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Effects of chronic ethanol consumption on aortic constriction in male and female rats

Christopher W. Stewart, Richard H. Kennedy *

Department of Pharmacology and Toxicology, Mail slot 611, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR 72205-7101, USA

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Abstract

This study was designed to determine if gender influences the effects of chronic ethanol intake on vasoconstrictive responsiveness. Ethanol-preferring rats were allowed ad libitum access to tap water or tap water containing 20% or 30% ethanol for 16 weeks. All of the ethanol groups consumed more daily calories than their respective controls, and female rats consumed more ethanol calories per unit body mass than their male counterparts. Following treatment, endothelium-intact and endothelium-denuded thoracic aortic rings were used to examine the contractile response to phenylephrine. Ethanol consumption did not alter vasoconstriction in endothelium-intact aortae from either gender. In contrast, males, but not females, demonstrated an ethanol-associated increase in the maximum response to phenylephrine in endothelium-denuded preparations. Aortae from male rats that consumed 20% and 30% ethanol showed an increased contractility of 37% and 85%, respectively. These data indicate that gender influences the vasoconstrictive effects elicited by chronic ethanol consumption and suggest that males may be more susceptible to the associated hypertension.

Keywords: Ethanol; Vascular Response; Phenylephrine; (P-rat); Gender

1. Introduction

Chronic consumption of ethanol is known to produce several adverse cardiovascular effects including hypertension and alcoholic heart muscle disease (Davidson, 1989). Epidemiological data suggest that the risk of ethanol-associated cardiovascular disease is greater in men than women (Manolio et al., 1991), but the mechanisms underlying this proposed gender difference are unknown. Gender-related variation in vascular responsiveness may play a role. For example, Maddox et al. (1987) reported that the endothelium of female rats has a greater capacity for regulating the tension generated by vascular smooth muscle. Later studies by others demonstrated that estrogen receptors are present within the cardiovascular system of female rats (Perrot-Applanat, 1996), and that estrogen produces a gender-specific relaxation of the vasculature in female rats (McNeill et al., 1996) and in women (Kawano et al., 1997). The current study was conducted to determine if the enhanced vasoconstrictive responsiveness elicited by chronic ethanol consumption is affected by gender. Experiments examined the vascular response to α-adrenoceptor stimulation in thoracic aortae isolated from control and ethanol-treated male and female rats.

2. Materials and methods

2.1. Animals and housing

Two-month old male ($n = 16$) and female ($n = 16$) ethanol-preferring rats (P-rats, University of Indiana) were housed on-site for 2 weeks prior to beginning experiments. While the animals were acclimating to our facilities, they were group housed (4 per cage) by gender. Food (Harlan Rat Chow # 8640) and tap water were available ad libitum. The temperature of the animal room was maintained at 22 ± 1°C with a 12-h light/dark cycle.

* Corresponding author. Tel.: +1-501-686-8041; Fax: +1-501-686-5521; E-mail: kennedyrichardh@exchange.uams.edu

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2.2. Ethanol treatment

For the duration of the 16-week treatment period, all animals were housed individually to facilitate monitoring of body weight, food consumption and fluid intake. Each gender group was equally divided into 8 control and 8 treated animals. The treated animals were equally subdivided into groups that were ultimately provided tap water containing 20 and 30% (v/v) ethanol ($n = 4$ per group). Ethanol was added to the drinking water starting at 10% (v/v). Each week the concentration of ethanol was increased by 10% until the level of long-term treatment was established.

2.3. Experimentation

After the 16-week treatment period, animals were anesthetized for removal of the thoracic aortae which were bathed in a Krebs–Henseleit (KH) solution (30°C). The composition of the KH solution was as follows (in mM): 118.0 NaCl, 25.0 $NaHCO_3$, 3.7 KCl, 1.0 KH_2PO_4, 1.4 $CaCl_2$, 1.2 $MgCl_2$, and 11.0 dextrose. The solution was buffered to pH 7.4 by saturation with 95% O_2/5% CO_2 gas. Vessels were dissected into two ring segments (3–4 mm in length) with the endothelial lining being removed from one of the segments by gentle physical means. Each ring segment was suspended vertically between two 21 gauge stainless-steel hooks. The top hooks were connected to force–displacement transducers (Type FT03, Grass Instrument, Quincy, MA) for continuous recording of isometric tension (Grass Model 7 D Polygraph). The vessels were maintained at a resting tension of 2 g and equilibrated for 90 min. A stable contractile response was acquired by repeatedly increasing the buffer KCl concentration to 80 mM with subsequent washout after each steady-state contraction was obtained. Endothelial integrity was monitored near the end of the equilibration period by examining the response to acetylcholine. Preparations were contracted by 10^{-6} M phenylephrine, and 10^{-5} M acetylcholine was added after a stable contraction was obtained. An intact endothelium was denoted by relaxation $> 50\%$; absence of endothelium was denoted by no relaxation or by contraction.

After equilibration, concentration–response curves for phenylephrine were obtained by cumulative addition; each concentration of agonist was added to the medium only after the tissues reached a steady-state response at the previous level. After completing the concentration–response curves, tissues were dried to constant weight at 100°C and weighed. Developed tension was expressed per unit tissue dry weight. EC_{50} values were obtained by graphical evaluation of individual concentration–response curves.

2.4. Statistics

Results are presented as means ± S.E.M. Data were compared by Student's *t*-test or analysis of variance (ANOVA) with post hoc analysis by Duncan's multiple range test. The criterion for significance was a P value < 0.05.

3. Results

3.1. Effects on caloric intake and body weight

Animals receiving ethanol in their drinking water had a decrease in food intake but consumed more daily calories that controls (Table 1). There were no statistically significant differences in food consumption in the 20% and 30% ethanol groups for males or females. Calories from ethanol accounted for 50–66% of daily intake in all ethanol groups, and males and females in the 30% ethanol groups consumed more daily ethanol calories than males and females in the 20% ethanol groups. Furthermore, the female 20%

Table 1
Body weight and daily caloric intake (food and ethanol) in male and female P-rats

Measured parameters	Male control	20% EtOH Male	30% EtOH Male	Female Control	20% EtOH Female	30% EtOH Female
Final body weight (g)	576 ± 4	547 ± 14[c]	534 ± 11[c]	327 ± 8[d]	321 ± 7[d]	303 ± 10[c,d]
Food (kcal/day)[a]	75.0 ± 1.8	53.3 ± 1.1[c]	47.4 ± 2.9[c]	56.4 ± 3.0[d]	39.6 ± 1.3[c,d]	34.6 ± 1.7[c,d]
Food (% control group)	100	71	63	100	70	61
EtOH (kcal/day)[b]	0	53.0 ± 4.8	68.4 ± 1.2[e]	0	44.2 ± 8.6	67.7 ± 5.4[e]
EtOH (% daily kcal)	0	50	59	0	53	66
EtOH (kcal day^{-1} g^{-1} body wt.)	0	0.097 ± 0.008	0.128 ± 0.003[e]	0	0.137 ± 0.016[d]	0.223 ± 0.011[d,e]
Total intake (kcal/day)	75.0 ± 1.8	106.3 ± 5.8[c]	115.7 ± 3.1[c]	56.4 ± 3.0[d]	83.7 ± 4.8[c,d]	102.3 ± 5.7[c,e]
Total intake (% control group)	100	142	154	100	148	181

(Control, $n = 8$/group; ethanol-treated, $n = 4$/group).
[a]Calculated based on metabolizable energy/g food.
[b]Calculated based on total volume of ethanol consumed.
[c]Significantly different than corresponding control.
[d]Significantly different than corresponding male group.
[e]Significantly different than corresponding 20% EtOH group.

and 30% ethanol groups consumed 42% and 76% more ethanol calories per unit body mass than their male counterparts. Body weight at the end of the treatment period was lower than controls in all ethanol groups with the exception of the 20% female group.

3.2. Effects on contractile responsiveness of thoracic aortae

As shown in Fig. 1, there was a pronounced effect of chronic ethanol consumption on male thoracic aortae that was detectable only in the absence of the endothelium. In male endothelium-intact preparations, neither the maximum tension elicited by phenylephrine nor the EC_{50} value for the agonist was altered by ethanol intake (Fig. 1, Table 2). The response to phenylephrine was enhanced by removal of the endothelium in preparations isolated from control male rats; the maximum tension elicited by the agonist was increased, and the EC_{50} value was decreased. Chronic ethanol intake resulted in even greater values for maximum tension in endothelium-denuded preparations but no further change in EC_{50} values. This effect of ethanol seemed to be dose-dependent as indicated by the fact that the maximum response tended to be greater in preparations from male rats consuming 30% ethanol as compared to 20% ethanol.

Similar ethanol-associated changes in male aortae were observed when examining the response to a high concentration of extracellular K^+ (80 mM). As shown in Table 2, endothelium-intact male preparations generally responded to K^+ depolarization with a tension that was greater than the maximum elicited by phenylephrine with no differences observed in tissues isolated from control and ethanol-consuming rats. In endothelium-denuded male aortae, the phenylephrine-induced maximum tension was greater than that produced by 80 mM extracellular K^+, and the response to 80 mM K^+ was greater in preparations isolated from ethanol-treated as compared to control animals.

When compared to males, endothelium-intact preparations from control female rats showed no difference in the response to phenylephrine (Table 2). However, endothelium-denuded aortae from control females responded with a significantly greater maximum tension when compared to male controls. The reduction in the EC_{50} value elicited by removal of the endothelium was not significantly different in control males and females. In contrast to the male preparations, chronic ethanol intake did not affect the phenylephrine responsiveness of either endothelium-intact or endothelium-denuded female aortae (Table 2). Similarly, the high extracellular K^+-induced tension was not affected by ethanol intake in either the endothelium-intact or -denuded female groups. As in male aortae, the K^+-induced tension was greater than the maximum elicited by phenylephrine in the female intact group but less than the phenylephrine-maximum in the female denuded group.

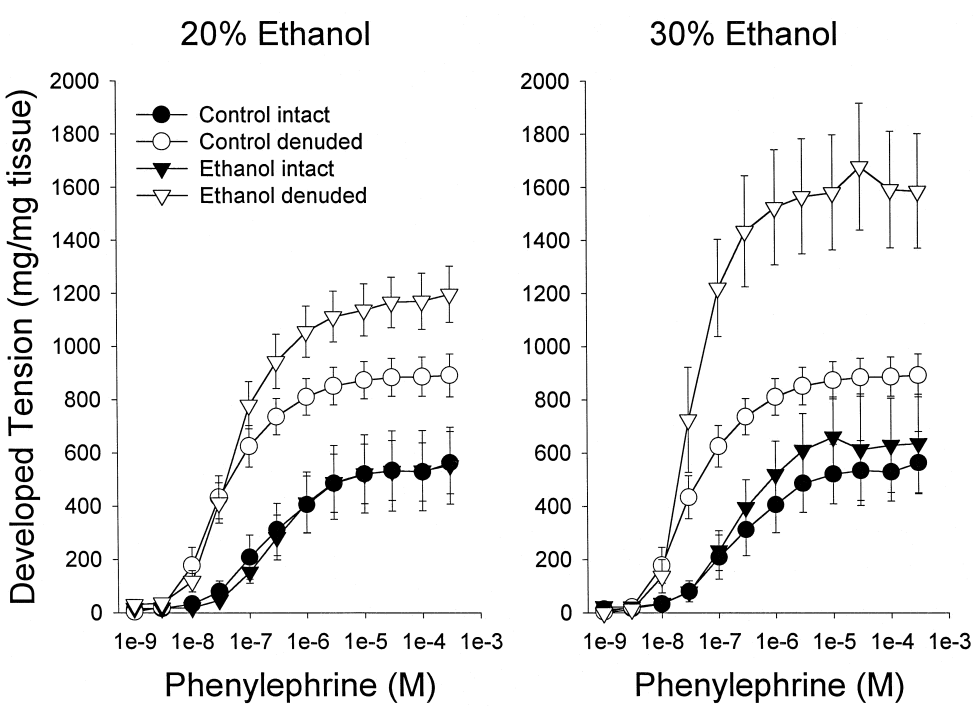

Fig. 1. Concentration-dependent effects of phenylephrine on endothelium-intact and endothelium-denuded thoracic aortae from male rats. Preparations were isolated after the animals had been maintained on drinking water or drinking water containing ethanol (20 or 30%) for 16 weeks. Effects of phenylephrine were examined by cumulative addition. Data are expressed as means with the vertical bars representing S.E.M. (control groups, $n = 8$; ethanol-treated groups, $n = 4$).

Table 2
Responses to phenylephrine and KCl in isolated thoracic aorta

Group	*N*	Tissue wt.[a] (mg)	Maximum tension[b] (phenylephrine)	Phenylephrine EC_{50} (10^{-7}M)	Maximum tension[b] (80 mM KCl)
Male intact					
Control	8	2.8 ± 0.1	543 ± 110	3.67 ± 0.90	865 ± 46[e]
20% EtOH	4	3.0 ± 0.2	553 ± 145	4.36 ± 1.94	828 ± 82
30% EtOH	4	2.7 ± 0.1	679 ± 166	2.19 ± 0.38	982 ± 103
Male denuded					
Control	8	2.9 ± 0.2	895 ± 72[c]	0.53 ± 0.24[c]	585 ± 55[c,e]
20% EtOH	4	2.8 ± 0.1	1200 ± 106[c]	0.59 ± 0.14	878 ± 85[e]
30% EtOH	4	2.3 ± 0.2	1593 ± 219[c,d]	0.39 ± 0.11[c]	1129 ± 178[d]
Female intact					
Control	8	2.1 ± 0.1	633 ± 82	2.71 ± 1.01	1014 ± 52[e]
20% EtOH	4	2.0 ± 0.1	620 ± 180	3.19 ± 1.32	956 ± 117
30% EtOH	4	1.9 ± 0.1	535 ± 102	2.43 ± 0.82	1030 ± 137[e]
Female denuded					
Control	8	2.0 ± 0.1	1502 ± 142[c,d]	0.68 ± 0.30	1036 ± 91[d,e]
20% EtOH	4	2.0 ± 0.1	1637 ± 257[c]	0.53 ± 0.10	993 ± 143
30% EtOH	4	1.7 ± 0.1	1509 ± 63[c]	0.46 ± 0.12	1103 ± 16[e]

[a] Mediated in part by dissection length (3–4 mm).
[b] Shown as mg tension/mg tissue dry weight.
[c] Significantly different than corresponding endothelium-intact group.
[d] Significantly different than male denuded control.
[e] Significantly different than phenylephrine-induced maximum tension in corresponding group.

Unlike male preparations, the response to K^+ depolarization was not affected by removal of the endothelium in female aortae isolated from control animals.

4. Discussion

Current results indicate that chronic ethanol consumption acts to enhance the smooth muscle contractile response to α-adrenoceptor stimulation and K^+ depolarization in male thoracic aortae. This effect was detected in endothelium-denuded ring preparations but not when the endothelium was left intact, suggesting that the enhanced smooth muscle contractile responsiveness was antagonized by a parallel increase in endothelial vasodilatory actions. Previous experiments by Strickland and Wooles (1988) in ethanol-fed male Sprague–Dawley (SD) rats showed a slight rightward shift in the dose–response curve for phenylephrine in endothelium-intact aortic rings with no obvious change in maximum response. Current data in endothelium-intact preparations from ethanol-consuming male P-rats showed no significant difference in the EC_{50} values for phenylephrine; however, available information provides little insight into the cause of this disparity. In contrast to the male aortae, results of the current study indicated that chronic ethanol consumption has no detectable effect on the contractile responses of endothelium-intact or endothelium-denuded female thoracic aortae. This gender-related difference in vascular smooth muscle responsiveness, if elicited in resistance vessels, could result in a greater blood pressure in male rats. Similarly, if observed in humans, this variation would help explain the reported gender-associated variation in the cardiovascular effects of ethanol (Manolio et al., 1991).

Future studies are required not only to further characterize the proposed differing pressor effects of ethanol in males and females but also to determine: (1) the cellular alterations and mechanisms of the ethanol-induced changes in smooth muscle responsiveness and endothelial actions in male rats; and (2) why the female does not show the altered vasoconstrictive response to α-adrenoceptor stimulation and K^+ depolarization following chronic ethanol intake. In terms of effects observed in male aortae, studies need to define the changes in Ca^{2+} handling or myofilament activation that underlie the enhanced smooth muscle contractile responsiveness. The parallel increase in the effect of high extracellular K^+ suggests that the enhanced responsiveness to phenylephrine is not mediated by changes in α-adrenoceptors or associated signalling pathways. Studies focusing on the endothelium should be designed to determine if the proposed enhanced vasodilatory action elicited by ethanol intake in males is associated with increased production/release of nitric oxide (NO) or other vasorelaxant agents. Alternatively, there could be an enhanced vasodilatory action of these mediators on the smooth muscle. In contrast to current results, previous work by Mayhan (1992) suggests that chronic ethanol intake diminishes the release of or response to an endothelium-derived relaxing factor in male SD rat cerebral arterioles. Future work is required to determine if this disparity

is due to the different vascular beds being studied or to differences in experimental design.

Although other possibilities obviously exist, the observed gender-related variation suggests that estrogen may play a protective role by antagonizing the ethanol-induced enhanced vasoconstrictive responsiveness. Estrogen has been shown to affect several neurohumoral modulators that control vascular tone. For example, estrogen has been reported to alter glucocorticoid metabolism (Brem et al., 1997), increase NO production (Gordodeski et al., 1995; Weiner et al., 1994; Wellman et al., 1996), decrease norepinephrine synthesis and release (Du et al., 1995; Huikuri et al., 1996), decrease endothelin levels (Polderman et al., 1993), and antagonize the in vitro response to norepinephrine and other vasoconstrictors (Cohen and Susemichel, 1996; McNeill et al., 1996; Garcia-Vallalón et al., 1996). Men have been shown to respond to exogenous epinephrine with a greater pressor response (Webber and Macdonald, 1993). Continued studies are required to determine if the gender-related differences in the chronic vascular action of ethanol is associated with any of these known effects of estrogen, or if the difference in gender is mediated by other actions of estrogen or by effects of other sex hormones such as progesterone or testosterone.

It is interesting to compare the current results from control (non-ethanol-treated) rats with previous reports of gender-related differences in vasoresponsiveness. Current data show: (1) no difference in the maximum response to phenylephrine when compared in endothelium-intact thoracic aortic rings isolated from 5–6-month-old male and female P-rats, and (2) that the increased contractile response to phenylephrine elicited by removal of the endothelium is significantly greater in female (average values of 633 and 1502 mg/mg dry weight in endothelium-intact and -denuded, respectively) than male (average values of 543 and 895 mg/mg dry weight in endothelium-intact and -denuded) preparations. In contrast, Maddox et al. (1987) examined the response to prostaglandin $F_{2\alpha}$ in aortic rings isolated from 10 week-old SD rats and found that removal of the endothelium had no effect on the response in male tissue but resulted in a significant increase in the maximum contractility in females. They also reported that endothelium-intact male aortae showed a greater maximum contractile response than corresponding female preparations. However, their data was reported as tension rather than tension per unit tissue mass, and it is possible that the variation in contractility reflects differences in tissue weight similar to those observed in the current study. In fact, Li et al. (1997) showed that the response to norepinephrine in rat tail artery was significantly greater in male than female preparations but that this difference was eliminated when tension was expressed relative to tissue weight. Still other investigators found that the contractile response was greater in male vasculature even when compared relative to tissue mass. For example, Stallone (1993) examined the contractile response to phenylephrine in thoracic aortae isolated from 8–10-week-old male and female SD rats, and observed a greater maximum contraction per unit tissue dry-weight in endothelium-intact male preparations bathed in a physiological solution containing L-arginine. Similar to the present study, he also found that the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) enhanced the contractile response in both genders, but that this effect was greater in female than male aorta. Thus, there seems to be no simple answer for the disparity among results regarding possible gender-related differences in the contractile responsiveness of endothelium-intact vessels. Variations in tissue weight can explain some discrepancies; however, it would appear that differences in experimental conditions or the age and strain of rat may also play a role.

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