP production. On the other hand, excessive NO synthesis and the subsequent generation of reactive oxygen species have also been reported under chronic ethanol ingestion and may contribute to endothelial dysfunction (Zima et al., 2001). It has been speculated that the endothelial function under chronic ethanol ingestion may be regulated by the levels of Mg^{2+}. Recently, Alturas' group demonstrated

that extracellular Mg^{2+} ions induce both endothelium-dependent and -independent relaxation in rat aorta through a NO-dependent pathway (Altura and Altura, 1987b; Yang et al., 2000). Their findings indicated that Ca^{2+}-activated K^+ channels and elevation of intracellular Ca^{2+} as well as release of NO are responsible for the Mg^{2+}-evoked vasorelaxation.

One of the limitations of the current study is that the aorta is essentially a large conduit vessel with a minor contribution to the total peripheral resistance and, therefore, blood pressure regulation. Although the use of aortic rings to examine vascular responsiveness has long been a generally accepted practice, caution must be taken for data interpretation. Future investigations using models of small resistance vessels, such as tail and mesenteric arteries, are warranted.

In summary, chronic ethanol consumption alters both vasoconstrictive and vasorelaxant responses, while dietary Mg^{2+} supplementation attenuates such impairment. The loss and restoration of Mg^{2+} may affect the Mg^{2+}-regulated intracellular Ca^{2+} mobilization, ion channel function, protein synthesis, and contractile protein sensitivity, leading to altered vascular response. In addition, little evidence is available regarding the role of ethanol metabolites such as acetaldehyde and acetate in chronic ethanol ingestion-induced vascular dysfunction (Altura and Altura, 1982). Further studies are warranted to define the intracellular Ca^{2+} mobilization mechanism, the pathogenic significance of ethanol metabolic products, and therapeutic potential involving Mg^{2+} in the pathogenesis and prevention of vascular dysfunctions in alcoholism.

Acknowledgements

References

Altura, B.M., Altura, B.T., 1978. Magnesium and vascular tone and reactivity. Blood Vessels 15, 5–16.

Altura, B.M., Altura, B.T., 1982. Microvascular and vascular smooth muscle actions of ethanol, acetaldehyde, and acetate. Fed. Proc. 41, 2447–2451.

Altura, B.M., Altura, B.T., 1984. Magnesium, electrolyte transport and coronary vascular tone. Drugs 28 (Suppl. 1), 120–142.

Altura, B.M., Altura, B.T., 1986. Magnesium and cardiovascular diseases. In: Berthon, G. (Ed.), Handbook of Metal Ligand Interactions in Biological Fluids, vol. 2. Marcel Dekker, New York, pp. 822–842.

Altura, B.M., Altura, B.T., 1987a. Peripheral and cerebrovascular actions of ethanol, acetaldehyde, and acetate: relationship to divalent cations. Alcohol Clin. Exp. Res. 11, 99–111.

Altura, B.M., Altura, B.T., 1987b. Endothelium-dependent relaxation in coronary arteries requires magnesium ions. Br. J. Pharmacol. 91, 449–451.

Altura, B.M., Altura, B.T., 1994. Role of magnesium and calcium in alcohol-induced hypertension and strokes as probed by in vivo television microscopy, digital image microscopy, optical spectroscopy, ^{31}P-NMR, spectroscopy, and a unique magnesium ion-selective electrode. Alcohol Clin. Exp. Res. 18, 1057–1068.

Altura, B.M., Altura, B.T., 1995. Role of magnesium in the pathogenesis of hypertension updated: relationship to its actions on cardiac, vascular smooth muscle, and endothelial cells. In: Laragh, J.H., Brenner, B.M. (Eds.), Hypertension: Pathophysiology, Diagnosis and Management. Raven Press, New York, pp. 1213–1242.

Altura, B.M., Altura, B.T., Gebrewold, A., Ising, H., Gunther, T., 1984. Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes in situ. Science 223, 1315–1317.

Altura, B.M., Altura, B.T., Gebrewold, A., Ising, H., Gunther, T., 1992. Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium. J. Appl. Physiol. 72, 194–202.

Altura, B.M., Gebrewold, A., Altura, B.T., Gupta, R.K., 1995. Role of brain $[Mg^{2+}]_i$ in alcohol-induced hemorrhagic stroke in a rat model: a 31P-NMR in vivo study. Alcohol 12, 131–136.

Altura, B.M., Zhang, A., Cheng, T.P.O., Altura, B.T., 2001. Extracellular magnesium regulates nuclear and perinuclear free ionized calcium in cerebral vascular smooth muscle cells: possible relation to alcohol and central nervous system injury. Alcohol 23, 83–90.

Brautbar, N., Altura, B.M., 1987. Hypophosphatemia and hypomagnesemia result in cardiovascular dysfunction: theoretical basis for alcohol-induced cellular injury. Alcohol Clin. Exp. Res. 11, 118–126.

Brown, R.A., Savage, A.O., 1996. Effects of acute acetaldehyde, chronic ethanol, and pargyline treatment on agonist responses of the rat aorta. Toxicol. Appl. Pharmacol. 136, 170–177.

Brown, R.A., Crawford, M., Natavio, M., Petrovski, P., Ren, J., 1998. Dietary magnesium supplementation attenuates ethanol-induced myocardial dysfunction. Alcohol Clin. Exp. Res. 22, 2062–2072.

Chan, T.C.K., Sutter, M.C., 1983. Ethanol consumption and blood pressure. Life Sci. 33, 1965–1973.

De Carli, L.M., Leiber, C.S., 1967. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. J. Nutr. 91, 331–336.

Delpiano, M.A., Altura, B.M., 1996. Modulatory effect of extracellular Mg^{2+} ions on K^+ and Ca^{2+} currents of capillary endothelial cells from rat brain. FEBS Lett. 394, 335–339.

Ema, M., Gebrewold, A., Altura, B.T., Zhang, A., Altura, B.M., 1998. Alcohol-induced vascular damage of brain is ameliorated by administration of magnesium. Alcohol 15, 95–103.

Flink, E.B., 1986. Magnesium deficiency in alcoholism. Alcohol Clin. Exp. Res. 10, 590–594.

Flink, E.B., Stutzman, F.L., Anderson, A.R., Konig, T., 1953. Magnesium in delirium tremens. J. Clin. Invest. 32, 568.

Kalbfleish, L., Linderman, R.D., Ginn, H.E., Smith, W.O., 1963. Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. J. Clin. Invest. 42, 1471–1475.

Keane, B., Leonard, B.E., 1989. Rodent models of alcoholism: a review. Alcohol Alcohol. 24, 299–309.

Moore, R.D., Levine, D.M., Southard, J., Entwisle, G., Shapiro, S., 1990. Alcohol consumption and blood pressure in the 1982 Maryland Hypertension Survey. Am. J. Hypertens. 3, 1–7.

Morrill, G.A., Gupta, R.K., Kostellow, A.B., Ma, G.Y., Zhang, A., Altura, B.T., Altura, B.M., 1997. Mg^{2+} modulates membrane lipids in vascular smooth muscle: a link to atherogenesis. FEBS Lett. 408, 191–194.

Morrill, G.A., Gupta, R.K., Kostellow, A.B., Ma, G.Y., Zhang, A., Altura, B.T., Altura, B.M., 1998. Mg^{2+} modulates membrane sphingolipid and lipid second messenger levels in vascular smooth muscle cells. FEBS Lett. 440, 167–171.

Murakawa, T., Altura, B.T., Carella, A., Altura, B.M., 1988. Importance of

magnesium and potassium concentration on basal tone and 5-HT-induced contractions in canine isolated coronary artery. Br. J. Pharmacol. 94, 325–334.

Murphy, E., 2000. Mysteries of magnesium homeostasis. Circ. Res. 86, 245–248.

Nishio, A., Ishiguro, S., Matsumoto, M., 1988. Ex vivo study of the vascular reactivity to some vasoactive agents in isolated thoracic aorta from dietary magnesium-deficient rats. Magnesium Res. 1, 169–175.

Patel, V.B., Why, H.J., Richardson, P.J., Preedy, V.R., 1997. The effects of alcohol on the heart. Adverse Drug React. Toxicol. Rev. 16, 15–43.

Resnick, L.M., 1995. Ionic disturbances of calcium and magnesium metabolism in essential hypertension. In: Laragh, J.H., Brenner, B.M. (Eds.), Hypertension: Pathophysiology, Diagnosis and Management. Raven Press, New York, pp. 1169–1191.

Romani, A.M., Scarpa, A., 2000. Regulation of cellular magnesium. Front Biosci. 5, D720–D734.

Shi, J., Cui, J., 2001. Intracellular Mg^{2+} enhances the function of BK-type Ca^{2+}-activated K^+ channels. J. Gen. Physiol. 118, 589–606.

Turlapaty, P.D., Altura, B.M., 1980. Magnesium deficiency produces spasms of coronary arteries: relationship to etiology of sudden death ischemic heart disease. Science 208, 198–200.

Walker, G.M., 1994. The role of magnesium in biotechnology. Crit. Rev. Biotechnol. 14, 311–354.

Yang, Z.W., Altura, B.T., Altura, B.M., 1999. Low extracellular Mg^{2+} contraction of arterial muscle: role of protein kinase C and protein tyrosine phosphorylation. Eur. J. Pharmacol. 378, 273–281.

Yang, Z.W., Gebrewold, A., Nowakowski, M., Altura, B.T., Altura, B.M., 2000. Mg^{2+}-induced endothelium-dependent relaxation of blood vessels and blood pressure lowering: role of NO. Am. J. Physiol. 278, R628–R639.

Yang, Z.W., Wang, J., Zheng, T., Altura, B.T., Altura, B.M., 2001. Ethanol-induced contractions in cerebral arteries: role of tyrosine and mitogen-activated protein kinases. Stroke 32, 249–257.

Zhang, A., Cheng, T.P., Altura, B.T., Altura, B.M., 1997. Chronic treatment of cultured cerebral vascular smooth cells with low concentration of ethanol elevates intracellular calcium and potentiates prostanoid-induced rises in $[Ca^{2+}]_i$: relation to etiology of alcohol-induced stroke. Alcohol 14, 367–371.

Zima, T., Fialova, L., Mestek, O., Janebova, M., Crkovska, J., Malbohan, I., Stipek, S., Mikulikova, L., Popov, P., 2001. Oxidative stress, metabolism of ethanol and alcohol-related diseases. J. Biomed. Sci. 8, 59–70.