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Acute and chronic effects of ethanol on papillary muscles from spontaneously hypertensive rats

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Abstract

The effects of chronic ethanol ingestion (12 weeks) on the mechanical properties of hypertrophied papillary muscle and the in vitro effects of ethanol (80–640 mg/dl) was studied. Papillary muscles from spontaneously hypertensive rats (SHRs) and their normotensive controls, the Wistar–Kyoto rat (WKY), were used in this study. Peak- developed tension was significantly less in muscles obtained from SHR compared with WKY even when normalized for muscle cross-sectional area. Chronic ethanol ingestion resulted in a significant shortening of both contraction and relaxation duration in muscles from SHR and WKY. In muscles from SHR and WKY, acute in vitro ethanol exposure produced concentration-dependent negative inotropic effects that were associated with a reduction in the duration of contraction and relaxation and marked slowing in the maximum velocities of tension development and decay. These findings suggest that the contractile response to ethanol exposure, in vitro, is not modified by either chronic ethanol ingestion or hypertension.

Keywords: Ethanol, chronic; Spontaneously hypertensive rat (SHR); Hypertension; Papillary muscle

1. Introduction

Chronic hypertension and long-term alcohol consumption in humans and experimental animals each independently alters cardiac electromechanical function, resulting in an inability of myocardial tissue to develop normal force (Gallardo-Carpentier and Carpentier, 1987, Carpentier and Gallardo-Carpentier, 1987; Posner et al., 1984, 1987). The long-term effects of each disorder leads to the development of a specific myopathic state characterized by diminished output and dysrhythmias (Regan et al., 1977). Chronic ethanol ingestion and hypertension-induced alterations in cardiac performance have been attributed, in part, to biochemical and ultrastructural changes within individual myocytes and/or the surrounding connective tissue matrix (Ferrans et al., 1965; Schrieber et al., 1974; Sarma et al., 1976; Kawamura et al., 1976; Sharma et al., 1986; Conrad et al., 1995). These degenerative changes in myocardial function may be caused by imbalances in autonomic outflow, postsynaptic responsiveness or the result of a direct action on heart muscle (Adams and Hirst, 1983; Posner et al., 1984, 1985; Sharma et al., 1986).

Although each condition has been studied extensively, very little is known regarding the effects of chronic ethanol ingestion on the mechanical properties of papillary muscle from hypertensive animal models. Therefore, the present investigation was designed to determine whether the negative inotropic effects of prolonged hypertension is potentiated, depressed or unaltered in response to chronic ethanol ingestion. In addition, the in vitro contractile response of isolated papillary muscles to ethanol following long-term ethanol ingestion was also studied.

2. Materials and methods

2.1. Experimental animals

The experimental procedures and protocols outlined in this study were approved by Wayne State University's School of Medicine Animal Investigation Committee in accordance with principles outlined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85–23, revised in 1985). To deter-

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mine whether chronic hypertension alters the inotropic response to acute in vitro ethanol exposure following chronic ethanol ingestion adult male spontaneously hypertensive rats (SHRs) and their normotensive counterpart, the Wistar–Kyoto rat (WKY) were obtained as pairs of litter mates weighing ~ 50 g. All animals were individually housed in a temperature-controlled room under a 12-h light/dark illumination cycle and allowed tapwater ad libitum. Animals were initially maintained on standard rat chow for a 1-week quarantine period.

2.2. Chronic ethanol treatment

Following the quarantine period, all animals were introduced to a liquid diet (Shake and Pour, BioServ) for a 1-week acclimation period. This liquid diet has been used extensively in chronic ethanol studies and has been determined to be nutritionally complete (Gallardo-Carpentier and Carpentier, 1987; Carpentier and Gallardo-Carpentier, 1987; Regan et al., 1977; De Carli and Lieber, 1967). Upon completion of acclimatization period, one of each of the litter mates for both groups was maintained on the liquid diet without ethanol (SHR-control animals or WKY-control animals). The remaining litter mate 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 of the diet 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 were 36% of the total calories were derived from ethanol which marked the beginning of the 12-week experimental period of chronic ethanol exposure. As with most chronic ethanol studies, an isocaloric pair-feeding regimen was employed to eliminate the possibility of nutritional deficits (De Carli and Lieber, 1967; Walker and Freund, 1971). For example, non-ethanol consuming animals (SHR-control or WKY-control animals) were offered the same quantity of diet ethanol-consuming (SHR-ethanol animals or WKY-ethanol animals) drank the previous day. The liquid diet consumed throughout the experimental period and body weight were measured daily and weekly, respectively. Prior to sacrifice systolic blood pressure was determined by the tail-cuff method under mild barbiturate sedation (sodium pentobarbital, Sigma, 30 mg/kg i.p.).

At the end of the experimental period, animals were sacrificed under barbiturate sedation (sodium pentobarbital, Sigma, 60 mg/kg i.p.) and the hearts were rapidly excised and immersed in oxygenated (95% O_2–5% CO_2) Tyrode's solution at 37°C. Left-ventricular papillary muscles were dissected and mounted horizontally in a temperature-controlled bath superfused with oxygenated Tyrode's solution (mM: KCl 5.4, NaCl 136.9, $NaHCO_3$ 11.9, $MgCl_2$ 0.50, Ca Cl_2 2.70, NaH_2PO_4 0.45 and glucose 5.6, pH 7.4) flowing at 10 ml/min at 30°C. Plasma glucose and lactate concentrations were determined with a YSI 2300 STAT-glucose/lactate analyser. Blood ethanol concentration was determined via gas chromatography (Perkin-Elmer) equipped with a flame ionization detector using samples collected at random. The mean blood ethanol concentration was significantly higher among the ethanol-consuming SHR compared with WKY animals, 179.4 ± 12.3 vs. 113.0 ± 35.3, respectively, $P < 0.01$.

2.3. Recording equipment

Preparations were allowed to equilibrate in Tyrode's solution for 90 min while electrically driven by a Grass stimulator (S-88) at a frequency of 0.5 Hz, to establish baseline isometric peak-tension development. Square wave pulses, 2-ms duration and 50% suprathreshold were delivered through a pair of platinum electrodes in close contact with one end of the muscle. Length–tension curves were constructed for each preparation and isometric tension recorded at ~ 90% of L_{max} using a force transducer (Hugo Sachs, F-30). Signals were amplified, differentiated and displayed on a chart recorder (Grass-79). The output of the chart recorder was coupled to the input stage of a digital storage oscilloscope (Nicolet-310).

2.4. Acute in vitro ethanol exposure

Following equilibration, preparations were exposed to various concentrations of ethanol (80–640 mg/dl; Ethanol, Aaper Chemical, Shelbyville, KY) for 10 min at a time when the maximum effects on developed tension were apparent. The addition of ethanol to the superfusing solution did not modify pH or alter osmolarity. The following parameters were measured: developed tension; time-to-peak tension; time-to-90% relaxation and the maximum velocities of tension developed and decline in tension.

2.5. Data analysis

For each experimental series, data are reported as the mean ± S.E.M. Difference between means within groups for each variable was calculated by repeated measures ANOVA. When an overall significance was determined a Dunnett's posthoc analysis was used to compare conditions to control. Difference between groups was assessed using a two-way ANOVA. A P value of < 0.05 was considered statistically significant.

3. Results

3.1. Diet consumption / body weight gain

The pattern of weekly diet consumption for SHR and WKY animals is shown in Fig. 1. As expected, since a pair-feeding protocol was used in this study, the diet consumption of ethanol-consuming animals essentially par-

alleled that of control animals in both groups. However, diet consumption was significantly higher in SHRs compared with WKYs during the acclimatization period 61 ± 4.8 vs. 50 ± 3.7 ml/day, respectively. During the ethanol introduction period, ethanol-consuming animals drank more than their pair-fed control counterpart. Subsequently, a sharp decrease in diet consumption occurred at a time (week 1) when 36% of the total caloric intake was derived from ethanol. In the ensuing weeks, there was a steady increase in weekly diet consumption throughout the remainder of the experimental period. At the end of the experimental period, control and ethanol-consuming animals drank between 500–600 ml/week. By group, diet consumption was significantly higher in hypertensive compared with normotensive controls, 86 ± 7.1 vs. 71 ± 5.9 ml/day, respectively. The progressive increase in body weight over the experimental period is shown in Fig. 1. There was no difference in the rate of weight gain observed between ethanol-consuming and non-ethanol-consuming animals.

3.2. General features of control and experimental animals

The effects of hypertension and chronic ethanol ingestion on blood pressure, body weight, heart, liver and kidney weight as well as plasma glucose and lactate concentrations are shown in Table 1. SHR-control animals exhibited a significantly higher systolic blood pressure than WKY controls, 143.2 ± 7.0 vs. 123.0 ± 8.2, $P < 0.01$. The hypertensive state was also associated with a higher body weight and cardiomegaly. Interestingly, in SHRs, chronic ethanol ingestion reduced systolic blood pressure, body weight, heart/body weight ratio and was associated with both hepatomegaly and renal hypertrophy. Although, in WKYs chronic ethanol ingestion did not alter systolic blood pressure or mean body weight, similar changes in heart/body weight ratio as well as both hepatomegaly and renal hypertrophy occurred as seen in SHR-ethanol animals.

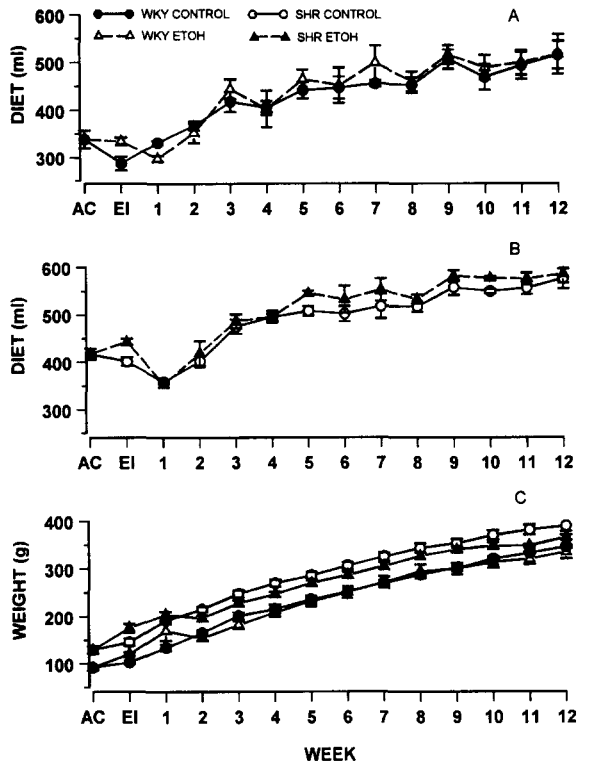

Fig. 1. Mean weekly diet consumption and effect of long-term ethanol feeding on body weight gain in WKY and SHR. Ethanol, ethanol; AC, acclimatization period; EI, ethanol introduction period. Panel A, mean weekly diet consumption in WKY animals; panel B, mean weekly diet consumption in SHR animals; panel C, mean weekly body weight for WKY and SHR animals. Open circles, WKY control rats; filled circles, WKY ethanol-consuming rats; open triangles, SHR control rats; filled triangles, SHR ethanol-consuming rats. Values, mean ± S.E.M. collected from five animals in each group.

3.3. Baseline mechanical properties

Isolated papillary muscles from SHR-control animals exhibited less peak-tension development than their normotensive counterparts (1.30 ± 0.14 vs. 1.73 ± 0.24 g, respectively) even when normalized for muscle cross-sec-

Table 1
General characteristics of WKY and SHRs

	WKY		SHR	
	Control	ETOH	Control	ETOH
Systolic BP (mm Hg)	123 ± 8.2	112.5 ± 8.7	143.2 ± 7.0 [a]	112.5 ± 2.9 [b]
Body weight (g)	343.4 ± 6.4	342.1 ± 9.7	389.2 ± 10.2 [a]	363.3 ± 5.1 [b]
Heart weight (mg)	1151 ± 31.7	1072 ± 38.0	1466 ± 37 [a]	1304 ± 43 [b]
Heart/body weight (mg/g)	3.35 ± 0.06	3.13 ± 0.05 [b]	3.77 ± 0.06 [a]	3.59 ± 0.08 [b]
Liver weight (g)	9.66 ± 0.70	11.33 ± 0.50 [b]	11.76 ± 0.59 [a]	14.46 ± 0.78 [b]
Liver/body weight (mg/g)	28.0 ± 1.6	33.3 ± 1.9 [b]	30.2 ± 1.2	39.7 ± 1.8 [b]
Kidney/body weight (mg/g)	6.69 ± 0.24	7.25 ± 0.29 [b]	6.42 ± 0.11	6.79 ± 0.08 [b]
Glucose (mg/dl)	171 ± 12.4	173.5 ± 4.8	167.2 ± 12.2	151.2 ± 5.5
Lactate (mg/dl)	51.3 ± 11.0	39.8 ± 6.7	37.5 ± 8.7	34.7 ± 8.2

BP, blood pressure. Values are means ($n = 5$) ± S.E.M., $P < 0.05$. [a] Significantly different from WKY Control. [b] Significantly different from pair-matched control drinker.

Table 2
Acute effects of ethanol on TPT, RT90 and ±VT of muscles from WKY and SHRs

	Control	80 mg/dl	240 mg/dl	640 mg/dl	Recovery
TPT (ms)					
WKY-C	106 ± 3.3	104 ± 2.4	101 ± 2.2	91 ± 2.7 [b]	101 ± 2.4
WKY-E	99 ± 4.6	97 ± 3.5	90 ± 3.2 [b]	78 ± 3.9 [b]	95 ± 3.0
SHR-C	105 ± 7.0	104 ± 6.7	97 ± 6.4 [b]	89 ± 7.6 [b]	105 ± 7.0
SHR-E	88 ± 2.6 [a]	91 ± 4.7	84 ± 4.0 [b]	76 ± 4.9 [b]	88 ± 2.6
RT90 (ms)					
WKY-C	133 ± 9.1	131 ± 10.4	122 ± 6.7 [b]	116 ± 6.4 [b]	127 ± 6.5
WKY-E	116 ± 7.1 [a]	112 ± 7.3	113 ± 5.0	97 ± 3.7 [b]	118 ± 7.4
SHR-C	129 ± 12.9	126 ± 10.8	122 ± 10.3	116 ± 10.8 [b]	124 ± 9.9
SHR-E	106 ± 10.1 [a]	110 ± 8.1	103 ± 10.4	93 ± 8.4	117 ± 14.3
+VT (g/s)					
WKY-C	30.3 ± 4.2	29.4 ± 4.3	26.4 ± 3.7 [b]	28.7 ± 2.4 [b]	31.8 ± 3.6
WKY-E	26.3 ± 3.3	24.7 ± 3.6	21.7 ± 2.9 [b]	14.5 ± 2.3 [b]	26.3 ± 3.1
SHR-C	26.1 ± 3.1	25.6 ± 3.2	22.4 ± 3.6	16.5 ± 3.1 [b]	27.7 ± 3.5
SHR-E	29.0 ± 3.4	27.6 ± 3.3	23.6 ± 4.0 [b]	16.0 ± 3.5 [b]	27.2 ± 4.6
−VT (g/s)					
WKY-C	25.4 ± 4.0	21.8 ± 4.4	20.3 ± 3.8 [b]	14.9 ± 2.7 [b]	23.5 ± 4.2
WKY-E	19.5 ± 2.1	18.1 ± 1.9	14.9 ± 1.9 [b]	9.8 ± 1.4 [b]	18.2 ± 2.2
SHR-C	18.6 ± 2.6	19.1 ± 3.0	14.9 ± 2.2 [b]	10.5 ± 2.0 [b]	18.3 ± 1.9
SHR-E	22.8 ± 2.4	20.8 ± 3.3	16.6 ± 2.9 [b]	10.2 ± 2.2 [b]	19.8 ± 3.4

TPT, time-to-peak tension; RT90, time-to-90% relaxation; +VT, maximum velocity of tension development; −VT, maximum velocity of decline in tension. C, control diet; E, ethanol diet. Values are means ($n = 5$) ± S.E.M., [a] Significantly different from respective control drinker. [b] Significantly different from respective control response, $P < 0.05$.

tional area (1.30 ± 0.30 vs. 1.96 ± 0.61 g/mm^2, respectively). Chronic ethanol ingestion did not modify baseline tension development in preparations from SHR animals (1.30 ± 0.14 vs. 1.35 ± 0.21 g; control vs. ethanol). However, isolated papillary muscles from WKY-ethanol animals exhibited less baseline peak-developed tension than WKY controls (1.38 ± 0.2 vs. 1.73 ± 0.24 g, respectively). The lower peak-developed tension in SHR-control animals was not associated with any change in the velocity or duration of contraction and relaxation (Table 2). In papillary muscles from SHRs, chronic ethanol ingestion resulted in a significant shortening in the duration of contraction and relaxation represented as time-to-peak tension and time-to-90% relaxation, respectively. Papillary muscles from WKYs chronically ingesting ethanol exhibited a similar shortening of relaxation duration.

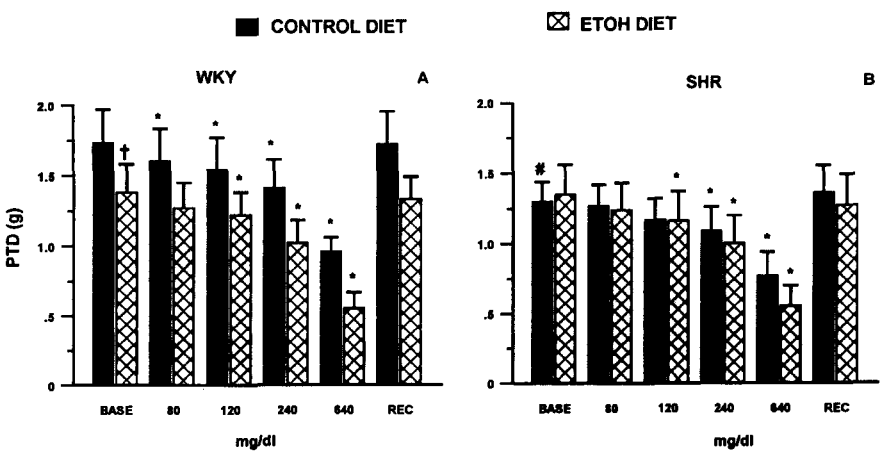

Fig. 2. Acute inotropic effects of ethanol on developed tension in muscles from WKY and SHR animals. PTD, peak developed tension. Panel A, WKY developed tension (g); panel B, SHR developed tension (g). Filled columns, control diet; cross-hatched columns, ethanol diet. BASE, baseline responses in absence of ethanol; REC, responses obtained 15 min after washout. Values, mean ± S.E.M. collected from seven muscles in each group. * Significantly different from respective baseline responses; # significantly different from responses in WKY group; †significantly different from responses within respective non-ethanol consuming group, $P < 0.05$.

3.4. Acute inotropic effects of ethanol

The acute inotropic effects of clinically relevant concentrations of ethanol (80–640 mg/dl) on papillary muscles from normotensive and hypertensive animals chronically exposed to ethanol is shown in Fig. 2. Acute exposure to ethanol elicited a negative inotropic effect on peak-developed tension in all groups. When expressed in absolute values of force, peak-developed tension was significantly reduced by a low concentration of ethanol (80 mg/dl) in preparations from WKY-control animals whereas, in muscles from WKY-ethanol animals, a higher concentration was required to appreciably attenuate tension (Fig. 2a). In muscles obtained from SHR-control animals and SHR-ethanol animals, a higher concentration was required to appreciably attenuate peak-developed tension (< 240 mg/dl) compared with WKY control (Fig. 2b). The magnitude of the negative inotropic effect of ethanol in WKY-ethanol and SHR-ethanol muscles was nearly identical to WKY-control and SHR-control preparations (data not shown). In muscles from WKY-ethanol animals, the highest dose tested (640 mg/dl) caused a significantly higher negative inotropic effect compared with WKY control, $-61.8 \pm 4.4\%$ vs. $-42.1 \pm 5.0\%$, $P < 0.01$, respectively. However, in muscles from SHR-ethanol animals, the magnitude of the negative inotropic effect of ethanol 640 mg/dl, was not different from the non-ethanol-consuming control group, $-62.1 \pm 4.7\%$ vs. $-44.1 \pm 8.8\%$. In all instances, the negative inotropic effect of ethanol was completely reversible upon removal of the drug from the superfusate.

3.5. Acute effects of ethanol on time-to-peak tension and time-to-90% relaxation

The acute effects of ethanol on time-to-peak tension and time-to-90% relaxation in muscles from WKY and SHRs is shown in Table 2. In muscles from WKY-control animals, acute in vitro ethanol exposure exerts a dose-dependent shortening of time-to-peak tension that was significant at the highest dose tested (640 mg/dl). By contrast, a lower concentration of ethanol (240 mg/dl) reduced time-to-peak tension in muscles obtained from WKY-ethanol animals. A significant ethanol-induced shortening effect on time-to-peak tension in muscles from both SHR-control and SHR-ethanol animals occurred at 240 and 640 mg/dl. Acute in vitro ethanol exposure also exerts a dose-dependent shortening of time-to-90% relaxation in preparations from WKY animals. However, the dose which resulted in a significant reduction in time-to-90% relaxation was lower in WKY-control (240 mg/dl) than in WKY-ethanol animals (640 mg/dl). Preparations from SHR-control animals were less sensitive to the ethanol-induced shortening effect on time-to-90% relaxation compared with WKY, whereas SHR-ethanol muscles were completely insensitive.

3.6. Acute effects of ethanol on $\pm VT$

The acute effects of ethanol on maximum velocity of tension development and maximum velocity of tension decay in muscles from WKY and SHRs is shown in Table 2. Acute exposure to ethanol caused a dose-dependent slowing of both maximum velocity of tension development and maximum velocity of tension decay. With the exception of SHR-control preparations, the threshold of this action occurred between 180 and 240 mg/dl in muscles from both ethanol-consuming and non-ethanol-consuming animals

4. Discussion

It is well-established that acute in vitro ethanol exposure elicits concentration-dependent negative inotropic effects on isolated myocardial tissue (Fisher and Kavaler, 1975). This action has been attributed, in part, to a depletion of high energy phosphates and/or inhibition of excitation–contraction coupling due to a depletion of Ca^{2+} from the sarcoplasmic reticulum (Danzinger et al., 1991; Schulman et al., 1991; Guarnieri and Lakatta, 1990). It has also been suggested that ethanol may alter myofilament Ca^{2+} sensitivity which leads to a reduction in contractile responses. Chronic ethanol exposure also leads to an elevation in blood pressure in humans (Klatsky et al., 1977) and experimental animals (Chan and Sutter, 1982, 1983). However, in some instances, chronic ethanol ingestion elicits a hypotensive action (Hatton et al., 1991, 1992; Howe et al., 1985; Sanderson et al., 1983; Capasso et al., 1991a, 1991b). In this study, chronic ethanol ingestion lowered systolic blood pressure in both normotensive and hypertensive animals. The hypotensive response to chronic ethanol ingestion has been, in part, attributed to both ultrastructural and electrophysiological alterations that attenuates the force generating capacity of the heart (Polimeni and Posner, 1990; Koga et al., 1993; Thomas et al., 1989; Zhang et al., 1992; Altura and Altura, 1994; Capasso et al., 1991b; Posner et al., 1984, 1987). The present study characterized the baseline mechanical properties of hypertensive animals following chronic ingestion of ethanol and the effect of acute exposure to clinically relevant concentrations of ethanol. Our results indicate that chronic ethanol ingestion reduces heart size in both normotensive and hypertensive animals. Whereas, with hypertension alone the overall heart size is increased. The hypertensive state was also associated with a reduction in developed tension which was not further modified by chronic ethanol ingestion. By contrast, chronic ethanol ingestion significantly reduces developed tension in normotensive animals. Chronic ethanol ingestion also reduced both contraction and relax-

ation duration in SHRs, whereas only relaxation duration was attenuated in WKYs. Lastly, acute in vitro ethanol exposure elicited a concentration-dependent negative inotropic effect in all groups studied.

Previous studies have reported the development of myocardial hypertrophy in response to chronic ethanol ingestion (Capasso et al., 1991b; Posner et al., 1984). Interestingly, our results indicate an ethanol-induced reduction in heart size-to-body weight ratio. Sanderson et al., 1983, also observed a decrease in left ventricular body weight index following 16 weeks of chronic ethanol ingestion using SHRs. Therefore, this difference may be attributed to the strain of rat used. This enlargement of the heart, in hypertension, may be due to an increase in individual myocyte size and/or the number of fibrotic foci (Kawamura et al., 1976; Conrad et al., 1995).

Chronic hypertension is also characterized by a reduction in developed tension with a significant prolongation of time-to-peak tension and a depression of force-velocity characteristics (Capasso et al., 1981; Conrad et al., 1991, 1995; Heller, 1978). In the present study, the baseline force generating capacity of cardiac muscle from non-ethanol-consuming hypertensive animals was significantly reduced. Contraction and relaxation duration as well as the velocity of force development were not altered. The increased synthesis of type II and type V collagen in hypertensive myocardial tissue results in an increase in ventricular stiffness which decreases the force generating capacity of the heart (Honda et al., 1993; Conrad et al., 1995). Alternatively, the reduced force generating capacity cardiac muscle from hypertensive animals may be directly related to alterations in Ca^{2+} entry through sarcolemmal calcium channels and/or Ca^{2+} release from intracellular stores. In the hypertrophic heart there is an increase in the calcium transient (Lakatta, 1990, Lakatta, 1991; Pérez et al., 1993; Sharma et al., 1986). The increase in intracellular Ca^{2+} may be the result of inhibition of Na^{+}/K^{+}-ATPase activity. It has also been shown that the caffeine-induced release of Ca^{2+} mechanism from the sarcoplasmic reticulum is attenuated in hypertension.

Chronic ethanol ingestion as with essential hypertension is also associated with a reduction in baseline mechanical properties (Capasso et al., 1991b; Posner et al., 1987; Polimeni and Posner, 1990). The ethanol-induced decrease in contractile force and prolongation of relaxation kinetics was thought to be due to a reduction in transmembrane Ca^{2+} movement and/or a decrease in sarcoplasmic reticulum binding of Ca^{2+}. In the present study, baseline mechanical properties of normotensive animals were depressed following chronic ethanol ingestion. In hypertensive animals chronically ingesting ethanol, developed tension was not attenuated compared with their non-ethanol-consuming counterparts. However, as expected chronic ethanol ingestion significantly reduced both contraction and relaxation duration.

It is well-established that acute exposure to ethanol results in a concentration-dependent depression of contractile function (Thomas et al., 1989; Schulman et al., 1991; Danzinger et al., 1991; Guarnieri and Lakatta, 1990). In our study, acute in vitro ethanol exposure resulted in a concentration-dependent negative inotropic response in muscles from both groups. While the threshold of the negative inotropic effect of ethanol was decreased in muscles from normotensive animals chronically ingesting ethanol, the magnitude of the negative inotropic effect was greater at higher ethanol doses. The time-to-peak tension and the time to 90% relaxation were not appreciably attenuated until tension was reduced by $\sim 25\%$ in normotensive animals which occurred at higher ethanol concentrations. Papillary muscles from ethanol-consuming SHRs were slightly more sensitive to the negative inotropic effect of acute in vitro ethanol exposure than muscles from ethanol-consuming WKYs. However, in general, their response to ethanol was similar to that obtained in muscles from ethanol-consuming WKYs. This disparity between the threshold for the depression in contractile force and the marked slowing of tension development and decay may be due to ethanol acting at more than one locus at higher doses. It has been shown that low doses of ethanol depress myofilament response to Ca^{2+} and at higher doses there is an impairment of excitation–contraction coupling at the sarcoplasmic reticulum and myofilaments (Danzinger et al., 1991). Acute in vitro ethanol exposure has also been shown to result in a net efflux of Ca^{2+} with a reduction in sarcoplasmic reticulum Ca^{2+} stores (Thomas et al., 1989; Schulman et al., 1991). The net efflux of Ca^{2+} has been attributed to an inhibition of Ca^{2+} influx through L-type Ca^{2+} channels and/or an enhanced efflux of Ca^{2+}. There is also an abbreviation of the action potential due to a possible alterations at the calcium or sodium channel level (Guarnieri and Lakatta, 1990; Schulman et al., 1991; Danzinger et al., 1991).

The cardiac muscle from non-ethanol-consuming hypertensive animals exhibited an attenuated response to the negative inotropic effect of acute in vitro ethanol exposure, with the threshold dose shifted to a higher concentration. Therefore, papillary muscles from hypertensive animals are less responsive to the negative inotropic effect of acute in vitro ethanol exposure.

In conclusion, chronic ethanol ingestion in hypertensive animals resulted in the normalization of blood pressure. This hypotensive effect of chronic ethanol ingestion may have prevented the development of concentric hypertrophy seen in hypertensive animals. However, chronic ethanol ingestion in normotensive animals and hypertensive animals still resulted in a decrease in myocardial contractility in conjunction with the development of tolerance to acute in vitro ethanol exposure which still places this group at risk of developing alcohol-induced cardiomyopathy. Clinically, the extent of hypertension might be masked and the risk of developing cardiomyopathy enhanced among hypertensive individuals chronically ingesting ethanol.

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