Biochemical and Biophysical Research Communications **261,** 202–208 (1999)
Article ID bbrc.1999.0944, available online at http://www.idealibrary.com on IDEAL®

Hypertension Augments Ethanol-Induced Depression of Cell Shortening and Intracellular Ca^{2+} Transients in Adult Rat Ventricular Myocytes

Jun Ren* and Ricardo A. Brown†

†*Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201; and* **Department of Physiology, University of North Dakota School of Medicine, Grand Forks, North Dakota 58202*

Received June 2, 1999

Ethanol, a risk factor for myocardial dysfunction, depresses myocardial contraction. This study was to determine whether ethanol-induced myocardial depression is affected by hypertension. Mechanical properties of ventricular myocytes isolated from both normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were evaluated using a video edge-detection system. Myocytes were electrically stimulated to contract at 0.5 Hz. Contractile properties analyzed include peak twitch amplitude (PTA), time-to-PTA (TPS), time-to-90% relengthening (TR_{90}), and maximal velocities of shortening/relengthening (±dL/dt). Intracellular Ca^{2+} transients were measured as fura-2 fluorescence intensity (ΔFFI) changes. Acute ethanol exposure (80-640 mg/dl) caused a concentration-dependent inhibition of PTA and ΔFFI in both WKY and SHR myocytes. The extent of maximal inhibition of PTA and FFI was significantly greater in SHRs (53.7 and 38.9%) compared to the WKY group (21.0 and 25.4%). Ethanol did not affect TPS but shortened TR_{90} and slowed ±dL/dt at high concentration ranges. Interestingly, the augmented ethanol-induced inhibition of cell shortening in hypertension was greatly attenuated by Ca^{2+} channel opener BayK 8644 (1 μM). These results suggest that ethanol-induced myocardial depression may be augmented in hypertension, possibly due to mechanism(s) involving sarcolemmal Ca^{2+} channels. © 1999 Academic Press

Key Words: **ethanol; hypertension; ventricular myocyte; shortening; intracellular Ca^{2+} transient.**

Alcoholism has been known to be associated with congestive alcoholic cardiomyopathy, a clinical condition characterized by ventricular hypertrophy, depressed contractile performance and electromechanical abnormalities (1–4). These ethanol-induced cardiac dysfunctions are believed to be mediated by direct depressive actions on cardiac muscle and cardiac myocytes, such as alteration in myofibril Ca^{2+} sensitivity, sarcolemmal Ca^{2+} channel activity and intracellular Ca^{2+} levels (5–7).

While the etiology of alcoholic cardiomyopathy may be multifactorial, chronic ethanol consumption has been commonly associated with an increased incidence of hypertension. Although this may be a result of an enhanced vascular tone or sympathetic activity (8–9), the exact nature regarding the relationship of ethanol-induced myocardial dysfunction and hypertension remains only partially understood. The aim of the present study was to examine the impact of hypertension on ethanol-induced myocardial depression using ventricular myocytes isolated from spontaneously hypertensive rats (SHR) and its normotensive control, Wistar-Kyoto (WKY) rats.

MATERIALS AND METHODS

Experimental animals. The experimental procedures outlined in this investigation were approved by Wayne State University Animal Investigation Committee and have been previously described (10). Weight-matched WKY and SHR rats were obtained at 4 weeks of age (50 g) (Harlan Bioproducts., Indianapolis, IN) and individually housed in a temperature controlled room under a 12/12-hour light/dark illumination cycle. The animals were allowed access to standard rat chow and tap water *ad libitum*. Systolic blood pressures and body weights were measured on a weekly basis. The animals were sacrificed at 25 weeks of age.

Cell isolation. Single ventricular myocytes were isolated using the method described previously (11). Briefly, animals were sacrificed and hearts were rapidly removed and perfused (at 37°C) with oxygenated (5%CO_2-95%O_2) Krebs-Henseleit bicarbonate (KHB) buffer (mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, N-[2-hydro-ethyl]-piperazine-N′-[2-ethanesulfonic acid] (HEPES) 10, glucose 11.1. Hearts were subsequently perfused with a nominally Ca^{2+}-free KHB buffer for 2–3 mins until spontaneous contractions ceased followed by a 20 min perfusion with Ca^{2+}-free KHB containing 176 U/ml collagenase (Worthington Biochemical

Abbreviations: PTA, peak twitch amplitude, TPS, time-to-PTA; TR_{90}, time-to-90% relengthening; ±dL/dt, maximal velocities of shortening/relengthening; FFI, fura-2 fluorescence intensity; FDT, fluorescence decay time.

TABLE 1

General Features of WKY and SHR Animals

Rat group	Blood pressure (mm Hg)	Body wt (g)	Heart wt/body wt (mg/g)	Liver wt/body wt (mg/g)	Kidney wt/body wt (mg/g)
WKY (10)	129 ± 1	417 ± 15	3.20 ± 0.07	28.9 ± 0.8	8.09 ± 0.36
SHR (10)	152 ± 1*	374 ± 21*	3.76 ± 0.15*	43.1 ± 3.3*	10.01 ± 0.70*

Note. Mean ± SEM, *$p < 0.05$ vs WKY.

Corp., Freehold, NJ) and 0.1 mg/ml hyaluronidase (Sigma Chemical, St. Louis, MO). After perfusion, ventricles were removed and minced, under sterile conditions, and incubated with the above enzymatic solution for 3–5 min. The cells were further digested with trypsin and deoxyribonuclease before being filtered through a nylon mesh (300 μm) and subsequently separated from the enzymatic solution by centrifugation (60 × g for 30 sec). Myocytes were resuspended in a sterile filtered, Ca^{2+}-free KHB buffer containing (mM): NaCl 131, KCl 4, $MgCl_2$ 1, HEPES 10, and glucose 10, supplemented with 2% bovine serum albumin (BSA), with a pH of 7.4 at 37°C. Isolated myocytes were plated on glass coverslips pre-coated with laminin (10 μg/ml, Collaborative Biochemical Products, Bedford, MA) and maintained in a serum-free medium consisting of Medium 199 (Sigma). Mechanical properties of myocytes remained relatively stable for the first 12–24 hr. Cell were not used if they had any obvious sarcolemmal blebs or spontaneous contractions.

Cell shortening/relengthening. Mechanical properties of ventricular myocytes were assessed using a video based edge-detection system (Crescent Electronics, Sandy, UT) (11). In brief, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Olympus X70) and superfused (~2 ml/min at 37°C) with a buffer containing (in mM): NaCl 131, KCl 4, $CaCl_2$ 1, $MgCl_2$ 1, glucose 10, HEPES 10, at pH 7.4. The cells were field stimulated at frequency of 0.5 Hz, 3 msec in duration. A video based edge-detector was used to capture and convert changes in cell length during shortening and relengthening into an analogue voltage signal (IonOptix Corp, Milton, MA). The myocyte being studied was displayed on a Sony monitor using a Pulnix camera. Cell shortening and relengthening were assessed using the following indices: peak twitch amplitude (PTA), time-to-PTA (TPS) and time-to-90% relengthening (TR_{90}), maximal velocities of shortening/relengthening (±dL/dt).

Intracellular fluorescence measurement. In these experiments, myocytes were loaded with fura-2/AM (0.5 μM) for 5 min at 30°C, and fluorescence measurements were recorded with an IonOptix dual-excitation fluorescence photomultiplier system (11). Myocytes were placed on an Olympus inverted microscope and imaged through a fluor 40x oil objective. Cells were exposed to light emitted by a 75W lamp and passed through either a 360 or a 380 nm filter (bandwidths were ±15 nm), while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480-520 nm by a photomultiplier tube (333 Hz sampling rate). The 360 nm excitation scan was repeated at the end of the protocol and qualitative changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was inferred from the ratio of the fura-fluorescence intensity (FFI) at both wavelengths. Fluorescence decay time (FDT) was also measured as an indication of the intracellular Ca^{2+} clearing rate.

Data analysis. Data are presented as mean ± SEM. Differences between and within groups were evaluated by two-way analysis of variance (ANOVA) with repeated measures (SYSTAT). A Tukey test was used as a follow-up for the multiple comparisons. To determine significant differences in the repeated measures factor (concentration of IGF-1), the 'within subjects' MSerror and dferror terms from the parent ANOVA were used. To determine significant differences between strains at a given concentration of IGF-1, the 'between subjects' MSerror and dferror terms from the parent ANOVA were used. Statistical significance was considered to be $p < 0.05$.

RESULTS

General features of WKY and SHR rats. The effects of hypertension on blood pressure, body, heart, liver, and kidney weights are shown in Table 1. As expected, SHR exhibited a significantly greater systolic pressure than WKY. However, SHR failed to gain weight as readily as their WKY counterparts. The hypertensive state was also associated with cardiomegaly, hepatomegaly and renal hypertrophy.

Baseline mechanical and fluorescent properties of WKY and SHR myocytes The prolonged hypertensive state lead to cardiac hypertrophy. The average resting cell length (RCL) of ventricular myocytes isolated from WKY and SHR was 103 ± 3 and 135 ± 3 μm, respectively ($p < 0.05$). SHR myocytes exhibited considerably larger cell surface area (5104 ± 592 μm^2), compared to that of WKY (2196 ± 171 μm^2, $p < 0.05$). The peak twitch amplitude (PTA) was greater in SHR myocytes (7.0 ± 0.6 vs 5.8 ± 0.6% in WKY, $p < 0.05$). However, the enhanced ability to contract was not asociated with increased maximal velocities of shortening/relengthening (±dL/dt) in SHR myocytes. SHR myocytes exhibited a prolonged relengthening duration (TR_{90}) associated with similar shortening duration (TPS), compared to their WKY counterparts (Fig. 1A & Table 2). Finally, fluorescent measurements indicated that SHR myocytes possessed an elevated resting intracellular Ca^{2+} level compared to their WKY counterpart, as reported elsewhere (12). The intracellular Ca^{2+} clearing rate measured by fluorescence decay time (FDT) was not different between the two cell types (Table 3).

Effect of ethanol on myocyte shortening (PTA). Acute ethanol exposure did not affect myocyte RCL over the range of concentrations tested (80–640 mg/dl). Representative traces depicting the typical effect of ethanol (240 mg/dl) on WKY or SHR myocyte shortening is shown in Fig. 1A. At the end of a 10 min exposure to this concentration of ethanol, PTA was depressed by 17.1 and 51.2%, in WKY and ShR myocytes, respectively. Acute ethanol exposure (80–640 mg/dl) caused a concentration-dependent inhibition of PTA in both

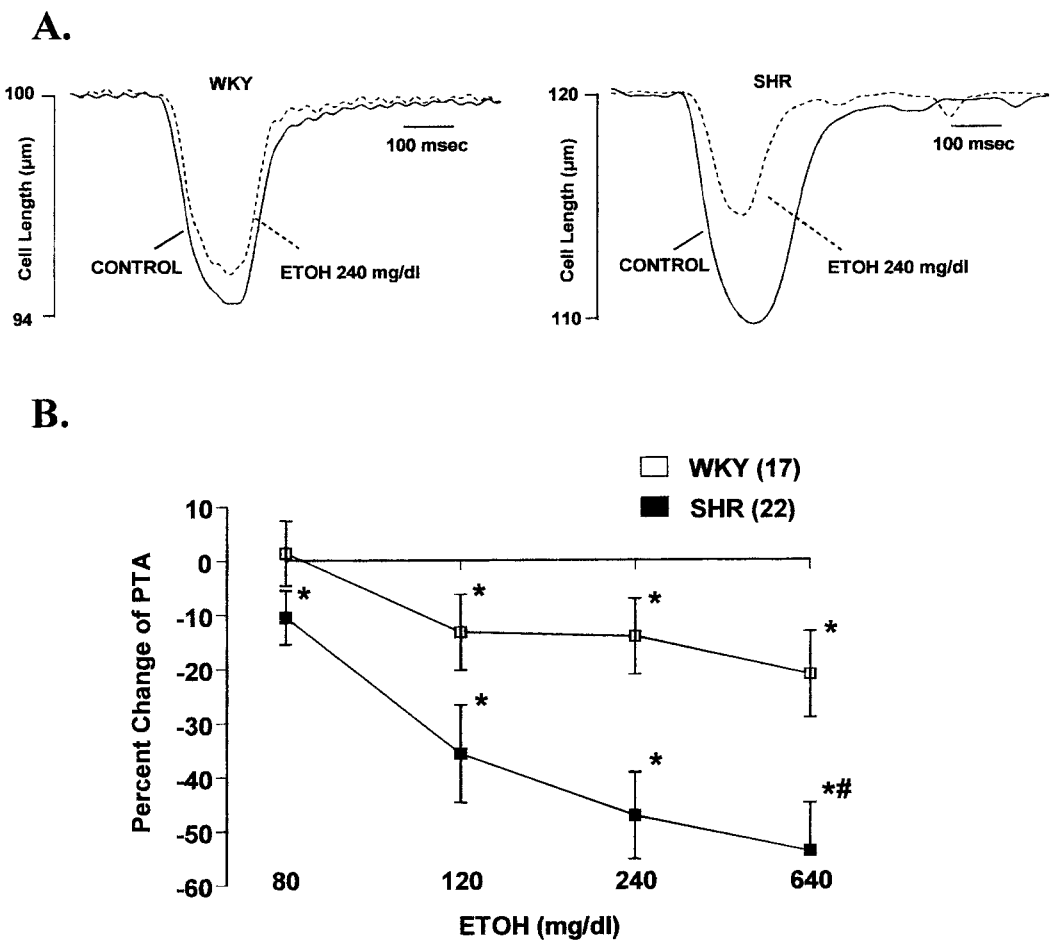

FIG. 1. (A) Typical experiment showing the effect of ethanol (ETOH) on myocyte shortening in a WKY (left) and a SHR (right) myocyte. Myocyte shortening and relengthening were recorded with a high-resolution video-edge detection system at 37°C before and 10 min after ethanol (240 mg/dl) administration. (B) Concentration-dependent response to ethanol (80–640 mg/dl) on myocyte shortening in both WKY and SHR myocytes. Mean ± SEM, $*p < 0.05$ vs control, $\#p < 0.05$ vs WKY.

WKY and SHR myocytes (Fig. 1B). The maximal inhibition was 21.0% with a threshold between 80 and 120 mg/dl in WKY cells. However, SHR myocytes were more sensitive to ethanol exposure with a maximal inhibition of 53.7% and a threshold between 0 and 80 mg/dl. The inhibitory effect on cell shortening was maximal within 6 min of exposure and was reversible. Ethanol exhibited little effect on TPS whereas shortened TR_{90} and slowed ±dL/dt at high concentration range in both WKY and SHR myocytes (Table 3).

Effect of ethanol on intracellular Ca^{2+} transients. To determine whether ethanol-induced inhibition of myocyte shortening was due to reduced intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), we used fura-2 to estimate changes in $[Ca^{2+}]_i$ in the absence or presence of ethanol. The time course of fluorescence signal decay (FDT) was evaluated to assess the rate of intracellular Ca^{2+} clearing. Representative traces of intracellular Ca^{2+} transients shown in Fig. 2 depict the typical actions of ethanol on ΔFFI in WKY and a SHR myocytes. Acute ethanol exposure caused a more pronounced inhibition on ΔFFI in SHR myocytes compared to WKY. The threshold of the ethanol-induced inhibition of ΔFFI was between 240 and 640 mg/dl in WKY myocytes and between 120 and 240 mg/dl in SHR myocytes, respectively. This suggests that ethanol-induced depression of intracellular free Ca^{2+} in response to electrical stimulation is substantiated in hypertension. Despite the elevated resting Ca^{2+} level, neither resting FFI (representing resting Ca^{2+} level) nor FDT was affected by ethanol, except for SHR myocyte at high ethanol concentrations (Table 3).

Relationship between ethanol-induced inhibitions of PTA and ΔFFI. Our results indicate that ethanol induced a substantially greater inhibition on cell shortening and intracellular Ca^{2+} transients in the hypertensive state, compared to the normotensive state. To further examine the relationship between ethanol-induced inhibition of PTA and ΔFFI in hypertension, we re-plotted data from Figs. 1 and 2 as a function of

TABLE 2

Influence of Ethanol on Duration and Velocity of Shortening and Relengthening in both WKY and SHR Myocytes

	Baseline	Ethanol (80 mg/dl)	Ethanol (120 mg/dl)	Ethanol (240 mg/dl)	Ethanol (640 mg/dl)	Recovery
TPS (msec)						
WKY	66 ± 6	66 ± 5	58 ± 5	60 ± 6	55 ± 5	62 ± 6
SHR	70 ± 4	75 ± 6	66 ± 7	54 ± 6*	43 ± 7*	77 ± 6
TR_{90} (msec)						
WKY	72 ± 9	73 ± 6	64 ± 5	69 ± 7	63 ± 6	64 ± 5
SHR	106 ± 15#	84 ± 8	80 ± 9	69 ± 10*	56 ± 10*	88 ± 10
+dL/dt μm/s)						
WKY	168 ± 30	171 ± 33	147 ± 36	143 ± 25	114 ± 20*	138 ± 12
SHR	211 ± 29	191 ± 23	120 ± 20*	139 ± 23*	114 ± 21*	168 ± 23
−dL/dt (μm/s)						
WKY	−197 ± 41	−139 ± 20	−143 ± 26	−129 ± 22*	−116 ± 17*	−118 ± 12*
SHR	−169 ± 17	−191 ± 19	−129 ± 23	−151 ± 24	−101 ± 19*	−174 ± 18

Note. Time-to-peak shortening (TPS), time-to-90% relengthening (TR_{90}), and maximal velocities of both shortening (+dL/dt) and relengthening (−dL/dt) of the twitch. Data represent means ± SEM. #$p < 0.05$ vs WKY, n = 17–21 cells/group.

ethanol concentration (Fig. 3). As shown in Fig. 3, the slopes of the two curves are similar in WKY myocytes whereas they are quite different in SHR myocytes, over the concentration range of ethanol tested. The inhibitory effect of ethanol on cell shortening was significantly greater than its effect on ΔFFI in the SHR group. This observation suggests that ethanol may affect cell shortening through a mechanism independent of reduction of intracellular Ca^{2+} transients in hypertension.

Effect of ethanol on cell shortening in the presence of BayK 8644. To explore the Ca^{2+} dependence of ethanol-induced effect on cell shortening, the effect of ethanol was examined in the presence of the dihydropyridine BayK 8644 (a voltage-dependent Ca^{2+} channel opener). As expected, BayK 8644 (1 μM) alone increased PTA by 114 ± 8 (n = 6) and 89 ± 6 (n = 8)%, in WKY and SHR myocytes, respectively. Interestingly, 240 mg/dl ethanol-induced inhibition on cell shortening was greatly attenuated in both WKY and SHR myocytes, in the presence of BayK 8644 (Fig. 4).

DISCUSSION

Acute cardiac depression by ethanol has been an important clinical issue in both healthy and diseased hearts (13, 14). Ethanol has been shown to be associated with depression of cardiac contractility at both cellular and tissue levels (1, 3, 6, 10, 15). Although ethanol is one of the oldest known substance of abuse, the mechanisms whereby this compound causes cardiac dysfunction remain only partially understood. The reduction in myocardial contraction induced by ethanol may be the result of direct or indirect effects of ethanol on various aspects of the excitation-contraction process such as Na^+/K^+- and Ca^{2+}-ATPase activity (16).

Epidermological evidence has long suggested that there is a significant interaction between ethanol ingestion, blood pressure and development of hypertension (17). Using the genetically inbred model of essential hypertension-SHR, we recently demonstrated the myocardial depressive effect of ethanol was not modified by hypertension (10, 15). However, our present

TABLE 3

Effect of Ethanol on Intracellular Ca^{2+} Transient Resting FFI and Fluorescence Decay Time (FDT) in Both WKY and SHR Myocytes

	Baseline	Ethanol (80 mg/dl)	Ethanol (120 mg/dl)	Ethanol (240 mg/dl)	Ethanol (640 mg/dl)	Recovery
Resting FFI						
WKY	0.84 ± 0.02	0.81 ± 0.01	0.80 ± 0.01	0.81 ± 0.01	0.82 ± 0.01	0.84 ± 0.01
SHR	0.98 ± 0.03#	0.97 ± 0.03	1.00 ± 0.03	0.95 ± 0.05	0.97 ± 0.03	1.01 ± 0.03
FDT (msec)						
WKY	226 ± 8	226 ± 8	231 ± 12	228 ± 9	218 ± 7	218 ± 9
SHR	263 ± 19#	264 ± 21	213 ± 15*	191 ± 18*	208 ± 9*	243 ± 22

Note. Data represent means ± SEM. *$p < 0.05$ vs WKY, n = 12/group.

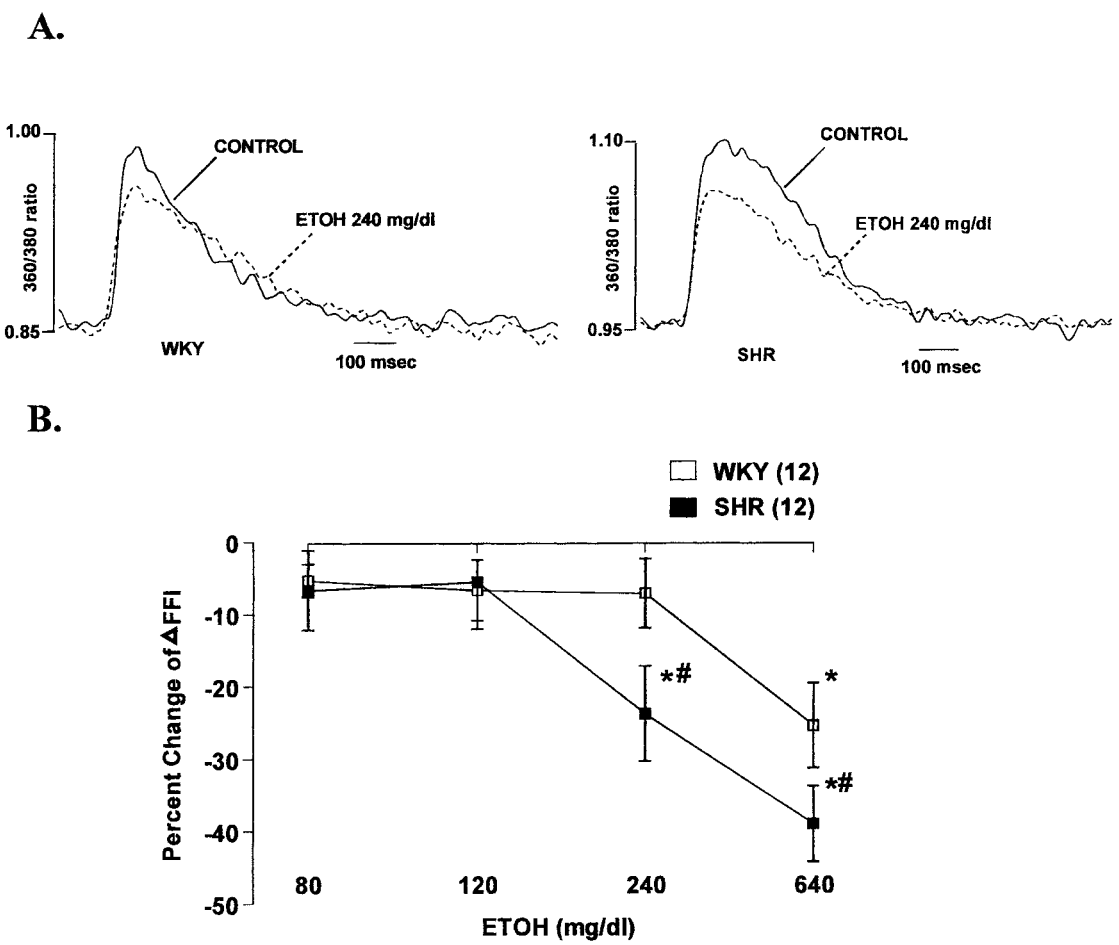

FIG. 2. (A) Typical experiments showing the effect of ethanol (ETOH) on intracellular Ca^{2+} transients in a WKY (left) and a SHR (right) myocyte. Solid and dashed traces show Ca^{2+} transients recorded from fura-2 loaded myocytes before and 10 min after ethanol (240 mg/dl) exposure. (B) Concentration-dependent response of ethanol (80–640 mg/dl) on intracellular Ca^{2+} transient changes (ΔFFI) in myocytes from both WKY and SHR animals. Mean ± SEM, $*p < 0.05$ vs control, $\#p < 0.05$ vs WKY.

study demonstrates that hypertension augments the ethanol-induced depression of myocardial contraction and intracellular Ca^{2+} concentration in ventricular myocytes, suggesting a discrepancy in the myocardial action of ethanol between the tissue and the cellular level. Our previous study showed that hypertension is associated with reduced force generating capacity of the ventricular papillary muscles (4, 10, 15). However the present study showed myocytes from SHR animals exhibit significantly greater shortening capacity at the single cell level, which may be the result of a higher afterload in hypertension. SHR myocytes also show prolonged relengthening duration, elevated diastolic intracellular Ca^{2+} level and slowed intracellular Ca^{2+} clearing. The prolonged relengthening time is correlated with a slower Ca^{2+} decay rate. It is possible that non-myocyte factors such as fibroblast or nerve terminals may contribute to the differential ethanol-induced contractile response.

Agents that reduce Ca^{2+} entry, deplete or block intracellular Ca^{2+} stores are expected to depress contractility. As such, ethanol is thought to affect a number of steps in the excitation-contraction coupling process. The most likely targets would include membrane ion channels and pumps responsible for action potential; sarcoplasmic reticulum (SR) Ca^{2+} release/resequestration; contractile proteins. Ethanol has been shown to depress SR Ca^{2+} binding (18), which was correlated with reduced contractile force and prolonged relaxation kinetics (14, 19). Recently, Danziger et al. (6) demonstrated that ethanol produces moderate contractile depression of adult rat myocytes without altering the magnitude of Ca^{2+} transient. Higher concentrations of ethanol severely depressed both contractile amplitude and magnitude of Ca^{2+} transient (6). In this study, the extent of depression of ethanol on myocyte shortening was similar to its effect on intracellular Ca^{2+} in the WKY group, suggesting that ethanol-induced inhibition of intracellular Ca^{2+} transients may account for its myocardial depression. In contrast, ethanol depresses cell shortening at concentrations that fail to inhibit intracellular Ca^{2+} transients in SHR

myocytes. This dissociation between PTA and ΔFFI indicates that ethanol may cause depression of myocyte contraction through a Ca^{2+}-independent mechanism(s), such as myofilament sensitivity. This led to the suggestion that the ethanol-induced reduction in contractile amplitude was possibly due to a reduced myofilament Ca^{2+} sensitivity at low concentrations, whereas higher concentrations causes a decrease of intracellular Ca^{2+}. Furthermore, the extent of reduction of Ca^{2+} sensitivity or intracellular Ca^{2+} may be substantiated in hypertension.

One interesting observation of the study was that ethanol-induced inhibition of cell shortening was greatly attenuated by the voltage-dependent Ca^{2+} channel opener, BayK 8644, to a similar extent in both WKY and SHR myocytes. This indicates that ethanol may interfere with the voltage-dependent Ca^{2+} entry and such interference may be more substantiated at hypertensive state. Kojima et al., (14) also reported that alcoholic cardiac depression in whole heart is partially antagonized by high extracellular Ca^{2+}, which would increase the Ca^{2+} influx. Although voltage-dependent Ca^{2+} channel activity is believed to be upregulated in hypertension (19), little information is known at this time regarding the influence of hypertension on ethanol-induced action on this type of Ca^{2+} channels. The fact that a high concentration of ethanol shortened TR_{90} and Ca^{2+} transient decay rate indicates that ethanol may interfere with mechanisms associated with Ca^{2+} sequestration/extrusion such as Na/Ca exchanger, Ca^{2+} pump or activation of β-adrenergic system.

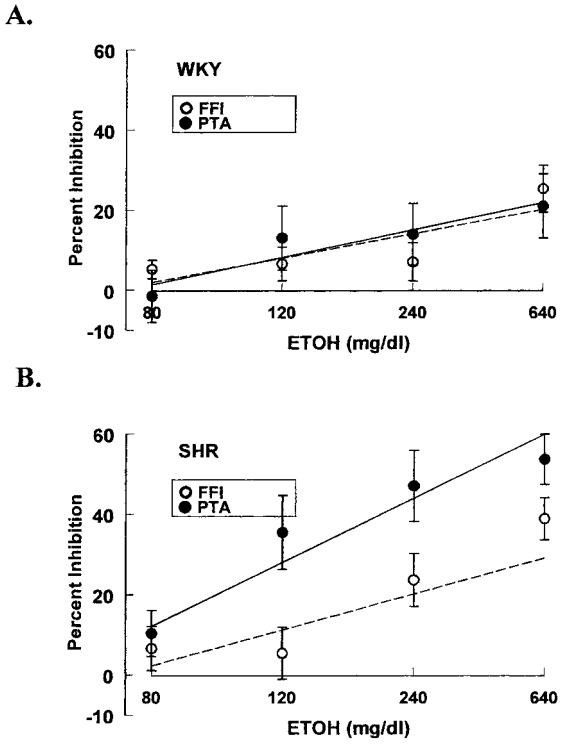

FIG. 3. Correlation of the depression of ΔFFI and reduction in PTA induced by ethanol (ETOH) in WKY (A) and SHR (B) myocytes. Each point represents the decrease of either ΔFFI (○) or PTA (●) plotted against concentration of ethanol. Data from both ΔFFI and PTA were expressed as percentage inhibition. Changes of the two parameters were closely correlated to each other in WKY group (PTA: slope = 8.07, r^2 = 0.87; ΔFFI: slope = 3.63, r^2 = 0.67). However, changes of these parameters were significantly different from each other in SHR group (PTA: slope = 8.94, r^2 = 0.88; ΔFFI: slope = 15.92, r^2 = 0.92).

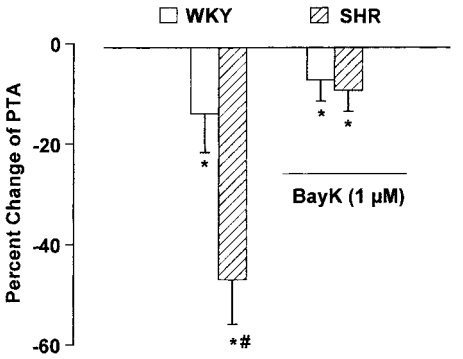

FIG. 4. Graph shows the effect of ethanol (ETOH) on myocyte shortening in the presence of voltage-dependent Ca^{2+} channel opener BayK 8644 (1 μM). Measurements were made before and 10 min after ethanol (240 mg/dl) administration. Data are expressed as means ± SEM, *$p < 0.05$ vs control. #$p < 0.05$ vs WKY.

In conclusion, the present study reveals that hypertension affects ethanol-induced myocardial contractile dysfunction. Our findings indicate that ethanol disproportionately affects cell shortening and intracellular Ca^{2+} transients in hypertensive myocytes. This action may be associated with alterations in sarcolemmal Ca^{2+} channel function and via Ca^{2+}-independent mechanism(s).

ACKNOWLEDGMENTS

The authors gratefully acknowledge Nidas Undrovinas and LeQuishia Jefferson for technical assistance. This work was supported in part by National Institutes of Health/NHLBI: GM08167 and NIMH/MIRDP MH47181 (to R.A.B.).

REFERENCES

1. Guarnieri, T., and Lakatta, E. G. (1990) *J. Clin. Invest.* **85,** 1462–1467.
2. Preedy, V. R., and Richardson, P. J. (1994) *Br. Med. Bulletin* **50,** 152–163.
3. Brown, R. A., Robinson, E., and Dunbar, J. C. (1993) *Alcohol* **10,** 231–235.
4. Brown, R. A., Crawford, M. L., Natavio, M., Petrovski, P., and Ren, J. (1998) *Alcoholism Clin. Exp. Res.* **22,** 2062–2072.
5. Kobayashi, M., Furukawa, Y., and Chiba, S. (1979) *Arch. Int. Pharmacodyn. Ther.* **239,** 109–120.
6. Danziger, R. S., Sakai, M., Capogrossi, M. C., Spurgeon, H. A.,

Hansford, R. G., and Lakatta, E. G. (1991) *Circ. Res.* **68,** 1660–1668.

7. Schulman, S. P., Lakatta, E. G., Weiss, R. G., Wolff, M. R., Hano, O., and Gerstenblith, G. (1991) *J. Mol. Cell Cardiol.* **23,** 417–426.
8. Klatsky, A. L., Freidman, G. D., and Armstrong, M. A. (1986) *Circulation* **73,** 628–636.
9. Potter, J. F., Watson, R. D. S., Skan, W., and Beevers, D. G. (1986) *Hypertension* **8,** 625–631.
10. Brown, R. A., Savage, A. O., and Lloyd, T. C. (1996) *Eur. J. Pharmacol.* **302,** 61–67.
11. Ren, J., Davidoff, A. J., and Brown, R. A. (1997) *Cell Mol. Biol.* **46,** 825–834.
12. Zhang, X. Q., Moore, R. L., Tenhave, T., and Cheung, J. Y. (1995) *Am. J. Physiol.* **269,** C632–C640.
13. Kelbaek, H. (1990) *Prog. Cardiovasc. Dis.* **32,** 347–364.
14. Kojima, S., Wu, S. T., Wikman-Coffelt, J., and Parmley, W. W. (1993) *Cardiovasc. Res.* **27,** 811–816.
15. Brown, R. A., Savage, A. O., and Lloyd, T. C. (1996) *Hypertension* **28,** 872–879.
16. Thomas, A. P., Rozanski, D. J., Renard, D. C., and Rubin, E. (1994) *Alcohol Clin. Exp. Res.* **18,** 121–131.
17. Hanna, E. Z., Chou, S. P., and Grant, B. F. (1997) *Alcoholism Clin. Exp. Res.* **21,** 111–118.
18. Bing, R. J., Tillmans, H., Fauvel, J., Seeler, K., and Mao, J. C. (1974) *Circ. Res.* **35,** 33–38.
19. Fisher, V. I., and Kavaler, F. (1975) *Adv. Stud. Card. Struct. Metab.* **5,** 415–422.
20. Asano, M., Nomura, Y., Ito, K., Uyama, Y., Imaizumi, Y., Watanabe, M. (1995) *J. Pharmacol. Exp. Ther.* **275,** 775–783.