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Role of endothelial adenosine receptor-mediated vasorelaxation in ethanol-induced hypotension in hypertensive rats

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Abstract

Our previous findings showed that chronic ethanol feeding lowers blood pressure in spontaneously hypertensive rats. The present study investigated the role of the adenosine receptor–endothelial nitric oxide (NO) pathway in the hypotensive response to ethanol. Changes in blood pressure were evaluated in radiotelemetered pair-fed rats receiving liquid diet with or without ethanol (2.5% or 5%, w/v) for 12 weeks. The vasorelaxant activity of the adenosine analogue 5′-*N*-ethylcarboxamidoadenosine (NECA) in isolated aortic rings obtained from ethanol and control rats were evaluated. Ethanol (2.5% and 5%) lowered blood pressure in a dose-dependent manner. The hypotension started at week 1, reached its maximum at week 4 and remained so thereafter. In aortas with intact endothelium, NECA (10^{-10} to 10^{-4} M) produced a concentration-dependent relaxation of the phenylephrine-precontracted aortas. Compared with control rats, ethanol (2.5% and 5%) caused significant and concentration-related increases in NECA responses. This effect of ethanol was attenuated by the adenosine receptor antagonist 8-sulfophenyltheophylline and the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA). Further, endothelium denudation abolished the ethanol-evoked enhancement of NECA responses. The vasorelaxant responses to acetylcholine or sodium nitroprusside in aortic rings were not influenced by ethanol. In conclusion, the present findings suggest that chronic ethanol enhances the NO-dependent vasorelaxant responses to adenosine receptor activation and this may explain, at least partly, the mechanism of the hypotensive effect of ethanol in spontaneously hypertensive rats.

Keywords: Ethanol; NECA (5′-*N*-ethylcarboxamidoadenosine); Nitric oxide (NO); Blood pressure; Radiotelemetry; Aorta

1. Introduction

Clinical findings have shown that the effect of ethanol on blood pressure follows a J-shaped relationship depending on the amount of ethanol consumed (Klatsky et al., 1977). Clinical studies have shown that ethanol elevates blood pressure (Arkwright et al., 1982; Gruchow et al., 1985) and that ethanol cessation restores blood pressure to pre-drinking levels (Aguilera et al., 1999). Experimental findings suggest that ethanol-evoked hypertension involves sympathoexcitation (Chan et al., 1985; Russ et al., 1991) and impairment of baroreflex function (Abdel-Rahman et al., 1985; Abdel-Rahman and Wooles, 1987). Paradoxically, ethanol feeding has also been shown to elicit hypotension in hypertensive rats (Howe et al., 1989; El-Mas and Abdel-Rahman, 2000b). A hypotensive effect for ethanol has also been demonstrated in normotensive rats in some studies (Beilin et al., 1992; Hatton et al., 1992) but not in others (El-Mas and Abdel-Rahman, 2000b). The discrepancy in the blood pressure effect of ethanol cannot be accounted for by differences in the amounts of ethanol consumed or in the duration of feeding (Chan et al., 1985; Abdel-Rahman and Wooles, 1987; Beilin et al., 1992; Hatton et al., 1992). Hatton et al. (1992) suggested a role for the nutrient intake in the blood pressure response to ethanol because hypotension develops when ethanol is administered in liquid diet (Hatton et al., 1992) compared to a hypertensive response when given in drinking water (Chan et al., 1985; Abdel-Rahman and Wooles, 1987). The hypotensive effect of ethanol may also relate to alterations in the cardiovascular autonomic control (Howe et al., 1989). In a recent study from our laboratory (El-Mas and Abdel-Rahman, 2000b), ethanol reduced blood pressure, pressure variability, and circadian fluctuations in radiotelemetered spontaneously hypertensive rats but not in their normotensive counterparts. The lack of a hypotensive response to ethanol in normo-

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tensive rats has been attributed to the concomitant increase in sympathetic activity (El-Mas and Abdel-Rahman, 2000b).

The mechanism(s) by which ethanol lowers blood pressure is not clear. Nonetheless, evidence is available to implicate the neuromodulator adenosine in mediating some of the neurobiological effects of ethanol. For instance, adenosinergic pathways are involved in the ethanol-induced motor incoordination (Phan et al., 1997), reductions in myocardial ischemia–reperfusion injury (Miyamae et al., 1997), and increases in gastric mucosal blood flow (Nagata et al., 1996). Signal transduction studies demonstrated that ethanol inhibits adenosine uptake with consequent increases in extracellular adenosine and intracellular cAMP contents (Nagy, 1994; Sapru et al., 1994). Adenosine is known to lower blood pressure (Robertson et al., 1988; Casati et al., 1995) and to cause vasodilation of some vascular beds (Biaggioni, 1992; Herrmann and Feigl, 1992). The vasodilatory effect of adenosine may be attributed to the reduction in renin release (Kuan et al., 1990), inhibition of noradrenergic neurotransmission (Illes et al., 1989), or facilitation of nitric oxide (NO) release from vascular endothelium (Smits et al., 1990; Bassenge, 1992). Evidence is also available that implicates endothelium-independent mechanisms, smooth muscle adenosine receptors, and non-adenosinergic sites in the vasorelaxant effects of adenosine and its analogs as shown in previous studies including our own (Abebe et al., 1994, 1995; Prentice et al., 1995, 2001; Prentice and Hourani, 1996). The adenosine receptor antagonists have been shown to elevate blood pressure in rats (Azevedo and Osswald, 1992).

The primary objective of this study was to investigate the possible role of vascular adenosinergic mechanisms in the hypotensive response to chronically administered ethanol in spontaneously hypertensive rats. The hypothesis was tested that ethanol elicits hypotension via enhancement of the adenosine–NO pathway in vascular endothelium. This assumption warrants investigation because of the established role of adenosine in mediating cardiovascular responses to ethanol (Nagata et al., 1996; Miyamae et al., 1997) and the similar facilitatory effects of both substances on endothelial nitric oxide synthase activity (Smits et al., 1990; Abebe et al., 1995; Hendrickson et al., 1999; Venkov et al., 1999). Furthermore, several studies established a cause–effect relationship between enhanced endothelial nitric oxide activity and hypotension (Kishi et al., 2001; Gompf et al., 2002). To accomplish this goal, we evaluated the effects of a 3-month ethanol feeding (2.5% or 5% w/v liquid diet) on blood pressure and on adenosine receptor-mediated vasorelaxation in aortas of spontaneously hypertensive rats. The radiotelemetry technique was used for blood pressure measurement in conscious animals. The adenosine–NO relationship was assessed by evaluating the relaxant responses to the adenosine analogue 5′-*N*-ethylcarboxamidoadenosine (NECA) in aortas with intact and denuded endothelium. The effects of adenosine receptor blockade or NO synthase inhibition by 8-sulfophenyltheophylline and N^G-monomethyl-L-arginine (L-NMMA), respectively, on NECA responses were also determined.

2. Methods

A total of 25 male spontaneously hypertensive rats (10–11 weeks, 250–300 g, Taconic, Germantown, NY) was used in the present study. Upon arrival, the rats were housed individually in standard plastic cages and allowed free access to water and purina chow and were maintained on a 12–12-h light–dark cycle with light off at 7:00 PM. The room temperature was maintained at 22 ± 1 °C. After 1-week acclimatization, rats were fed a regular Lieber-DiCarli liquid diet (Dyets, Bethlehem, PA) for 7 days before implantation of the telemetry device. Rats received the diet daily at 8:30 AM. All experiments were performed in strict accordance with institutional animal care and use guidelines.

2.1. Telemetry system

The telemetry system (Data Sciences, St. Paul, MN) used in this study has been described in previous studies including ours (Webb et al., 1998; El-Mas and Abdel-Rahman, 2000a,b). The system consists of five major components: (i) implantable transmitter unit for measurement of blood pressure, (ii) radio receiver to receive telemetered signals, (iii) ambient pressure monitor to measure absolute atmospheric pressure, (iv) a consolidation matrix to multiplex multiple cage signals to the computer, and (v) a PC-based data acquisition system to process signals. The implanted sensor consisted of a fluid-filled catheter (0.7 mm diameter, 15 cm long, Model TA11PA-C40) connected to a highly stable low-conductance strain-gauge pressure transducer, which measured the absolute arterial pressure relative to a vacuum, and a radio-frequency transmitter. The tip of the catheter was filled with a viscous gel that prevented blood reflux and was coated with an antithrombogenic film to inhibit thrombus formation and maintain patency. The distal 1 cm of the catheter consisted of a thin-walled thermoplastic membrane while the remainder of the catheter was composed of a thick-walled low-compliance urethane. The implants (2.5 cm length and 1.2 cm diameter) weighed 9 g and had a typical battery life of 6 months. Implants were gas sterilized and provided precalibrated (relative to vacuum) by the manufacturer and calibrations were verified to be accurate within 3 mm Hg (Brockway et al., 1991). A radio receiver platform (RLA1010, Data Sciences Int.) connected the radio signal to digitized input that was sent to a dedicated personal computer (Compaq, Pressario 9548). Arterial pressures were calibrated by using an input from an ambient-pressure monitor (C11PR, Data Sciences Int.).

2.2. Transmitter implantation

The method described in our previous studies (El-Mas and Abdel-Rahman, 2000a,b) was adopted. The rats were anesthetized with intraperitoneal injection of a mixture of ketamine (90 mg/ml; Ketaject) and xylazine (10 mg/ml; Xyla-ject). The abdomen was opened with a midline incision (4 cm). Another incision (1.5 cm) was made along the inner thigh to expose the femoral artery. The abdominal wall was pierced with a large bore syringe needle (15 gauge) from the femoral side into the peritoneal cavity. The implant body was placed in the peritoneal cavity and the catheter (15 cm) was passed caudally through the syringe needle into the thigh area. A 5-cm portion of the catheter was inserted into the femoral artery and secured in place with sutures. The abdominal muscle was closed with non-absorbable suture incorporating the implant suture rib with alternating stitches. The skin (abdomen and thigh) was closed with surgical clips. Each rat received a subcutaneous injection of the analgesic ketorolac tromethamine (2 mg/kg; Toradol) and an intramuscular injection of 60 000 U of penicillin G benzathine and penicillin G procaine in an aqueous suspension (Durapen). Rats were allowed to recover for 2 weeks prior to the start of experimentation.

2.3. Isolated aortic ring preparation

At the end of the 3-month period of the study, rats were killed by decapitation and thoracic aortas were removed immediately, trimmed free of connective tissue, cut into ring segments 3 mm in length, and placed into cold Krebs–Henseleit solution of the following composition (in mM): 118 NaCl, 4.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$ and 11 glucose, and pH 7.4. Aortic rings with intact or denuded endothelium were mounted in organ baths containing 10 ml of Krebs–Henseleit solution, gassed with a mixture of 95%O_2–5%CO_2 and maintained at 37 °C. The endothelium was removed mechanically by gentle rubbing of the intimal surface of the aorta with a fine forceps. The forceps was inserted into the lumen of the ring and rolled back and forth on a filter paper moistened with the physiological solution (El-Mas et al., 1997). Proper removal of the endothelium was tested by the reduction (at least 45%) in the vasorelaxant responses to acetylcholine in rings precontracted with phenylephrine (El-Mas et al., 1997). The rings were equilibrated for 90 min under a resting tension of 1.5 g with the bath fluid being replaced every 15 min. Changes in isometric tension were measured with force transducers (TSD125C from BIOPAC Systems, Santa Barbara, CA) connected to the MP100WSW (BIOPAC Systems), a data acquisition system that converts incoming signals into digital signal processed with a PC computer. Data acquisition process and post-acquisition calculations were performed with *AcqKnowledge* software for windows 95 (BIOPAC Systems).

2.4. Measurement of plasma ethanol concentration

A blood sample was taken from each rat at the end of the study and its ethanol content was determined by the enzymatic method described by Bernt and Gutmann (1974) as in our previous studies (El-Mas and Abdel-Rahman, 2000a,b).

2.5. Protocols and experimental groups

2.5.1. Hemodynamic effects of chronic ethanol feeding

Three groups of telemetered rats ($n = 8–9$ each) matched for body weight and baseline blood pressure and heart rate were used in the present study to investigate the hemodynamic responses to chronic ethanol feeding. All rats were fed control liquid diet 7 days before implantation of the telemetry transmitter. Two weeks after transmitter implantation, two groups were provided a regular Lieber-DiCarli liquid diet containing 2.5% or 5% ethanol (w/v, 18 and 36% of total caloric intake, respectively). The third group of rats (controls) was pair-fed and received isocaloric amount of dextrin/maltose (89.6 g/l) in place of ethanol, which allowed similar nutrient intake and fluid consumption to that of ethanol-fed rats. Fresh diets were prepared every other day and stored in the refrigerator until dispensed. Rats were maintained on the ethanol or control diet for 12 weeks. Individual rat cages were placed on the top of the radio receivers and all data were collected using a computerized data acquisition system (Dataquest ART, Data Sciences Int.). The system is designed to cycle from animal to animal.

2.5.2. Vasorelaxant responses

After 90-min equilibration period, cumulative concentration–response curves to stepwise cumulative addition of NECA (10^{-10} to 10^{-4} M) were established in aortic rings, obtained from ethanol (2.5% or 5%) or pair-fed control spontaneously hypertensive rats, precontracted with phenylephrine (1 μM) as described elsewhere (Fahim et al., 1994). Each new addition was made after the response to the previous addition had attained a steady state. Changes in NECA relaxations by ethanol were measured in aortic rings with intact or denuded endothelium. The effects of ethanol on relaxant responses to cumulative addition of acetylcholine (10^{-9} to 10^{-5} M) and sodium nitroprusside (10^{-10} to 10^{-5} M) were also determined.

2.5.3. Effect of L-NMMA and 8-sulfophenyltheophylline on the vasorelaxant responses

This experiment investigated the effect of nitric oxide synthase inhibition and adenosine receptor blockade by L-NMMA (30 μM) and 8-sulfophenyltheophylline (10 μM), respectively, on the vasorelaxant responses to NECA in aortic rings with intact or denuded endothelium obtained from ethanol or control groups. Aortic rings were contracted with 1 μM phenylephrine and then incubated with 8-sulfophenyltheophylline or L-NMMA for 30 min before establishing the concentration–response curve to NECA.

Neither 8-sulfophenyltheophylline nor L-NMMA had any significant effect on phenylephrine contractions.

2.6. Drugs

Phenylephrine hydrochloride, sodium nitroprusside, acetylcholine chloride, N^G-monomethyl-L-arginine (Sigma, St. Louis, MO), 8-sulfophenyltheophylline, 5′-*N*-ethylcarboxamidoadenosine (Research Biochemicals, Natick, MA), Ketaject (ketamine), Xyla-ject (xylazine) (Phoenix Pharmaceuticals, St. Joseph, MI), Toradol (ketorolac tromethamine, Abbott Labs, Chicago, IL), Durapen (Penicillin G benzathine and penicillin G procaine, Vedco, Overland Park, KS), and ethanol (Midwest Grain Products, Weston, MO) were purchased from commercial vendors.

2.7. Data analysis

All values are expressed as means ± S.E.M. Waveforms of blood pressure for each rat was sampled at a rate of 500 Hz for 10 s every 10 min. The data were averaged in 60-min blocks for analysis. All parameters (mean arterial pressure and heart rate) were averaged over a 7-day period for weekly values as in our previous studies (El-Mas and Abdel-Rahman, 2000a,b). Vasorelaxant responses to NECA, acetylcholine and nitroprusside were calculated as a percent decrease in steady state contraction obtained with 1 μM phenylephrine. The agonist concentration–response curves were transformed to log–logit plots as described in our previous studies (Abebe et al., 1994). Changes in adenosine receptor sensitivity to NECA were evaluated by computing the slopes of the regression lines of NECA concentration–response curves for individual tissues (El-Mas et al., 1997). Analysis of variance (ANOVA) followed by a Newman–Keuls post-hoc analysis was used to analyze the effects of ethanol on hemodynamic parameters and vasorelaxant responses, respectively. Probability levels less than 0.05 were considered significant.

3. Results

3.1. Hemodynamic effects of chronic ethanol feeding

Baseline (week 0) body weight, mean arterial pressure, and heart rate were similar in rats subsequently receiving ethanol (2.5% or 5%) or control diet (Table 1). Blood ethanol concentration measured at the end of the study was 61 ± 14 and 194 ± 36 mg/dl in spontaneously hypertensive rats that received 2.5% and 5% ethanol, respectively. These values correlated well with the ethanol intakes that amounted approximately to 4 and 9 g/kg/day in both groups, respectively. Ethanol was not detectable in the blood of control rats.

Table 1
Baseline values of body weight (g), mean arterial pressure (MAP, mmHg), and heart rate (HR, beats/min) in ethanol (2.5% and 5%) and pair_fed control spontanoeusly hypertensive rats

Group	Body weight	MAP	HR
Control	299.2 ± 7.7	130.1 ± 2.0	363.8 ± 6.1
Ethanol 2.5%	298.1 ± 7.8	129.7 ± 2.0	365.3 ± 2.8
Ethanol 5%	298.1 ± 7.7	135.3 ± 2.1	358.2 ± 4.8

Values are mean ± S.E.M.

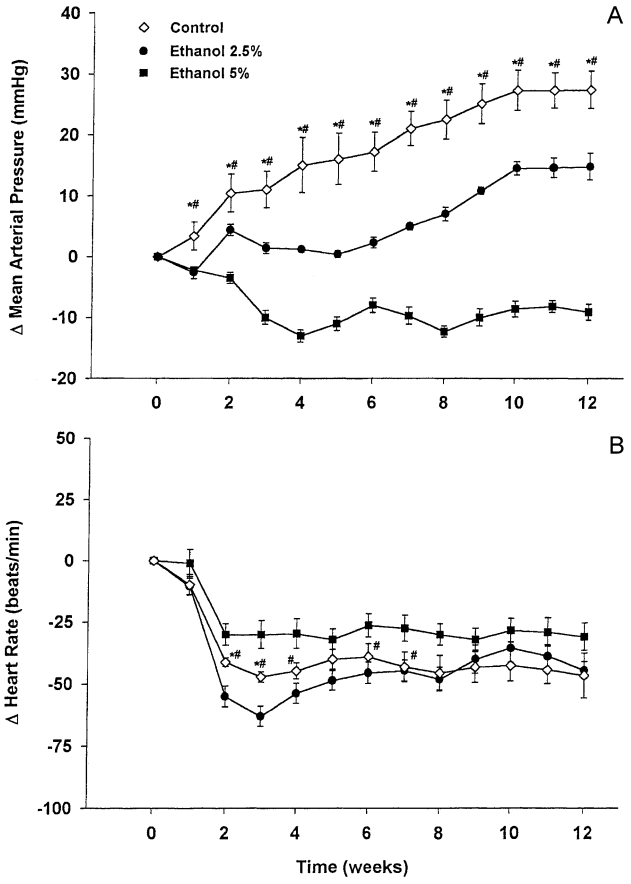

Fig. 1. Changes in mean arterial pressure and heart rate evoked by chronic ethanol feeding (2.5% and 5%, 3 months) in radiotelemetered spontaneously hypertensive rats. Values are mean ± S.E.M. of eight to nine observations. * and #$P<0.05$ vs. corresponding 2.5% and 5% ethanol values, respectively.

Weekly changes in mean arterial pressure and heart rate evoked by chronic ethanol feeding, as compared to control pair-fed rats, are shown in Fig. 1. In control rats, the mean arterial pressure showed gradual and progressive increases over the duration of the study (Fig. 1). Ethanol feeding produced significant ($P<0.05$) and dose-dependent decreases in mean arterial pressure compared with the control group (Fig. 1A). The hypotensive effect of the higher dose (5%) of ethanol started as early as week 1 after ethanol feeding (Fig. 1A). The maximum fall in mean arterial pressure was obtained at week 4 and continued throughout the 12-week treatment period (Fig. 1). The mean arterial pressure of the ethanol (2.5%) group showed a slight increase over time but this increase was significantly

($P<0.05$) less than that observed in the pair-fed control rats (Fig. 1A). The heart rate was decreased in all rat groups during the first 2 weeks and then remained stable till the end of the study. However, ethanol (2.5%) produced significantly ($P<0.05$) greater decreases in heart rate at weeks 2 and 3 compared with control rats, whereas ethanol (5%) caused significantly smaller decreases in heart rate at weeks 2, 3, 4, 7 and 8 (Fig. 1B).

3.2. Effect of chronic ethanol administration on the vasorelaxant responses

Changes evoked by ethanol feeding in the relaxant responses to NECA in aortic rings with intact or denuded endothelium are shown in Fig. 2. In rings with intact endothelium, cumulative addition of NECA (10^{-10} to 10^{-4} M) resulted in concentration-related relaxations of phenylephrine precontracted aortic rings. Ethanol (2.5% and 5% w/v) caused significant ($P<0.05$) and concentration-related increases in the responsiveness of adenosine receptors to NECA

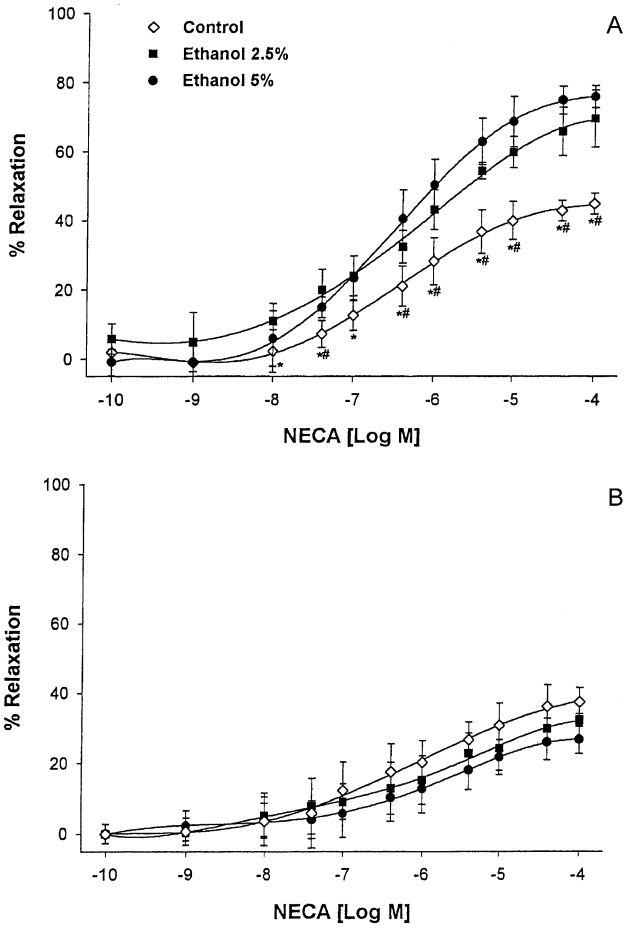

Fig. 2. Effect of chronic ethanol feeding (2.5% and 5%, 3 months) to spontaneously hypertensive rats on the vasorelaxant responses to 5′-*N*-ethylcarboxamidoadenosine (NECA) in aortic rings with intact (panel A) or denuded (panel B) endothelium. Values are mean ± S.E.M. of six to eight observations. * and #$P<0.05$ vs. corresponding 2.5% and 5% ethanol values, respectively.

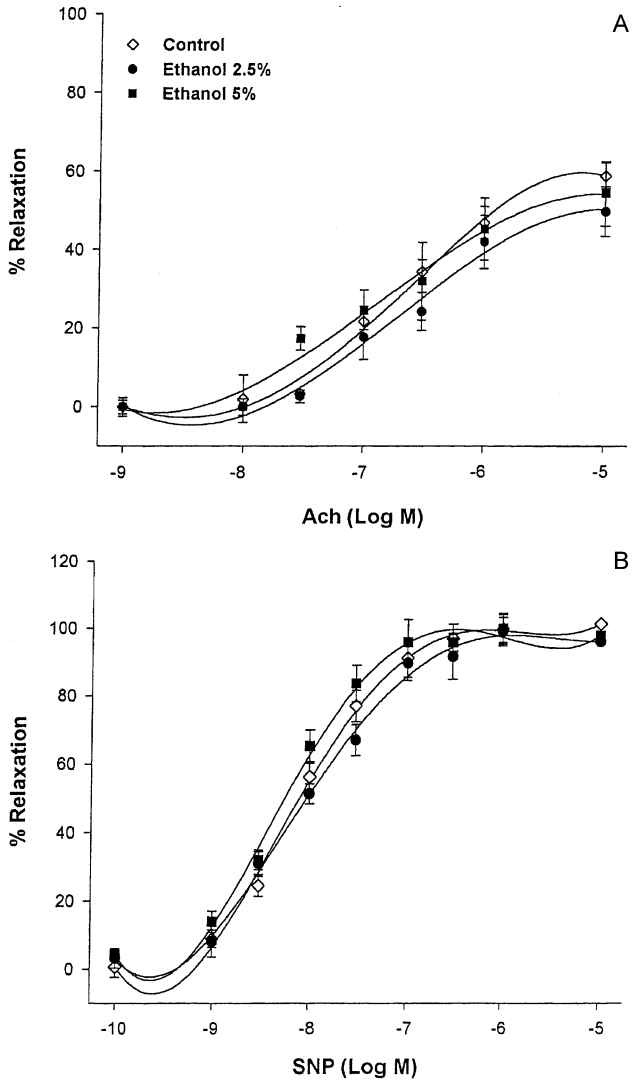

Fig. 3. Effect of chronic ethanol feeding (2.5% and 5%, 3 months) to spontaneously hypertensive rats on the vasorelaxant responses to acetylcholine (Ach, panel A) and sodium nitroprusside (SNP, panel B) in aortic rings. Values are mean ± S.E.M. of five to eight observations.

(Fig. 2A). The slopes of the regression lines, which represented receptor sensitivity, were increased from 8.0 ± 1.2% relaxation/log M in aortas of control rats to 14.1 ± 1.2% and 20.1 ± 1.7% relaxation/log M in ethanol (2.5% and 5%) groups, respectively. The maximum relaxant responses evoked by NECA (10^{-4} M) showed significant and similar increases by the two ethanol concentrations (2.5% and 5%) compared with control values (69.7 ± 5.2%, 76.5 ± 3.1% and 45.2 ± 1.1%, respectively). The ethanol-evoked enhancement of NECA relaxation was completely abolished in endothelium-denuded aortic rings. As shown in Fig. 2B, NECA produced similar relaxations in endothelium-denuded rings obtained from ethanol (2.5% or 5%) or control spontaneously hypertensive rats.

Cumulative addition of acetylcholine (10^{-9} to 10^{-5} M) or sodium nitroprusside (10^{-10} to 10^{-5} M) to phenylephrine precontracted aortic rings with intact endothelium

produced concentration-dependent relaxations (Fig. 3). The responses to either vasorelaxant were not altered by long-term feeding of 2.5% or 5% ethanol (Fig. 3).

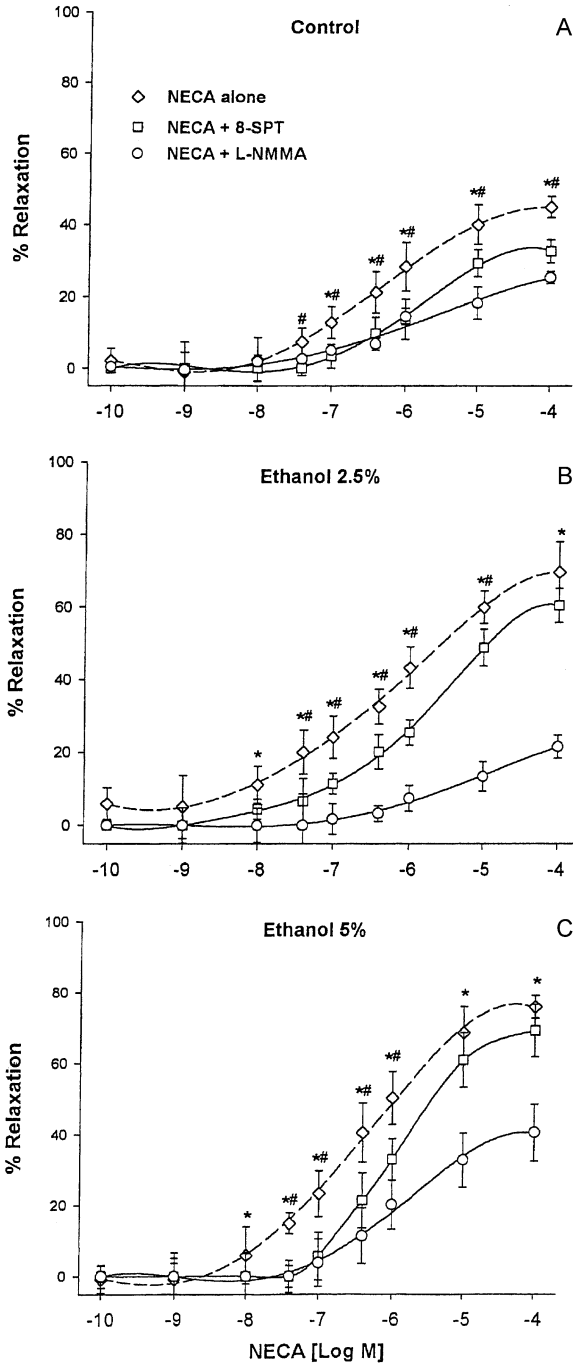

Fig. 4. Effects of adenosine receptor blockade by 8-sulfophenyltheophylline (8-SPT, 10 μM) and nitric oxide synthase inhibition by N^G-monomethyl-L-arginine (L-NMMA, 30 μM) on the vasorelaxant responses to 5′-*N*-ethylcarboxamidoadenosine (NECA) in aortic rings with intact endothelium obtained from ethanol (2.5% and 5%, 3 months) or pair-fed control spontaneously hypertensive rats. Values are mean ± S.E.M. of five to seven observations. * and #$P<0.05$ vs. corresponding L-NMMA and 8-SPT values, respectively.

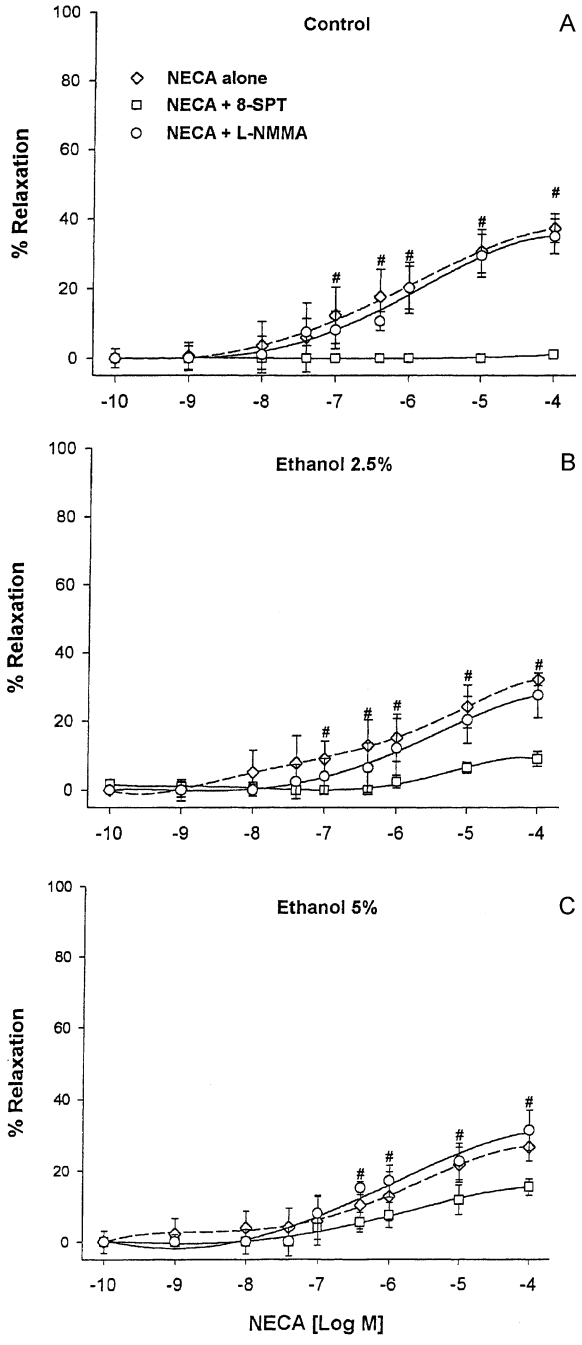

Fig. 5. Effects of adenosine receptor blockade by 8-sulfophenyltheophylline (8-SPT, 10 μM) and NO synthase inhibition by N^G-monomethyl-L-arginine (L-NMMA, 30 μM) on the vasorelaxant responses to 5′-*N*-ethylcarboxamidoadenosine (NECA) in aortic rings with denuded endothelium obtained from ethanol (2.5% and 5%, 3 months) or pair-fed control spontaneously hypertensive rats. Values are mean ± S.E.M. of six to seven observations. #$P<0.05$ vs. corresponding 8-SPT values.

3.3. Effect of L-NMMA and 8-sulfophenyltheophylline on the vasorelaxant responses

The effect of nitric oxide synthase inhibition (by L-NMMA) or adenosine receptor blockade (by 8-sulfophe-

nyltheophylline) on vasorelaxant responses to NECA in aortic rings with intact and denuded endothelium obtained from ethanol and pair-fed control rats are illustrated in Figs. 4 and 5. In rings with intact endothelium, pretreatment with L-NMMA (30 μM) or 8-sulfophenyltheophylline (10 μM) for 30 min caused significant ($P<0.05$) decreases in the relaxant responses and downward shifts in the concentration–response relationships of NECA in ethanol (2.5% and 5%) and control groups (Fig. 4). L-NMMA was more effective in reducing NECA responses in preparations obtained from ethanol-fed rats. The L-NMMA-induced reductions in the slopes of NECA concentration–response curves amounted to 41.2 ± 3.5% in control rats compared to 60.3 ± 7.7% and 57.1 ± 5.7% in ethanol (2.5% and 5%) groups, respectively. In endothelium-denuded preparations, the NECA relaxations were not affected by L-NMMA in aortas obtained from all rat groups (Fig. 5). In contrast, 8-sulfophenyltheophylline abolished NECA responses in control aortas and caused partial attenuation of the responses in aortas obtained from ethanol-fed rats (Fig. 5).

4. Discussion

The current study presents important findings that implicate the endothelial adenosine receptor–NO pathway in the hypotensive effect of long-term ethanol feeding in spontaneously hypertensive rats. Ethanol feeding to spontaneously hypertensive rats for 3 months produced a sustained fall in blood pressure that was associated with dose-related increases in the vasorelaxant responses of aortic smooth muscle to the adenosine receptor analogue NECA but not to acetylcholine or sodium nitroprusside. Ethanol appears to facilitate the adenosine-dependent NO activity in vascular endothelium because the potentiation of NECA-evoked relaxations by ethanol was abolished after the inhibition of nitric oxide synthase and also by endothelium denudation. The finding that 8-sulfophenyltheophylline effectively reduced ethanol potentiation of NECA responses provides further support for the involvement of adenosine receptors in ethanol-evoked enhancement of the NO-dependent relaxation.

The present finding that chronic ingestion of ethanol lowered blood pressure in spontaneously hypertensive rats is consistent with previous findings including ours (Howe et al., 1989; Beilin et al., 1992; El-Mas and Abdel-Rahman, 2000b). However, in contrast to our previous study (El-Mas and Abdel-Rahman, 2000b), which employed a single concentration of ethanol (5%), the present study tested the effect of 2.5% and 5% ethanol and demonstrated a dose-related hypotensive effect for ethanol. The findings obtained from the in vitro studies of the present investigation support a role for the endothelial adenosine–NO pathway in mediating the hypotensive effect of ethanol in spontaneously hypertensive rats. This is supported by the finding that ethanol hypotension was associated with a dose-related increase in the vasorelaxant responses to the adenosine analogue NECA in isolated aortas. The maximal relaxant response to NECA was approximately 50% and 70% greater in rats receiving the 2.5% and 5% ethanol, respectively, compared with the pair-fed controls. This effect of ethanol seems to be selective and involves only adenosinergic mechanisms as the vasorelaxant responses to acetylcholine and sodium nitroprusside were not altered by chronic ethanol feeding.

The modulation by ethanol of the adenosinergic-endothelial NO pathway is supported by two observations. First, the potentiation of NECA vasorelaxant activity by ethanol was completely abolished in endothelium-denuded aortas; a finding that clearly implicates the endothelium and its relaxing factors in the ethanol–adenosine interaction. Second, the inhibition of nitric oxide synthase activity by L-NMMA caused a significant attenuation of NECA responses in control as well as in ethanol groups. However, L-NMMA evoked attenuation of the relaxant responses to NECA was remarkably greater in ethanol-fed rats as compared with control rats. These findings are consistent with previous reports including our own that the vasorelaxant responses to adenosine are endothelium-dependent (Fahim et al., 1994; Prentice and Hourani, 1996) and that the cellular signal pathway mediating the dilation of isolated arteries by NECA involves the production of NO by endothelial cells (Abebe et al., 1995; Hein and Kuo, 1999). Taken together, the present findings suggest that enhancement of the NO-dependent adenosine responsiveness in vascular endothelium contributes, at least in part, to the hypotensive action of chronically administered ethanol in spontaneously hypertensive rats. Notably, the blood ethanol concentrations achieved in the present study are comparable to those attained in humans following consumption of moderate to intoxicating amounts of ethanol (Abdel-Rahman et al., 1987; Potter and Beevers, 1984).

The adenosine receptor antagonist 8-sulfophenyltheophylline was used in the present study to evaluate the role of adenosine receptors in the ethanol–endothelial NO interaction. The results showed that 8-sulfophenyltheophylline exhibited quite variable effects on NECA responses that depended on the tissue preparation (control vs. ethanol) and the status of the endothelium (intact vs. denuded). 8-Sulfophenyltheophylline caused partial but significant attenuation of NECA-evoked responses in aortas with intact endothelium from all groups of rats. However, in endothelium-denuded aortas, 8-sulfophenyltheophylline virtually abolished NECA responses in aortas of control rats while a residual relaxant response was evident in aortas obtained from the ethanol groups. The reason for these discrepancies is not clear. The possibility should be considered, however, that adenosine receptor agonists relax rat aorta via the activation of multiple adenosine receptors located on the smooth muscle and endothelial cells (Prentice and Hourani, 1996). Further, adenosine and some of its analogues produce relaxations that are resistant to 8-sulfophenyltheophyl-

line and other xanthines in rat aorta (Prentice and Hourani, 1996) and in other smooth muscle preparations (Brackett and Daly, 1991; Prentice et al., 1995). Whether chronic ingestion of ethanol modifies the adenosine receptor identities at endothelial and smooth muscle levels and their subsequent responsiveness to adenosine agonists and antagonists remains to be investigated.

The findings of the present study that facilitation of endothelial adenosinergic pathways mediates, at least in part, the blood pressure lowering effect of ethanol in spontaneously hypertensive rats are consistent with reported findings that highlighted the importance of vascular endothelium and adenosinergic mechanisms in circulatory control. Endothelium dysfunction is known to play a fundamental role in the pathogenesis of hypertension (Taddei et al., 2002). The activation of adenosine receptors has been shown to lower blood pressure whereas their blockade causes hypertension (Biaggioni, 1992; Azevedo and Osswald, 1992; Abdel-Rahman and Tao, 1996). Hypertension has also been demonstrated in adenosine A_{2a} receptor-knockout mice (Ledent et al., 1997). Interestingly, previous studies from our laboratory have shown that acutely administered ethanol elicited hypotension in conscious female rats (El-Mas and Abdel-Rahman, 1999) and this effect was abolished by prior administration of L-NMMA, thus implicating NO in ethanol hypotension (unpublished observation). Future studies will be undertaken to determine whether chronic inhibition of NO synthesis or blockade of adenosine receptors prevents ethanol-evoked hypotension in spontaneously hypertensive rats.

The reason(s) as to why chronic ethanol feeding enhanced vasorelaxant responses to the adenosine analogue NECA but not acetylcholine is not clear. Given the established role of vascular endothelium and its relaxing factors in responses evoked by either vasorelaxant (Fahim et al., 1994; Wu et al., 1997; Hein and Kuo, 1999), the present findings may suggest a selective interaction of ethanol with endothelial adenosinergic pathways. One possible explanation for the variability in the effect of ethanol on NECA and acetylcholine relaxations might relate to differences in the identity of relaxing factors mediating these responses. The present study highlights a predominant role for NO in NECA relaxations because they were largely blocked by L-NMMA (see Fig. 4). In contrast, vasorelaxant responses to acetylcholine in the vasculature of hypertensive rats are mediated via not only NO but also other endothelial factors such as vasodilator prostanoids are involved (Takase et al., 1994), which may be ethanol resistant. The notion also should be considered that acetylcholine releases endothelial-constricting prostanoids, an effect that is more evident in hypertensive rats (Takase et al., 1994; Kahonen et al., 1998) and may act to offset potential facilitation of cholinergically mediated vasorelaxations by ethanol. Moreover, evidence is available that the nitric oxide level is negatively related to muscarinic acetylcholine receptor density (Caron and Alling, 2001). It is conceivable, therefore, that the effect of ethanol on adenosinergic nitric oxide pathway may have resulted in downregulation of endothelial muscarinic receptors and subsequently their responsiveness to acetylcholine. Finally, the preferential facilitatory effect of ethanol on NECA responses may possibly include an increased density and/or affinity of endothelial adenosine receptors or enhancement of NECA-mediated release of nitric oxide. More studies are needed, therefore, to investigate these possibilities.

In conclusion, the present study investigated the possible interaction between ethanol, administered chronically, and the NO-dependent vasorelaxant responses elicited by the adenosine analogue NECA and its role in ethanol-evoked hypotension in radiotelemetered spontaneously hypertensive rats. The results indicate that the blood pressure lowering effect of ethanol was associated with enhanced vasorelaxant responses to NECA in aortic smooth muscle. The enhanced NECA responses were completely abolished in endothelium-denuded preparations and significantly attenuated by nitric oxide synthase inhibition and by adenosine receptor blockade. Collectively, these results suggest that ethanol lowers blood pressure in spontaneously hypertensive rats through a mechanism that is totally endothelium dependent and involves, at least partly, enhancement of the adenosine receptor–NO pathway.

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