Taurine Depletion and Excitation-Contraction Coupling in Rat Myocardium

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Abstract Although the sulfur-containing amino acid taurine is found in high concentrations in mammalian myocardium, its involvement in function of the cardiac myocyte remains unclear. To examine the effects of taurine depletion on cardiac mechanical function, rats were treated in vivo with the taurine transport antagonist guanidinoethane sulfonate (GES). After 6 weeks of treatment, myocardial taurine concentrations were decreased to <40% of control, with no change in tissue DNA content. Right ventricular trabeculae from taurine-depleted rats exhibited significant reductions (P<.05) in isometric twitch force (Ft) at all [Ca\(^{2+}\)], levels and systolic sarcomere lengths examined. Taurine-depleted trabeculae also exhibited increased passive compliance. A slight (P<.05) rightward shift in the Ft-[Ca\(^{2+}\)] relation suggested a decrease in the sensitivity of the transsarcolemmal Ca\(^{2+}\)-handling mechanisms remained unchanged. The fraction of Ca\(^{2+}\) recirculated through the sarcoplasmic reticulum, inferred from the decay of postextrasystolic potentiation, was also different in the taurine-depleted muscles. When force was expressed relative to the rate of stimulation, length of rest periods, or postextrasystolic potentiation, virtually all curves were superimposable for control and taurine-depleted muscles, suggesting that the deficit was not dependent on Ca\(^{2+}\) handling. Thus, we conclude that in taurine-depleted muscles the force-generating processes showed the same regulation as in control muscle. Furthermore, the substantial deficit in force development is consistent with a reduced population of force generators on the basis of three pieces of evidence. First, when Sr\(^{2+}\) was added to the perfusate to directly activate the contractile proteins, the substantial force deficit of taurine-depleted muscles persisted, indicating that the number of contractile elements was substantially smaller than in control muscles. Second, biochemical analysis revealed a significant reduction in the content of both actin and myosin. Third, the force induced by passive stretch was significantly smaller in taurine-depleted muscles, suggesting a loss of elastic elements. Our results offer new information regarding the etiology of cardiomyopathy induced by taurine deficiency. (Circ Res. 1994;74:1210-1219.)

Key Words • taurine • excitation-contraction coupling • cardiac muscle • rats

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The present studies were designed to further investigate the mechanisms underlying the deficits in contractility produced by chronic taurine deficiency by using right ventricular trabeculae from the rat heart, a preparation that has been used extensively for monitoring sarcomere function, excitation-contraction coupling, intracellular Ca\(^{2+}\) handling, and myofibrillar Ca\(^{2+}\) sensitivity.\(^{16,20-23}\) In addition to small alterations in Ca\(^{2+}\) handling and sensitivity expected from previous studies, the results provide new insight into the effects of taurine depletion, suggesting that the loss of force development is related to a reduction of the number of contractile elements.

Materials and Methods

Materials

Unless otherwise stated, all chemicals were of analytical grade and were purchased from BDH Chemicals. GES was produced as described previously.\(^3\) The antibodies against cardiac calsequestrin were supplied by Dr M. Michalak.

Taurine Depletion and Tissue Preparation

Male Sprague-Dawley rats (initial weight, 150 to 175 g) were given access to normal rat chow ad libitum, with treated rats receiving 1% GES in their drinking water.\(^3\) After a minimum of 6 weeks, the animals were anesthetized with ether, and their hearts were removed and perfused through the coronary arteries via the aorta with a modified Krebs-Henseleit buffer containing (mmol/L) NaCl 120, KCl 20, MgCl\(_2\) 1.2, NaSO\(_4\) 1.2, NaH\(_2\)PO\(_4\) 2.0, NaHCO\(_3\) 19, CaCl\(_2\) 0.7, and glucose 10, equilibrated with 95% O\(_2\)/5% CO\(_2\) (2.0, NaHCO\(_3\) 19, NaH\(_2\)PO\(_4\) 2.0, NaHCO\(_3\) 19, CaCl\(_2\) 0.7, and glucose 10, equilibrated with 95% O\(_2\)/5% CO\(_2\) to maintain a pH of 7.4 at 22°C. This high-K\(^+\) buffer facilitated further dissection by inhibiting myocardial contraction. Free-running right ventricular trabeculae, attached at the atrioventricular ring and the ventricular wall, were dissected as described previously.\(^20\) The remaining ventricular tissue was frozen and stored in liquid N\(_2\) for subsequent determination of tissue taurine, DNA, calsequestrin, and myofibrillar protein content.

Each trabecula was dissected with a block of ventricular wall to fit within a stainless-steel basket attached to a miniature strain gauge (model AE-801, SensoNor). The valvular end was fixed to a stainless-steel hook attached to the arm of a servomotor model 300 S, Cambridge Technology, to permit measurement of resting sarcomere length. The trabeculae were mounted horizontally in a glass-covered chamber (volume, 0.35 mL) and superfused at 5 mL/min with the Krebs-Henseleit buffer modified as follows: 5 mmol/L KCl and 1 mmol/L CaCl\(_2\) at 26°C. The muscles were observed with an inverted microscope (model Diaphot TMD, Nikon Inc) and a video system (Panasonic camera WV 3170 and recorder AG 2400). They were stimulated at 0.2 Hz with 5-millisecond pulses at 2× threshold voltage via platinum field electrodes and, under these conditions, were stable for the duration of the experiments (up to 5 hours). Except where noted, trabeculae contracted while their overall length remained constant. Each trabecula was measured with an ocular micrometer; the accuracy of the measurement was ±5 μm for width and ±10 μm for thickness. All force measurements were normalized to the cross-sectional area for comparisons between muscles. A video image of each trabecula was obtained through the microscope objective and was continuously monitored. Sarcomere length was measured at a nontranslating region by use of the optical diffraction technique described in detail previously.\(^20\) Force and sarcomere length signals were measured with a bridge amplifier connected to a chart recorder (model 2800S, Gould) and sampled at 900 Hz with an analog-to-digital converter (model DT 2801A, Data Translation Inc) and stored on an IBM AT computer.

Experimental Protocols

Force–Sarcomere Length Relation

Each trabecula was equilibrated for 45 minutes in Krebs-Henseleit buffer containing 1.6 mmol/L Ca\(^{2+}\) and stimulated at 0.2 Hz at a resting sarcomere length of 2.0 μm. Then the resting sarcomere length was adjusted in 0.05-μm steps from 1.8 to 2.3 μm, while passive force, twitch force (Ft), and systolic sarcomere length were recorded after a 30-second equilibration period at each new length. This protocol was repeated in Krebs-Henseleit buffer containing 0.4 mmol/L Ca\(^{2+}\). The total force generated during each twitch, calculated as active force plus any passive force at the same systolic sarcomere length, was related to the systolic sarcomere length attained at peak force development. On completion of this protocol, resting sarcomere length was set so that passive force was ±5% of developed force (ie, 2.1 to 2.2 μm).

Force-Ca\(^{2+}\) Relation

Each trabecula was equilibrated in Krebs-Henseleit buffer containing 0.1 mmol/L Ca\(^{2+}\) at a stimulation frequency of 0.2 Hz, and Ft was determined. The response to increasing [Ca\(^{2+}\)]\(_o\) was assessed by sequentially doubling [Ca\(^{2+}\)]\(_o\), to a maximum of 3.2 mmol/L, and recording Ft after a 15-minute equilibration period at each [Ca\(^{2+}\)]\(_o\) level. The six points for each trabecula were fitted to the Hill equation as follows:

\[
\text{Ft/Ft}_{\text{max}} = \frac{[\text{Ca}^{2+}]^{n}}{[\text{Ca}^{2+}]^{n} + K_d^{n}}
\]

where Ft\(_{\text{max}}\) is maximal Ft, n is the Hill coefficient, and K\(_d\) is the [Ca\(^{2+}\)]\(_o\) at half Ft\(_{\text{max}}\). Each curve was iteratively fit by use of SIGMAPLOT (Jandel Scientific), which generated values for Ft\(_{\text{max}}\), K\(_d\), and n for each trabecula separately. To determine differences in the response and sensitivity of the muscles to [Ca\(^{2+}\)]\(_o\), the K\(_d\) and n values for control and taurine-depleted trabeculae were averaged separately and compared. Each trabecula was then equilibrated for 15 minutes in Krebs-Henseleit buffer containing 0.7 mmol/L Ca\(^{2+}\) at 26°C and stimulated at 0.2 Hz. Individual stimulation protocols will be described in each figure legend.

Force–[S\(_{2}\)P\(_2\)]\(_o\), Relation in the Presence of 0.4 mmol/L [Ca\(^{2+}\)]\(_o\)

Trabeculae were equilibrated at 0.2 Hz for 20 minutes in Krebs-Henseleit buffer containing 0.4 mmol/L Ca\(^{2+}\) and then exposed sequentially to increasing concentrations of S\(_{2}\)P\(_2\) ranging from 1.5 to 15 mmol/L added to the buffer, while Ft and sarcomere length were recorded. [Ca\(^{2+}\)]\(_o\) remained constant at 0.4 mmol/L throughout this protocol.

Biochemical Assays

Ventricular taurine and DNA levels were determined by using the techniques described by Lake et al.\(^3\) Contractile proteins were isolated from each of six control and six taurine-depleted heart tissue samples by using a low-salt–high salt protamine sulfate crude extraction method.\(^24\) The equivalent of ~20 mg wet weight of frozen left ventricular tissue was ground to a fine powder in liquid N\(_2\), homogenized on ice for 5 minutes in 200 μL of chilled low-salt buffer containing (mmol/L) KCl 20, K\(_2\)HPO\(_4\) 2, and EGTA 1, along with 5 μmol/L leupeptin, pH 6.8, and centrifuged at −10°C for 45 seconds at 2500g. The supernatant was frozen in liquid N\(_2\) for subsequent determination of soluble proteins, and the pellet was resuspended for 30 minutes on ice in 250 μL of high-salt buffer containing (mmol/L) Na\(_4\)P\(_2\)O\(_7\) 40, MgCl\(_2\) 1, and EGTA 1, pH 9.5. After centrifugation at −10°C for 15 minutes, this second supernatant, containing the contractile proteins, was frozen and stored in liquid N\(_2\). Both supernatants and the final pellet were assayed for protein content by the method described by Lowry et al\(^{25}\) with bovine serum albumin as a
standard. Less than 5% of the total tissue protein remained in the final pellet. An aliquot of the high-salt supernatant containing either 5, 10, or 20 \( \mu \)g of total protein was added to each lane of an 8% sodium dodecyl sulfate (SDS)–polyacrylamide slab gel and the proteins were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The relative protein content for each band was approximated by an integrating laser densitometer (LKB).

A second sample of left ventricular tissue from each of six control and six taurine-depleted hearts was used for isomyosin determination by a modification of the technique described by Hoh et al. Briefly, membranes were removed from \( \approx 10 \) mg of frozen (in \( N_2 \)) tissue in a buffer containing (mmol/L) \( K_2HPO_4 \) 100, EDTA 5, and mercaptoethanol 2, pH 8.8. After a 5-minute centrifugation at 2500g, the supernatant was collected and mixed with an equal volume of 100% glycerol, and protein content was determined using the Bradford method with bovine serum albumin as a standard. A total of 12 \( \mu \)g protein was loaded onto a 4% polyacrylamide tube gel (containing 20 mmol/L Na_2HPO_4). The myosin heavy chain isoforms were separated overnight by an electrophoresis system (Pharmacia GE 2/4) with recirculating running buffer (containing 20 mmol/L Na_2HPO_4, 4 mmol/L cysteine, and 10% glycerol, pH 8.8) to prevent the generation of a pH gradient in the gels. Tube gels were also run on which were loaded 6 \( \mu \)g (each) of myosin from both control and taurine-depleted myocardium or 6 \( \mu \)g (each) of purified \( \alpha \) and \( \beta \) isoforms. The gels were stained with Coomassie blue.

The equivalent of 100 mg wet weight of frozen powdered ventricular tissue from each of six control and six taurine-depleted hearts was homogenized in 400 \( \mu \)L of chilled buffer containing (mmol/L) \( KH_2PO_4 \) 100, EDTA 1, and (NH_4)_2SO_4 2.44, plus phenylmethylsulfonyl fluoride (Sigma) 0.5, and benzamidine (Sigma) 0.01, and then centrifuged at 0°C for 30 minutes at 9000g. The supernatant was saved, the pellet was resuspended in 400 \( \mu \)L of the above buffer and centrifuged, and the second supernatant was combined with the first. To this was added 100 mg of (NH_4)_2SO_4, and the pH was brought to 4.7 by using 1N NaOH. The solution was stirred on ice for 3 hours before centrifuging for 30 minutes at 9000g. The pellet was resuspended in 500 \( \mu \)L of 0.1 mol/L \( KH_2PO_4 \) and 1 mmol/L EDTA, pH 7.1, and dialyzed overnight in 50 mmol/L NaCl, 0.1 mol/L \( KH_2PO_4 \), and 1 mmol/L EDTA. The sample was centrifuged for 10 minutes, and the supernatant was used for the determination of calsequestrin content. An aliquot containing 10 \( \mu \)g protein was separated using SDS-PAGE (10% gel) at neutral pH, and the proteins were transferred to nitrocellulose according to the method of Towbin et al and exposed sequentially to (1) rabbit anti-bovine cardiac calsequestrin antibody and (2) goat anti-rabbit peroxidase. Antibody binding was detected with a standard peroxidase color development. The mobility of calsequestrin on SDS-PAGE is pH sensitive, and under neutral pH conditions, the cardiac form of calsequestrin will migrate to the \( \approx 45 \) 500-D position.

**Statistics**

Data were analyzed by STATVIEW 512 (Abacus Concepts) or CLR ANOVA (Cleal Lake Research) software on a Macintosh SE30 computer. The significance of differences between experimental groups was determined using the Bonferroni correction for multiple samples. Values are expressed as mean±SEM, and significance was set at \( P<0.05 \).

**Results**

After 6 weeks of treatment with GES, all animals appeared healthy. Myocardial DNA levels were similar in control (0.346±0.037 mg/g wet weight, \( n=9 \)) and GES-treated (0.347±0.026 mg/g wet weight, \( n=14 \)) rats. The myocardial taurine content was reduced by 61% (\( P<0.01 \)), from 38.43±2.98 \( \mu \)mol taurine per milligram DNA in control (\( n=9 \)) to 15.13±1.10 \( \mu \)mol taurine per milligram DNA in GES-treated (\( n=14 \)) rats. No differences were observed in the heart weights (left ventricular wet weights were 816±31 mg for control muscles and 789±18 mg for taurine-depleted muscles, \( n=8 \) for each) or in the availability of right ventricular trabeculae between the two groups. Trabecular length (2.32±0.20 versus 2.41±0.22 mm) and cross-sectional area (0.041±0.009 versus 0.047±0.011 mm²) were not different between 12 control and 14 taurine-depleted muscles, respectively.

**Force–Sarcomere Length Relation**

The data on force development by trabeculae at varying sarcomere lengths are summarized in Fig 1. Although passive force increased in both groups of trabeculae at diastolic sarcomere lengths >2.2 \( \mu \)m, taurine-depleted muscles exhibited significantly greater compliance in response to passive stretch. The mean passive force in response to increasing sarcomere length was the same for Krebs-Henseleit buffer containing either 0.4 or 1.6 mmol/L \( Ca^{2+} \). In 1.6 mmol/L \( Ca^{2+} \), both taurine-depleted and control trabeculae exhibited a force–sarcomere length relation that was convex toward the ordinate, whereas in 0.4 mmol/L \( Ca^{2+} \), the relation was convex toward the abscissa (data not shown), as has been described previously for trabeculas of normal rats. Taurine-depleted trabeculae developed significantly less total force than control trabeculae at all systolic sarcomere lengths >1.7 \( \mu \)m.
nized sarcomere activity in buffer with a [Ca\(^{2+}\)]\(_{0}\) of 3.2 mmol/L.

Nonsynchronized sarcomere activity has been reported in trabeculas at elevated [Ca\(^{2+}\)]\(_{0}\) or under conditions that result in supermaximal Ca\(^{2+}\) loading of the sarcoplasmic reticulum.\(^{22,32}\) The nonsynchronized sarcomere activity observed in both control and taurine-depleted trabeculas is therefore taken to represent the spontaneous release of Ca\(^{2+}\) from a saturated sarcoplasmic reticulum.

Time to peak force was longer in taurine-depleted muscles (180±10 milliseconds) than in control muscles; time to peak force in the taurine-depleted muscles was independent of peak force. In contrast, time to peak force increased with peak force in control muscles (from 135±10 to 170±7 milliseconds for peak forces of 5% and 100% of Ft\(_{\text{max}}\), respectively). The rate of relaxation was similar in both groups of muscles (data not shown).

**Force-Frequency Relation**

The responses of control and taurine-depleted trabeculas to increasing stimulation frequency in Krebs-Henseleit buffer containing 0.7 mmol/L Ca\(^{2+}\) are summarized in Fig 3. In both groups, developed force increased with increasing stimulation frequency from 0.4 to 1.6 Hz. Whereas developed force in trabeculas from control hearts continued to increase until 3.2 Hz, Ft in taurine-depleted trabeculas declined slightly (P=NS) from 1.6 to 3.2 Hz. This resulted in a significant difference in percent force generation at 3.2 Hz between the taurine-depleted and control trabeculas. This decrease of Ft coincided with an increase in nonsynchronous sarcomere activity and spontaneous force generation between twitches (see inset, Fig 3), which appeared on the video image as small regional contractions along the length of the trabeculas. This is taken to represent the spontaneous release of Ca\(^{2+}\) from saturated sarcoplasmic reticulum Ca\(^{2+}\) stores.

**Force-Interval Relation**

At [Ca\(^{2+}\)]\(_{0}\). of 0.7 mmol/L, trabeculas from normal rats express a triphasic force-interval relation, with maximal peak force occurring when the interstimulus interval approaches 200 seconds.\(^{21}\) A downward shift in the late phase (ie, >5 seconds) of the force-interval curve reflects a reduced Ca\(^{2+}\) influx into the sarcoplasmic reticulum; an upward shift reflects a greater Ca\(^{2+}\) influx.\(^{21}\) As illustrated in Fig 4, no shift in the force-interval curve was observed in the taurine-depleted muscles compared with control muscles.

**Postextrasystolic Potentiation and the Rate of Decay**

The introduction of a series of extrastimuli (ie, 10 at 2 Hz) potentiated the first postextrasystolic steady-state beat (Ft\(_{\text{PESP}}\), where PESP is postextrasystolic potentiation) to the same extent in control and taurine-depleted trabeculas (191.8±23.4% [n=14] and 182.3±17.4% [n=10], respectively). Ft\(_{\text{max}}\) after potentiation was similar to Ft\(_{\text{max}}\) at elevated [Ca\(^{2+}\)]\(_{0}\), as has previously been shown for normal trabeculas.\(^{32}\) Since the taurine-depleted muscles responded in the same manner, there remained a significant disparity from control in maximal Ft\(_{\text{PESP}}\) generated per cross-sectional area.
When the contractility of the heart is potentiated by extrasystoles, the return to the steady-state force development is characterized by a nearly exponential decay (see Fig 5, inset). During this return to steady-state Ft, the slope of the relation between a potentiated twitch (ie, Ft\textsubscript{n+1}) and the immediately preceding contraction (ie, Ft\textsubscript{n}) is used as an approximation of the recirculation fraction (R\textsubscript{f}) of the total sarcoplasmic reticulum Ca\textsuperscript{2+} released during the nth contraction that is resealed by the sarcoplasmic reticulum and rereleased during the (n+1)th contraction.\textsuperscript{23,33} Both taurine-depleted and control trabeculae exhibited an exponential decay of potentiation with an R\textsubscript{f} comparable to previously reported values.\textsuperscript{23} Taurine-depleted muscles exhibited a slight (P=NS) tendency toward depression of R\textsubscript{f} compared with control muscles (0.73±0.04 [n=9] versus 0.84±0.04 [n=12]).

**Calsequestrin**

The difference in twitch kinetics between taurine-depleted muscles and control muscles was negligible. This suggested to us that the sum of Na\textsuperscript{+}-Ca\textsuperscript{2+}-mediated Ca\textsuperscript{2+} extrusion and Ca\textsuperscript{2+} reuptake by the sarcoplasmic reticulum was similar in the taurine-depleted

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**Fig 3.** Graph showing force-frequency relation for control and taurine-depleted (guanidinoethane sulfonate [GES]-treated) trabeculae in 0.7 mmol/L [Ca\textsuperscript{2+}]\textsubscript{o}. Each muscle was stimulated at the various frequencies, ranging from 0.2 to 3.2 Hz, for a minimum of 3 min before measurement of twitch force (Ft). No differences were found between control and GES-treated rat trabeculae (n=10 for both groups) up to 1.6 Hz. The insets show tracings of sarcomere length (SL, in micrometers) and twitch force (Ft, in millinewtons per square millimeter) from a control muscle (top) and GES-treated muscle (bottom) after stimulation at 3.2 Hz. Note the increase in spontaneous sarcomere activity and concomitant nonsynchronized force development in 4 of the 10 GES-treated muscles during the first 400 milliseconds. This increased spontaneous activity at 3.2 Hz accompanied a slight (not significant) decline in Ft in the GES-treated muscles. At 3.2 Hz, GES-treated muscles developed less force than did the control muscles (\textit{P}<.05). Two control and two taurine-depleted trabeculae exhibited slight increases (average of 14% and 4% of Ft, respectively) in resting force at a stimulation rate of 3.2 Hz.

**Fig 4.** Graph showing force-interval relation for control and taurine-depleted (guanidinoethane sulfonate [GES]-treated) trabeculae in 0.7 mmol/L [Ca\textsuperscript{2+}]\textsubscript{o}. Each muscle was stimulated at 0.2 Hz (steady state); then a rest interval ranging from 300 milliseconds to 300 seconds was imposed. The force developed by the first contraction after the rest interval (Ft\textsubscript{rest}) was normalized to the force developed by the last steady-state contraction (Ft) (see inset) (n=10 for both groups).

**Fig 5.** Scatterplot showing recirculation fraction (R\textsubscript{f}) for control (○) and taurine-depleted (○, guanidinoethane sulfonate [GES]-treated) trabeculae. Muscles were stimulated at 0.2 Hz in buffer containing 0.7 mmol/L Ca\textsuperscript{2+} at 26°C, and then a burst of 10 extrasystoles at 2 Hz was imposed followed by a 5-second rest period (see inset). Twitch force (Ft) from the initial eight contractions following a 5-second rest period was normalized to the maximal force developed at postextrasystole (F\textsubscript{PESP}) and plotted. R\textsubscript{f} was calculated for each muscle separately as the slope of the line relating F\textsubscript{n+1}/F\textsubscript{PESP} to F\textsubscript{n}/F\textsubscript{PESP} and the mean R\textsubscript{f} values (0.84±0.04 vs 0.73±0.04, n=10 for each group) for control and GES-treated muscles were compared. Regression lines (drawn) for both groups are indicated together with confidence intervals (dotted lines); no significant differences were observed between these mean R\textsubscript{f} values.
Fig 6. Sarcoplasmic reticulum proteins isolated with pH-neutral 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (A), then transferred onto nitrocellulose, and reacted with anti-cardiac calsequestrin antiserum (B). Lane 1 shows molecular weight markers (MMW); lane 2, sarcoplasmic reticulum proteins from guanidinoethane sulfonate (GES)-treated rat right ventricular tissue; and lane 3, sarcoplasmic reticulum proteins from control rat right ventricular tissue. No differences were observed in electrophoretic mobility of the proteins, or in their reactivity to the anticalcerein antibodies.

Fig 7. Graph showing force-strontium relation for control and taurine-depleted (guanidinoethane sulfonate [GES]-treated) trabeculas. Trabeculas were equilibrated in Krebs-Henseleit buffer containing 0.4 mmol/L Ca\(^{2+}\) with 0 mmol/L Sr\(^{2+}\), as represented by the dotted lines and first data points (black bar). Sr\(^{2+}\) was added to the perfusate at increasing concentrations, allowing 15-minute equilibration between additions, and steady state twitch force (Ft) was recorded. For the eight control muscles, 12 mmol/L [Sr\(^{2+}\)], induced a 74.8±13.1% increase in maximal force development over that produced by [Ca\(^{2+}\)]\(_0\), alone (66.6±10.6 mM/mg in 3.2 mmol/L [Ca\(^{2+}\)], vs 116.5±13.4 mM/mg in 12 mmol/L [Sr\(^{2+}\)], P<.05 by paired t test). For the seven GES-treated muscles, 12 mmol/L [Sr\(^{2+}\)], induced a 40.3±7.4% increase in maximal force development over that produced by [Ca\(^{2+}\)], alone (49.6±8.2 mM/mg in 3.2 mmol/L [Ca\(^{2+}\)], vs 70.7±11.3 mM/mg in 12 mmol/L [Sr\(^{2+}\)], P<.05 by paired t test). **P<.01 (n=6 for both groups).

**Force-[Sr\(^{2+}\)], Relation at 0.4 mmol/L [Ca\(^{2+}\)]\(_0\)**

Strontium-activated contractions were studied in Krebs-Henseleit buffer containing 0.4 mmol/L Ca\(^{2+}\) (required to maintain muscle integrity\(^{22}\)) and [Sr\(^{2+}\)]\(_0\) ranging from 1.5 to 15 mmol/L (see Fig 7). Aftercontractions were frequently observed in 15 mmol/L [Sr\(^{2+}\)]. Six of eight control muscles showed aftercontractions (average amplitude, 12% Ft\(_{\text{max}}\)), and four of seven taurine-depleted muscles developed aftercontractions (average amplitude, 5.5% Ft\(_{\text{max}}\)) under these conditions. For both groups, Ft\(_{\text{max}}\) achieved in Krebs-Henseleit buffer with 0.4 mmol/L Ca\(^{2+}\) and 12 mmol/L Sr\(^{2+}\) exceeded Ft\(_{\text{max}}\) achieved in buffer containing 3.2 mmol/L Ca\(^{2+}\) alone. The ratio between Ft\(_{\text{max}}\) developed with 12 mmol/L [Sr\(^{2+}\)], and Ft\(_{\text{max}}\) in 3.2 mmol/L [Ca\(^{2+}\)], for the six control muscles (1.75±0.13) was significantly higher than for the six taurine-depleted trabeculas (1.40±0.07).

**Contractile Proteins**

Exposure of the trabeculas to Sr\(^{2+}\) allows direct activation of the contractile filaments by bypassing the sarcoplasmic reticulum (see “Discussion”). This difference in maximally developed force in the presence of high [Sr\(^{2+}\)]\(_0\) of the taurine-depleted and control muscles could suggest a difference in either the structure or concentration of the contractile filaments.\(^{13}\) Fig 8 represents the separation of a crude extract of contractile proteins from control and taurine-depleted ventricular tissue. The major bands, consisting of myosin heavy and light chains, actin, troponin, and tropomyosin, showed no differences in either electrophoretic mobility or relative protein concentration, as determined by laser densitometry, between the two tissues. Similarly, no changes in the
distribution of V₁ and V₃ myosin isoforms were observed (Fig 8). Total tissue protein, expressed in milligrams protein per gram tissue dry weight, was slightly less (148.1±8.8 versus 167.5±7.3 mg/g, n=6, P=NS) in the taurine-depleted ventricular tissue compared with control tissue. Although there was no difference in the concentration of the proteins soluble in low-salt buffer (85.9±4.9 versus 88.2±4.8 mg/g), there was significantly less protein soluble in high-salt buffer (62.1±4.3 versus 80.2±4.6 mg/g) in taurine-depleted muscles compared with control muscles. Although the present study does not attempt to quantify the concentrations of actin or myosin directly, these results support those presented elsewhere showing that taurine-depleted myocardium expresses a reduced fraction of contractile proteins compared with control myocardium.

**Discussion**

Treatment of rats in vivo for 6 weeks with GES, a compound that antagonizes taurine transport, led to a loss of >60% of ventricular taurine, although the number of cells per unit volume (ie, DNA per wet weight) remained unchanged. These findings reproduced earlier reports indicating the dependence of myocardial taurine levels on specific transport processes. Earlier studies reported reduced force generation by papillary muscles excised from taurine-depleted rats, suggesting a link between cellular taurine and excitation-contraction coupling; however, the mechanisms involved remained unknown. The present studies made use of right ventricular trabeculae, which, because of their much smaller dimensions (ie, <100-μm diffusion distances), are less prone to the development of hypoxic regions. Further, the ability to simultaneously assess both force generation and dynamic sarcomere length ensures that force development was occurring at comparable sarcomere lengths. Thus, these trabeculae represent a superior model with which to examine the mechanisms of excitation-contraction coupling possibly affected by taurine deficiency.

Fₜmax that was developed by right ventricular trabeculae from taurine-depleted rats is only 50% to 60% of that developed by trabeculae from control rats (see Figs 1, 2, and 7). Several mechanisms could account for this loss of force generation: (1) a rightward shift in the length-dependent activation of the muscle, (2) decreased sensitivity of the myofibrils to available intracellular Ca²⁺, (3) curtailment of transsarcolemmal Ca²⁺ movement via Ca²⁺ channels or Na⁺-Ca²⁺ exchanger, (4) decreased activity of the sarcoplasmic reticulum Ca²⁺-ATPase, (5) decreased sarcoplasmic reticulum Ca²⁺ storage capacity, (6) impaired sarcoplasmic reticulum Ca²⁺ release, (7) change in myosin isoform from V₁ to V₃ or (8) a smaller population of force-generating units.

As shown in Fig 1, total force rises continually with sarcomere length over the range of sarcomere lengths at which the cardiac muscle operates. Although Fₜ developed by the taurine-depleted trabeculae is <60% of that developed by the control trabeculae at any given systolic sarcomere length, no shift in the relative force–sarcomere length relation was observed. This suggests that the reduction of developed force was not due to a reduced level of activation of the myofibrils, because this would change the curvature of the force–sarcomere length relation from convex toward the ordinate to convex toward the abscissa. For the control muscles, passive force increased with increasing diastolic sarcomere length, from negligible values at ~2.0 μm to values approaching 50% of active force by 2.3 μm. Taurine-depleted muscles exhibited significantly greater compliance in response to passive stretch. However, when force development was normalized for Fₜmax, the passive force–length relations of the taurine-depleted and the control muscles were not significantly different (Fig 1).
It seems, from the present study, reasonable to conclude that the effect of taurine depletion on the contribution of Ca²⁺ transport to excitation-contraction coupling is modest. Previous work has shown that the ventricular action potential duration is lengthened in taurine-depleted ventricular tissue, implying that the inward transsarcolemmal Ca²⁺ current was not curtailed. Using control rat trabeculae, Schouten et al. showed that blockage of the Ca²⁺ channels with nifedipine induced a significant decrease in the early phase of the force-interval relation. Their model further assumes that the late decaying phase of the force-interval relation—or rest depression—correlates with the leak of Ca²⁺ out of the cell via the Na⁺-Ca²⁺ exchange. Although we did not directly measure transsarcolemmal Ca²⁺ movements in the present study, the fact that the force-interval relations were identical between control and taurine-depleted trabeculae suggests that the transport of Ca²⁺ across the sarcolemmal membrane is not altered in taurine-depleted muscles.

It is impossible from either the present study or previous studies to separate changes in the ability of the sarcoplasmic reticulum to take up and release Ca²⁺ from a modified sensitivity of the contractile proteins to the available Ca²⁺. The addition of 30 mmol/L taurine to the activating solutions for skinned trabeculae from normal rats produces a slight (0.8%) increase in maximal Ca²⁺-activated force, whereas it decreases the [Ca²⁺]i needed for half-maximal tension by an average of 10.5%. However, Steele et al. also showed that taurine enhanced caffeine-induced contractures under conditions of submaximal sarcoplasmic reticulum Ca²⁺ loading but decreased the size of these contractures if sarcoplasmic reticulum Ca²⁺ loading was sufficiently high. Our data are consistent with the findings in the latter study: we observed a slightly decreased sensitivity of the trabeculae to [Ca²⁺]o and a slight (P=NS) decrease in sarcoplasmic reticulum recirculation fraction in the taurine-depleted muscles compared with control muscles. We failed to find any significant change in the content of sarcoplasmic reticulum Ca²⁺-binding protein calsequestrin, suggesting no change in the size of this pool of sarcoplasmic reticulum Ca²⁺ available for release, although we cannot rule out possible changes in the affinity or capacity of the calsequestrin for Ca²⁺.

One of the striking aspects of the present study was that despite the force deficit, the relation between force and several determinant parameters was the same for taurine-depleted and control muscles. When percent Ft max was expressed relative to the rate of stimulation up to 1.6 Hz, the length of rest periods, or postextrasystolic potentiation, the curves were nearly superimposable for control and taurine-depleted muscles, suggesting that the deficit is not dependent on Ca²⁺ handling. The only exception to this observation was a reduction of force at a high frequency of stimulation (3.2 Hz) in the taurine-depleted muscles, whereas the tendency of the muscles to develop spontaneous sarcomere-length fluctuations under these circumstances was increased. Increased spontaneous activity may explain reduced force development, but this explanation does not apply to the observed force deficit in the taurine-depleted muscles at the stimulus rate of 0.2 Hz and at the temperature at which these experiments were performed, because little or no spontaneous sarcomere-length fluctuations were observed under those conditions. Thus, we conclude that in taurine-depleted muscles, regulatory mechanisms of force generation show only minimal changes.

Even though we cannot judge the detailed cause of the minimal effects of taurine depletion on Ca²⁺ transport during excitation-contraction coupling, it was clear that the magnitude of these effects was insufficient to explain the nearly 50% reduction in maximal force development that we observed in taurine-depleted muscles. This led us to the hypothesis that the substantial deficits in force developed by taurine-depleted muscles reflect a reduced population of force generators. We have three pieces of evidence for this contention. First, when strontium was added to the perfusate to directly and maximally activate the contractile proteins, the substantial force deficit of taurine-depleted muscles persisted, indicating that the number of contractile elements was substantially less than in control muscles. It is not likely that the deficit in force development by the taurine-depleted muscles in the presence of strontium was due to a larger tendency to develop aftercontractions, because the data actually show the opposite; ie, control muscles generated the largest aftercontractions. Second, the hypothesis that the force deficit was due to a reduction of the number of force generators is supported by the biochemical assay, which revealed a significant reduction in the content of contractile proteins in the six samples analyzed. Third, consistent with a loss of elastic elements, it was noted that compliance of the taurine-depleted muscles was significantly higher. The increased compliance was commensurate with the decrease of active force development. The latter observation would be expected if the density of myosin filaments was decreased and thereby the density of the titin filaments was decreased. The titin filaments are at least in part responsible for the parallel elasticity of muscle; hence, a decrease of density of titin is expected to lead to increased compliance. One might consider the possibility that altered characteristics of the passive elements of the taurine-depleted muscle might underlie the increased compliance. This possibility cannot be ruled out on the basis of our data. However, it is unlikely that changes in the characteristics of the passive elastic structures underlie the reduction of active force development, because the latter has been measured at known sarcomere length and after correction for the contribution of passive elastic force at that sarcomere length.

Conclusion

In conclusion, although previous work in normal trabeculae and myocardial preparations has emphasized interactions of taurine and sarcoplasmic reticulum Ca²⁺ dynamics, our data suggest that the Ca²⁺-handling processes involved in excitation-contraction coupling are only mildly affected by taurine depletion under the conditions of our experiments. Instead, we suggest that the inability of the taurine-depleted myocardium to generate Ft max is closely related to a loss of contractile proteins. This is the first report of a direct link between functional changes and a loss of force-generating units, both passive and active, in taurine-depleted hearts. This loss of force-generating units in the rat myocardium may
explain the observation that systolic pressure development is reduced and that diastolic compliance is enhanced in the ventricles of taurine-deficient cats.9,10 Finally, and of relevance to congestive heart failure in general, several studies have suggested that taurine supplementation improves the clinical status of patients suffering from this syndrome,6,8 and improves cardiac function in animal studies of heart failure.4 The present study suggests a mechanism for a beneficial effect of taurine, meriting further investigation. Taurine has been shown to protect the myocardium by reducing Ca2+ overload in ischemia and on exposure to catecholamines, both of which are essential in the development of heart failure secondary to coronary artery disease. The protective effect requires a high intracellular taurine concentration to drive cotransport of taurine with Na+ and Cl− and thereby diminish intracellular Na+ and Ca2+ levels.4 It follows that repeated ischemic insults and excessive exposure to catecholamines are bound to cause taurine depletion and weaken this defense. Our data suggest that taurine depletion may worsen heart failure by causing reduced force generation that is due to its effect on the contractile apparatus.

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