EFFECTS OF TAURINE DEPLETION ON INTRINSIC CONTRACTILE CHARACTERISTICS OF RAT PAPILLARY MUSCLES

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ABSTRACT

The role of the high intracellular concentration of taurine in the heart is not known. The effects of taurine depletion in the heart were examined using the taurine transport antagonist guanidinoethyl sulfonate (GES). A six week treatment of rats with 1% GES in drinking water caused myocardial taurine levels to decrease to 25-30% that of control. Taurine depletion was found to be associated with specific changes in the mechanical characteristics of papillary muscles. While maximal muscle shortening velocity remained unaltered, relaxation time to 1/2 shortening length and the duration of isotonic contraction were prolonged. During isometric twitches, taurine-depleted muscles generated lower total tensions and the maximal rate of tension development was reduced, when compared to controls. The durations of isometric contractions of the treated muscles tended to be prolonged. These treatment-induced differences were not influenced by varying external calcium concentrations or stimulation rates. These results indicate that the intrinsic contractility of taurine-depleted hearts is depressed. The contractile changes observed may be a result of decreased intracellular calcium release and reuptake during muscle contraction. One function of taurine in the heart may be modulation of calcium homeostasis.

RÉSUMÉ

On ignore toujours le rôle que joue la présence d'une concentration élevée de taurine intracellulaire dans le coeur. Cette étude examine les effets, sur le coeur, de l'appauvrissement en taurine, en utilisant l'acide guanidinoéthyl sulfonique (AGS), un antagoniste au transport de la taurine. Le traitement de rats, pendant six semaines, avec 1% d'AGS ajouté à la ration d'eau, a causé une baisse des niveaux de taurine myocardiaux de 25 à 30% de la valeur contrôle. On a trouvé que cet appauvrissement en taurine est associé à des changements spécifiques des caractéristiques mécaniques des muscles papillaires. Alors que la vitesse maximale de raccourcissement demeure inchangée, le temps de relaxation nécessaire pour atteindre la moitié (1/2) de la longueur de raccourcissement et la durée de la contraction isotonique sont prolongés. Dans le cas des contractions isométriques, une comparaison aux valeurs contrôles a montré que les muscles appauvris en taurine génèrent des tensions totales plus faibles et que le taux maximal de développement de la tension est réduit. La durée des contractions isométriques des muscles traités a eu tendance à être prolongée. Ces différences, induites par le traitement, n'ont pas été influencées par la variation de la concentration externe de calcium ou la variation du taux de stimulation. Ces résultats indiquent que la contractilité intrinsèque du coeur pauvre en taurine est abaissée. Les changements contractiles observés peuvent être le résultat d'une diminution de la libération et de la recapture du calcium intracellulaire pendant la contraction musculaire. Une fonction de la taurine dans le coeur pourrait donc être la modulation de l'homéostase du calcium.

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ABBREVIATIONS

AP	action potential
ATPase	adenosine triphosphatase
c	centi
°C	degrees Celcius
Ca	calcium
Cl	chloride
cm	centimeter
CO_2	carbon dioxide
DC	duration of complete contraction
DNA	deoxyribonucleic acid
dT/dt	maximal rate of tension development
ECC	excitation-contraction coupling
EKG	electrocardiogram
ERG	electroretinogram
g	gram
G	gravitational force
GES	guanidinoethyl sulfonate
H	hydrogen
HCO_3	bicarbonate
Hz	hertz, cycles per second
Isi	second inward current
K	potassium
k	kilo
Km	Michaelis Menten constant
l	liter
Lmax	muscle length prior to contraction; preload
m	milli
M	molar
mg	milligram
ml	milliliter
mm	millimeter
mM	millimolar
ms	millisecond

Na	sodium
O_2	oxygen
pH	the logarithm of the reciprocal of the hydrogen ion concentration
PO_4	phosphate
QT	time interval between the QRS peak and the end of the T wave of an electrocardiogram
RL1/2	relaxation time to 1/2 muscle shortening length
RT1/2	relaxation time to 1/2 muscle tension decline
s	second
SEM	standard error of the mean
SO_4	sulphate
SR	sarcoplasmic reticulum
TPT	time to peak tension
TT	total tension
t-test	Student's t-test
TTX	tetrodotoxin
µ or u	micro
Vmax	maximal velocity of muscle shortening
Vs	velocity of muscle shortening, preload at Lmax
wt	weight

SUMMARY OF FINDINGS

* denotes original contribution to knowledge

In this study myocardial contractile parameters were evaluated in papillary muscles from taurine-depleted and taurine-repleted rats.

Biochemistry

1. The 6 week treatment of rats with GES resulted in 75% taurine depletion in ventricular tissue, and *68% taurine depletion in papillary muscle fibres.

2. *The 11 week GES-taurine treatment of taurine-depleted rats resulted in myocardial taurine repletion, while high intracellular GES content was maintained.

Electrophysiology

3. The QT intervals were prolonged in taurine-depleted rats. This effect was reversible with taurine repletion.

Contractility

Isometric, isotonic and unloaded contractions of rat papillary muscles were studied.

4. Isometric Twitch

   Changes from control in taurine-depleted muscles:

   *(A). Total tension was depressed.

   *(B). Maximal rate of tension development was depressed.

   *(C). Contraction duration tended to be prolonged.

5. Isotonic Twitch

   Changes from control in taurine-depleted muscles:

   *(A). The velocity of muscle contraction remained unaltered; Vs was unchanged.

   *(B). The relaxation of contraction was prolonged; RL1/2 was significantly longer.

   *(C). Contration duration was prolonged.

6. Unloaded Twitch

   *Maximal velocity of contraction remained unaltered in taurine-depleted muscles.

7. *Taurine-repleted muscles did not vary significantly from age-matched controls in all of the contractile parameters measured.

"...Samuel Pickwick burst like another sun from his slumbers; threw open his chamber window, and looked out on the world beneath. Goswell-street was at his feet, Goswell-street was on his right hand -- as far as the eye could reach, Goswell-street extended on his left; and the opposite side of Goswell-street was over the way. 'Such,' thought Mr. Pickwick, 'are the narrow views of those philosophers who, content with examining the things that lie before them, look not to the truths which are hidden beyond....'"

Charles Dickens, Pickwick Papers

INTRODUCTION

General Comments

Taurine (2-aminoethane sulfonic acid), synthesized mainly in the liver, is a sulfur-containing end product of methionine metabolism (Awapara, 1976). Taurine in the free form is distributed widely in the tissues of most mammalian species, including man (reviewed by Jacobsen and Smith, 1968). This remarkable ubiquity of taurine and its relatively high cytosolic concentrations is explained, in part, by transport processes. In many tissues taurine is not synthesized, but specific transport systems maintain and regulate its levels.

Despite the wide distribution of taurine, its only undisputed physiological function is conjugation with cholesterol in the formation of bile acids in the liver. Taurine does not act as a metabolic substrate because the sulfonate group replaces the carboxylic terminal found in most amino acids; hence taurine can not form peptide linkages (via its sulfonate terminal), and, it can not enter the Kreb's cycle. Thus the role of the millimolar concentrations of taurine in excitable tissues including the brain, the retina, skeletal muscle and the heart remains to be determined.

Taurine in Excitable Tissues

The action of exogenous taurine has been studied on many different tissues and preparations. As a result taurine is associated with a myriad of effects.

The effects of taurine on excitable tissues have been studied by both the pharmacologic approach and the analysis of taurine deficiencies. In the early sixties, exogenously applied taurine was shown to depress neuronal activity in the central nervous system (Curtis and Watkins, 1960). A few years later, intravenous infusion of taurine in millimolar concentrations was shown to prevent cardiac arrythmias induced by epinephrine, and to reverse those induced by digoxin (Read and Welty, 1963). More recently, the infusion of taltrimide, a lipophilic taurine

derivative, was shown to suppress chemically-induced seizures (Huxtable and Nakagawa, 1985). Taurine has been shown to have a diuretic effect (Dlouha and McBroom, 1986), and, at the behavioural level, to modify drinking and eating activities (Thut et al., 1976). It has also been found to modify aggressive responses (Mandel et al., 1985). These and many other studies, described in the books edited by Huxtable and Barbeau (1976), Schaffer et al. (1981), and Oja et al. (1985), focused on pharmacological manipulations of exogenous taurine and possible therapeutic applications, but did not contribute significantly to the general understanding of the physiological role of taurine in excitable tissues.

The most convincing evidence which shows that taurine plays an important physiological role in excitable tissues comes from deficiency studies. Prolonged absence of dietary taurine in the cat leads to a decrease in the taurine content of the retina, accompanied by abnormalities in the electroretinogram (ERG), degeneration of the photoreceptors and eventual blindness (Schmidt et al., 1976). A recent study which evaluated the effects of long-term parenteral nutrition (that does not provide taurine) in children, has shown ERG deficits in these patients. With the addition of taurine to the intravenous solution, the ERG defects disappeared (Geggel et al., 1985). These and other studies which have shown developmental deficits associated with low taurine diets and taurine deficiency (Lake, 1983; Neuringer et al., 1985) resulted in the recent addition of taurine to commercial infant formula, to the level found in breast milk. At the same time these studies suggested that high intracellular concentrations of taurine in the retina may be of critical importance to the normal function of this tissue.

Taurine in the Cardiovascular System

Taurine is the most abundant free amino acid in mammalian heart. In the rat, cardiac taurine levels are about 25-30 umol/g wet weight and constitute over 60% of the free amino acid pool (Huxtable, 1976). These levels are maintained and regulated by specific transport processes, while local metabolism is less important (Huxtable, 1976). Rat cardiac

taurine content can be specifically depleted in vivo to about 25% that of control values by the use of the transport antagonist, guanidinoethyl sulfonate (GES)(Huxtable et al., 1979).

Many effects of taurine on the cardiovascular system have been described (reviewed by Huxtable and Sebring, 1983), and include inotropic actions, effects on calcium metabolism, antiarrythmic actions, osmotic effects, and hypotensive actions. Most of these studies have evaluated the effects of exogenous administration of taurine in millimolar concentrations. Since plasma taurine levels are normally two orders of magnitude lower than the concentrations used in these studies, their conclusions should be interpreted with caution.

Superfusion of taurine has been shown to be 'cardio-protective' in hypoxia (Franconi et al., 1985). Kramer et al. (1981) reported a protective role of taurine in the calcium paradox paradigm: when a calcium-free buffer containing taurine was used to superfuse the hearts for various periods of time, the muscle was protected against an irreversible damage that is associated with calcium overload when calcium was later added back into the medium. Exogenous taurine has also been reported to antagonize the negative inotropic effects caused by decreased calcium concentration in the perfusate (Guidotti et al., 1971; Shaffer et al., 1978). Although the above studies did not evaluate the physiological function of myocardial taurine, they indicated that this amino acid may be useful, pharmacologically, during cardiac surgery (ie. transplantation), or possibly when reperfusion occurs during the treatment of an acute myocardial infarction.

In a recent clinical study, oral administration of taurine to young patients with borderline hypertension was shown to normalize blood pressure (Fujita et al., 1987). Also, in double blind randomized crossover studies, dietary supplements of taurine improved the clinical manifestations of patients with congestive heart failure (Azuma et al., 1983; Azuma et al., 1985). Taurine supplements have also been shown to effectively reverse the dilated cardiomyopathy associated with low plasma taurine levels in cats (Pion et al., 1987). The site(s) of action of these therapeutic effects of taurine, is, however, unknown.

One way to study the physiologic actions of taurine in the heart, is

to manipulate the intracellular content of this amino acid. In a recent study in our laboratory, cardiac taurine depletion in rats was highly correlated with selective prolongation of the QT interval (Lake et al., 1987). This prolongation was accompanied, and accounted for, by an increase in the duration of the ventricular muscle action potential (AP) (Lake et al., 1987). Since some of the currents which underlie ventricular APs may also be involved in the excitation-contraction coupling process (ECC), the present study was designed to evaluate any alterations of intrinsic myocardial contractile characteristics which may accompany the electrophysiologic changes in taurine-depleted rat hearts.

Hypothesis

The study conducted by Lake et al. (1987) indicated that GES-induced myocardial taurine depletion in the rat is associated with changes in cardiac electrophysiology. Other investigators reported that GES-induced taurine depletion in the rat heart resulted in decrease of the heart calcium content (McBroom and Welty 1985). Our hypothesis is that taurine depletion may also result in contractile changes, which may be reflective of altered calcium homeostasis in taurine-depleted hearts.

Outline

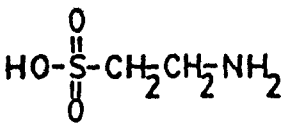
In this experiment the papillary muscle mechanics of GES-treated rats were studied and compared to the results obtained from control animals. In order to study how taurine may effect the ECC process, specific contractile parameters of unloaded, isotonic and isometric twitches were examined.

Rationale for the Use of GES-mediated Taurine Depletion

Taurine-free diets are ineffective in depleting the myocardial taurine stores of adult rats, because of compensatory changes in liver biosynthesis and renal excretion of this compound (Huxtable, 1976; Sturman, 1973), so GES treatment was used to induce taurine depletion

(Huxtable et al., 1979; Lake et al., 1987).

A



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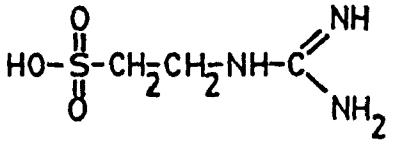

Figure 1. Chemical structures of taurine (A) and GES (B).

Rationale for the Use of Papillary Muscles

The term contractility describes the intrinsic performance of cardiac muscle. Evaluation of the contractile state of an intact heart, however, is difficult because of the interaction of many variables. Contractility itself is altered by catecholamines, glycosides or changes in heart rate (Brutsaert and Sonnenblick, 1973). On an absolute level, performance of the heart as a pump is affected by two major factors in addition to contractility: the volume of the ventricle prior to the contraction (preload) and the resistance to the ventricular emptying (afterload). On a practical level, the geometry of an intact heart is complicated, making calculations of maximal wall stress complex, and comparative measurements difficult to interpret.

Thus, in order to compare the mechanical performance of different cardiac muscles, contractile studies in vitro often rely on the use of papillary muscles. Papillary muscles, found both in left and right ventricles, are of a uniform, cylindrical shape. They project from the base of the ventricles to the ventricular valves, preventing the latter from inversion during contractions. Although papillary muscles do differ in their size and weight, their aligned fibres and cylindrical shape allow for normalization to cross-sectional area.

Contraction of the papillary muscle can be described by three inter-related variables: velocity of shortening, force of contraction, and length of displacement (Brutsaert and Sonnenblick, 1973). The force-velocity-length relation not only describes the contractile state of the cardiac muscle, but also serves as a direct analogy of the events occurring in the intact heart. Performance of the heart has been

expressed in hemodynamic terms as changes in pressures, flow rates and volumes (Brutsaert and Sonnenblick, 1973). The active force generated by the isolated papillary muscle translates into the ventricular pressure developed. The velocity of muscle shortening is analogous to the flow rate during the ejection phase of the pumping heart. Finally the changes in length of the contracting papillary muscle correspond to the dimensional changes of the working heart (end diastolic and end systolic volumes). Unlike the heart in situ, the isolated papillary muscle permits normalization of the variables measured. Furthermore, in isolated cardiac muscle, resting tension (preload) can be accurately adjusted and controlled, so that during the contraction the extent of shortening or the force developed are maximal (see Methods). It is important that contractile studies are conducted at the optimal resting tension (referred to as Lmax), since small deviations from Lmax produce large changes in the extent of shortening and force developed during contraction (Lakatta and Jewell, 1977).

The use of papillary muscles presents some technical difficulties. The muscles must be detached from the ventricular wall. During this procedure some fibres on the excised side may be damaged. Clamping the ends (for fixation) of the muscles results in lesions of the terminal ends of fibres, some change in series resistance, and some alteration in the natural arrangement of myofibres. The preparation is limited to muscle diameters that can be oxygenated by diffusion, as oxygen is provided via a buffering solution that bathes the muscles instead of from the vasculature. For this reason, the muscles used in this study were limited to those of small cross-sectional area (0.5-1.2 mm^2). Furthermore, to reduce metabolic demand, contractile studies were conducted at a temperature well below the physiological range (26°C), and low stimulation rates were used (0.1-0.6 Hz).

Rationale for the Use of the Rat as Species

The rat myocardial action potential (AP) differs from the myocardial APs of other mammalian species. The main difference is a shortened duration of the AP plateau (Josephson et al., 1984a; 1984b), which is

compatible with the high heart rates (around 400 beats per minute) encountered in the rat. The actual currents underlying the rat heart AP may be similar to those encountered in other species, but their relative magnitude may differ, so that some currents, like the sodium-calcium (Na/Ca) exchanger current, are more obvious in this species. In other species, the depolarizing effect of the Na/Ca exchanger is most likely disguised during their longer duration of AP plateau.

In fact, some investigators see the rat as a simplified model for the study of the myocardial ECC mechanism (Schouten and terKeurs, 1985). These investigators found that varied stimulus patterns led to specific mechanical changes. They hypothesized that consistent alterations in the shape of APs, observed during different stimulus patterns, may be caused by changes in sarcoplasmic reticulum (SR) calcium accumulation and release (Schouten and terKeurs, 1985). These studies gave rise to the formulation of a hypothesis of the ECC mechanism in the rat (terKeurs et al., 1987). By using the rat in our study this hypothesis could be used as a basis for interpretion.

Finally, the most immediate reason for using the rat animal model was that the previous study by Lake et al. (1987) demonstrated in the rat cardiac electrophysiological changes associated with taurine depletion.

The Measurements of Myocardial Muscle Mechanics

In order to quantitate the effects of taurine depletion on myocardial contractility, isometric, isotonic and unloaded contractions were studied. In isometric contractions, the afterload is set higher than the maximum tension that the muscle can generate, hence shortening does not occur but rather maximum isometric force is produced. The maximum (total) tension developed by the muscle is indicative of the number of activated contractile proteins, which is modulated by the concentration of free cytosolic calcium (Fabiato, 1983).

Isometric twitches were evaluated at varied stimulation frequencies. The force-frequency relationship is frequently used to assess the function of SR. In this protocol as the relative changes in contractile characteristics (from control) reflect the calcium turnover by the SR,

abnormalities in SR processing of calcium may be revealed.

Isometric twitches were also assessed during the paired-pulse stimulation. The paired-pulse stimulation paradigm produces contractile potentiation (ie. increased total tension and maximal rate of tension development) which is attributed to calcium release and accumulation by the SR (Wohlfart, 1982; Schouten, 1984). In rat myocardium, calcium influx during the action potential has little direct effect on contractile proteins (Bers,1985), but it triggers a release of intracellular calcium which activates these proteins (Fabiato, 1983). With paired stimuli, the extra calcium that enters during the second action potential is taken up into the SR, and more calcium is released in response to the first of the next pair stimuli.

In isotonic contractions the afterload is the same as the preload, while in 'unloaded' contractions the afterload is set lower that the preload. The study of contractile parameters of isotonic and unloaded twitches allows for the assessment of the kinetics of calcium turnover as well as the maximal velocity of muscle shortening (Vmax). The inverse relation between peak velocity of shortening and the force carried, the velocity-tension curve, is one of the fundamental and most frequently analyzed characteristics of active cardiac muscle (Brutsaert and Sonnenblick, 1973). When the load on the muscle is decreased to a near zero value, Vmax is obtained (Brutsaert and Sonnenblick, 1973). Vmax is thought to reflect the turnover rate of individual actin myosin crossbridges, and is positively correlated with myosin ATPase activity (Barany, 1967; Carey et al., 1979). It is also dependent on the ratio of myosin isozymes (Schwartz et al., 1981; Pagani and Julian, 1984; Rouleau et al., 1986), and the rate of rise of free cytosolic calcium (De Clerck et al., 1977). Vmax is also altered by phosphorylation of contractile proteins (Winegrad, 1983).

Contractile parameters obtained from unloaded, isotonic, and isometric contractions characterize the intrinsic contractile state of the muscle. When muscles obtained from animals from two different treatment groups are studied, specific and consistent differences in contractile properties between the groups may point to the mechanisms that underly these changes. Thus measurements of papillary muscle

contraction may provide, in addition to the assessment of the intrinsic contractile state, correlates of some of the molecular events that take place during muscle shortening.

Myocardial Mechanics in the Rat

Rat myocardial mechanical responses show large quantitative variations with different stimulus patterns. To test contractility at different rates of stimulation, often the force-frequency relationship is evaluated. Rat myocardium, unlike that of other mammals, does not show a positive force staircase with a stepwise increase in stimulation frequency. Instead a negative force-frequency relationship (Hoffman and Kelly, 1959; Forester and Manwood, 1974), or no change in force with increasing frequency of stimulation is observed (Schouten and terKeurs, 1986). This may be reflective of an ECC mechanism specific to rat cardiac muscle.

Contractions of the rat myocardium are highly dependent on the release and reuptake of intracellular calcium, and less dependent on the trans-sarcolemmal influx of calcium (Bers, 1985). Increasing stimulation frequency results in a change of contractile state of the muscle (Brutsaert and Sonnenblick, 1973) and may be accompanied by altered rates of intracellular calcium fluxes.

In the rat, changes in stimulation rates may result in altered kinetics of intracellular calcium recycling (ie. release and reuptake of calcium by the SR). Interruption of stimulation for a short period of time results in a potentiated contractile response of the twitch that follows the rest interval (Schouten et al., 1987). This 'mechanical restitution' response (contractile potentiation following a stimulus-free interval) may reflect a greater release of intracellular calcium (Schouten et al., 1987), since a greater amount of calcium could have been accumulated in the SR during the rest interval (terKeurs et al., 1987).

Other interpretations are possible. A change in stimulus frequency pattern can result in altered trans-sarcolemmal ionic fluxes during the AP; a rest interval being followed by an AP of increased duration

(Schouten, 1984). It is also possible that a rest interval may enhance the trans-sarcolemmal calcium influx during the subsequent AP, which could result in potentiated SR calcium release, and an enhanced force of contraction. A change in the stimulus frequency pattern may also alter the gain between the myocardial depolarization and the subsequent calcium release from the SR towards an enhanced force of contraction. At present, however, these questions are not yet resolved and future studies are required to address these alternate hypotheses.

METHODS

Male Sprague Dawley rats of initial weight 200-230 g were divided into two groups; treated and control. The treated group was subdivided into 'taurine-depleted' and 'GES-taurine' (taurine-repleted) groups. The taurine uptake antagonist, GES, was synthesized (Huxtable et al., 1979), purified by repeated crystallization from water, and its purity verified by assays for taurine (Troll and Cannan, 1953) and GES (Guidotti and Costalgi, 1970).

All animals were given free access to food and water. Treatment was by addition of 1% GES (weight/volume) to drinking water for a period of six weeks which results in approximately 70% depletion of the heart taurine content (Lake et al., 1987). These animals were the taurine-depleted group. After the six week treatment with 1% GES, 'taurine-GES' animals were treated with 1% GES and 0.1% taurine for five weeks, and then 1% GES and 1% taurine for an additional six weeks. During these last six weeks electrocardiograms (EKGs) were recorded every five to nine days (following the methods in Lake et al., 1987). In order to sedate the animals prior to recording, an intramuscular injection (0.44 ml/kg) of ketamine hydrochloride (90.0 mg/ml, Rogar, Montreal, Canada) mixed with acepromazine malate (2.3 mg/ml, Ayerst, Montreal, Canada) was given. At the end of the treatment period, each animal was anesthetized with halothane-nitrous oxide gas mixture, the chest cavity opened, and the heart removed. The atria were trimmed, and the papillary muscles excised after exposing them by cutting through the right ventricular wall and septum.

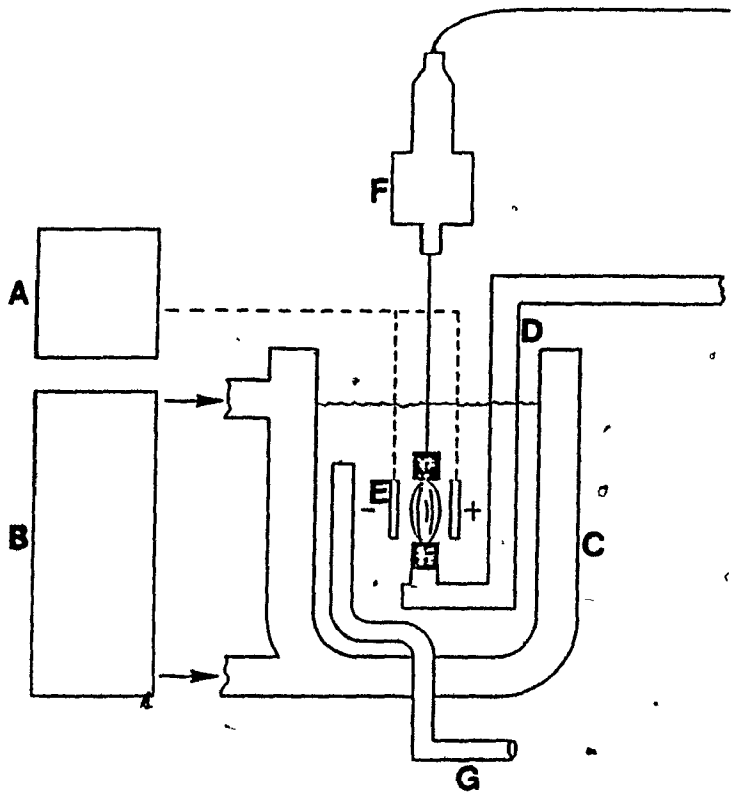

Figure 2. Schematic diagram of the experimental apparatus. A - stimulator, B - thermostat controlling the bath temperature, C - water jacket, D - stainless steel clamp holding the base of the papillary muscle, E - platinum field electrodes, F - electro-magnetic force/length transducer; G- air supply

Biochemistry

Freshly dissected left ventricular tissue and papillary muscles, as well as muscles used for the mechanical studies, were individually assayed for taurine, GES, and DNA content. The tissues were blotted to remove adhering medium, weighed, and homogenized in 85% ethanol. After a 12 hour incubation at -10°C, the tubes were centrifuged at 15,000 G. The resultant pellets were used for estimation of DNA content (Yates et al., 1974). The supernatants, which contain free amino acids and GES, were dried under a stream of nitrogen gas, and resuspended in 0.525 ml distilled water. A 0.5 ml aliquot was passed over a dual bed ion exchange column (0.8 x 2.0 cm of Dowex 50, H+ form, Biorad; layered over 2 cm of Dowex 1, Cl- form, Biorad). The column was washed with 3 ml distilled water and the effluent assayed for taurine (Troll and Cannan, 1953) and GES (Guidotti and Costalgi, 1970). In order to concentrate the effluent obtained from the papillary muscles, it was lyophilized and reconstituted in 0.65 ml of distilled water. This raised the concentration of taurine to a level detectable by our assay, however, the limited volume did not permit GES assays. Simultaneous taurine and GES assays were only possible with the ventricular tissues. All samples were assayed in triplicate and compared to standards.

Contractile Mechanics In Vitro

One papillary muscle from each animal was excised from the left ventricle and mounted in a bath in Krebs-Henseleit solution containing in millimoles: NaCl, 117.4; $CaCl_2$, 2.5; KCl, 3.6; $MgSO_4$, 1.2; $NaHPO_3$, 1.2; $NaHCO_3$, 25.0; and dextrose, 5.0. The pH was kept close to 7.4 by continuous aeration of the solution with 95% O_2 /5% CO_2. Temperature was kept at 29±0.5°C.

A schematic diagram of the apparatus is shown in Figure 2. The base of the muscle was held by a stainless steel clamp and the other end tied to a lever with an electromagnetic feedback system that controlled force, length, and velocity of contraction (Brutsaert et al. 1973). The

elastic damping of the lever feedback system was finely adjusted in order to compensate for electro-mechanical transients (Brutsaert et al. 1973). Two platinum field electrodes were arranged vertically on either side of the muscle in the bath, and they delivered a field stimulation from a Grass S88 stimulator using a 5 ms square-wave pulse at a voltage of 10% above threshold for muscle contraction and a frequency of 0.1 Hz. This rate was used in all experimental conditions except where otherwise stated. The amplified outputs from the force and length transducers were recorded on a Gould 2400s chart recorder, and simultaneously digitized by an IBM computer system which utilized a customized program, digitizing card DT2821, and 500 Hz sampling frequency.

Determination of Lmax

In order to ensure that optimal force was generated by each muscle, Lmax was determined by the following procedure: The preload was lowered to 0 grams. Subsequently the preload was increased in 0.1 gram steps. At each new preload, after two minutes of stabilization, an isometric twitch was recorded (ie. the contraction occurred against an afterload greater than the tension the muscle could generate, thus no change in length occurred). The preload was increased until the tension generated was maximal. This muscle length was called Lmax. The muscle was then allowed to stablilize for 90 minutes, contracting isotonically at a preload of less than Lmax. Then, Lmax was finely adjusted, and the muscle was allowed to stabilize for a further 30 minutes contracting isotonically at Lmax.

Experimental Procedures

There were two parts to this study. In the first part (Protocol A) contractility was evaluated in muscles from 14 taurine-depleted and 12 corresponding control rats, and in muscles from 5 taurine-repleted (GES-taurine) and 4 corresponding control rats by recording (I) isotonic and isometric contractions at a basal stimulation rate, (II) the isometric force-frequency relation, (III) isometric tension to a single

stimulus following a 3 minute stimulus-free period, and (IV) isometric tension generated by twin pulse stimuli. In order to assess the effects of extracellular calcium, protocol A was conducted at three different calcium concentrations. In the second part (Protocol B) a complete velocity-tension relationship was decribed for muscles from 8 taurine-depleted and 12 control rats. This protocol was conducted at four different calcium concentrations. In both protocols, at each calcium concentration, the muscles were allowed to stabilize for 30 minutes contracting isotonically, before beginning the contractility testing.

Protocol A

(I) Isotonic and isometric contractions (at Lmax preload) were examined sequentially in media containing 2.5 mM, 1.3 mM, 2.5 mM, and 6.3 mM calcium. In addition, at 1.3 mM calcium level and the subsequent 2.5 mM calcium level, muscle responses to varied frequency of stimulation and paired stimuli, were evaluated.

(II) The force-frequency relationship for isometrically contracting muscles at Lmax was examined at stimulation rates of 0.1, 0.2, 0.4, and 0.6 Hz. At each frequency, when the muscles stabilized, as judged by a plateau in total tension, a representative twitch was recorded.

(III) Then the muscles were allowed to stabilize while contracting isometrically at 0.1 Hz for at least fifteen minutes. Stimulation was then interrupted for three minutes. The first isometric contraction following the stimulus-free period was recorded.

(IV) At the end of the force-frequency protocol the muscles were left to stabilize for fifteen minutes while contracting isometrically at 0.1 Hz. Paired pulses with intervals of 200 or 300 ms were delivered to the muscles. When the contractions attained a stable tension plateau, a representative isometric contraction was recorded. The first peak of the two fused contractions was analyzed.

Protocol B

To obtain the maximum velocity of shortening at zero load (Vmax), the load on the muscle at the time of activation was abruptly decreased. A series of shortening velocities was obtained by clamping the load in eight to ten increments encompassing 5 to 100% of total tension. Between each tension step the muscles were permitted to restabilize while contracting isotonically at Lmax for at least 10 stimuli. The baseline response was established in medium containing 2.5 mM calcium, then the calcium content was changed successively to 1.3 mM, 1.9 mM, 2.5 mM, 3.8 mM, and 2.5 mM. The measurements at 2.5 mM were compared to check for stability throughout the protocol.

Analysis

Changes in isotonic and isometric twitch characteristics between the muscles from GES-treated rats and the rats from control groups were compared using unpaired t-tests. Some* values were normalized to the cross-sectional area of the muscle determined as follows: the papillary muscles were measured in length when removed from the bath. Their cross-sectional area (mm^2) was calculated by assuming that the muscle has a cylindrical shape, and dividing muscle weight by length using a specific gravity of 1.0. Tension is expressed in g/mm^2, and maximum rate of tension development is expressed in $g/mm^2/s$. Muscle shortening is expressed in %Lmax and velocity of shortening is expressed as Lmax/s. Data are presented as mean ± SEM.

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*For reference please consult Tables 3A and 4A.

RESULTS**

Treatment of animals with GES did not cause gross behavioural changes, overt sickness or death. After six weeks of treatment all the animals appeared healthy, and were in the same weight range as controls. The animals which were treated for six weeks with GES, and then an additional eleven weeks with GES and taurine, also remained in good health and in the same weight range as controls.

There were no significant differences in papillary muscle length, cross-sectional area, or preload at Lmax between the muscles obtained from the controls and those from the treated animals (Table 1).

Biochemical studies

As seen in Table 2A, ventricular tissue from untreated rats contained approximately 20 mM taurine and low amounts of GES. Rats treated with GES for six weeks lost about 75% of their ventricular taurine content and accumulated GES. Their DNA levels were unaffected, indicating that no cell death occurred. Papillary muscle taurine and DNA content mirrored that of the adjacent ventricular tissue for both control and treated rats. On this basis it is assumed that papillary GES contents were similar to the ventricular values, as the papillary muscle mass was insufficient to permit both taurine and GES assays.

Table 2B compares the taurine content of papillary muscles analyzed directly following dissection to those analyzed after three to four hours of in vitro study in the tissue bath ('perfused' group). The perfused muscles had taurine levels reduced by approximately 30% compared to the freshly dissected muscles, regardless whether they were obtained from control or GES-treated rats. No change was observed in DNA content (expressed as mg/g wet weight) which suggests that some taurine washout, rather than edema (which would dilute intracellular taurine), occured during the experiments. Since the fraction lost was about the same for

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**Please note: For convenience the Figures and Tables have been placed at the end of the Results section.

both groups, the relative taurine depletion of treated versus controls was the same in perfused as in freshly dissected muscle. Some muscles were assayed for biochemistry at intervals during the course of the experiment. These results show that after the initial taurine washout early during the muscle stabilization in the bath, the muscle taurine content remained constant over time. This suggests that the preparations were stable for the period of our evaluations.

Contractility studies

A representative recording of an isometric twitch is shown in Figure 4. For isometric twitches, there were no differences between the taurine-depleted and control muscles in time to peak tension or to half tension decline (Table 3A). However, muscles from taurine-depleted rats generated significantly lower total tensions, and had lower rates of maximal tension development at all calcium concentrations (Table 3A). These differences persisted at four stimulation rates (Figure 6). After a three minute stimulus-free interval, muscles from taurine-depleted or control rats generated higher total isometric tensions and maximal rates of tension development than during steady state contractions at 0.1 Hz. But the increases were such that taurine-depleted muscles still generated significantly lower total tensions and maximal rates of tension development than controls in 2.5 mM calcium (Figure 7; Table 5). The durations of isometric contractions had a tendency to be prolonged in the muscles from taurine-depleted rats. This trend attained statistical significance only in the low calcium condition (Table 3A).

Paired pulses, with intervals of 200 ms, delivered to isometrically contracting muscles established a higher total tension plateau in both groups in the low calcium condition, but there was little change using interval of 300 ms (Tables 6 and 7; Figure 8). None-the-less, muscles from taurine-depleted rats generated significantly lower total tensions, and maximal rates of tension development than controls at the two calcium concentrations tested.

For isotonic twitch characteristics there were no treatment effects on shortening velocity or the time to attain peak shortening (Table 4A).

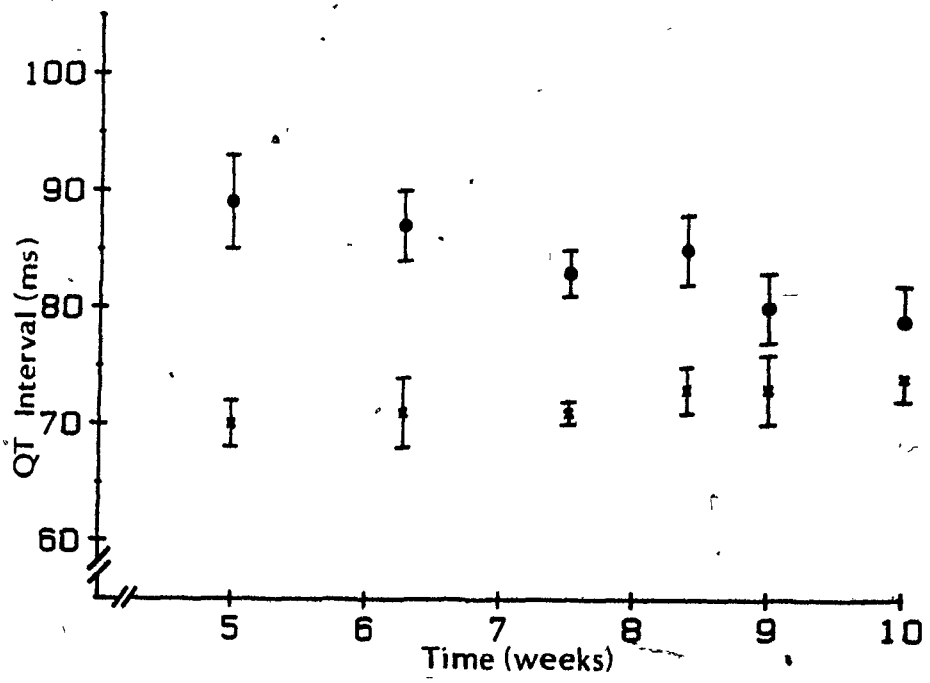

Figure 3 Effects of taurine depletion and repletion on the QT interval of the rat EKG. Data shown are group means ± SEM for 6 untreated age and weight-matched controls (X) and 6 rats (●) treated with GES for 6 weeks and then taurine and GES for an additional 11 weeks (weeks 5 to 10 of the additional 11 week period shown here). GES-induced taurine depletion resulted in significant prolongation of the QT intervals at 6 weeks (see also Lake et al., 1987). This effect was reversed by treatment with taurine and GES .

Significant differences were found, however, in the kinetics of contraction; taurine-depleted muscles having prolonged relaxation time to half shortening length, and increased duration of contraction (Table 4A). These differences persisted at the three calcium concentrations studied.

Representative isotonic and unloaded contractions are shown in Figure 9. There were no differences between control and taurine-depleted rats in the maximal velocity of shortening (Vmax) of papillary muscles. In both groups, the Vmax increased with increasing calcium concentrations (Figure 10).

A previous study from our laboratory indicated that GES-induced taurine depletion was associated with specific prolongation of the QT interval of the EKG (Lake et al., 1987). In the present study QT prolongation was reversed by subsequent treatment with GES and taurine. EKG recordings were made in a subgroup of rats at weekly intervals (Figure 3). The earliest recordings were done after five weeks of 1.0% GES with 0.1% taurine treatment which, we thought, may result in taurine repletion, however the QT intervals of the treated group remained prolonged. For the subsequent six weeks of treatment, GES concentration was held constant but taurine was raised to 1.0%. At week ten the treated rats (GES-taurine) had the QT intervals no different from control, and studies of muscle mechanics and biochemical assays were made one week later. As seen in Table 2A the ventricular and papillary muscles of GES-taurine rats had DNA contents indistinguishable from control, and taurine contents 90% of control, but they still contained substantial levels of GES. At this point evaluation of various intrinsic contractile indices showed no differences between GES-taurine rats and untreated age-matched controls (Tables 3B and 4B), in contrast to the marked depression of contractility associated with taurine depletion after six weeks of GES treatment (a description of which forms the main body of this thesis). It should be noted, however, that certain parameters (dT/dt, TPT, RL1/2; Tables 3B and 4B) were significantly different in these older animals (both control and GES-taurine) compared to the younger control group (see Tables 3A and 4A).

Table 1. Preload, length and cross-sectional area at Lmax of papillary muscles of control and treated rats

	N	Preload (g/mm^2)	Length (mm)	Cross-sectional area. (mm^2)
Control	28	1.0 ± 0.1	6.2 ± 0.2	1.0 ± 0.1
Treated	27	1.0 ± 0.1	5.9 ±0.2	1.0 ± 0.1

Data shown are group means ± SEM. N = number of samples

Table2 A. Effects of GES treatment on rat myocardial ventricular and papillary tissues

VENTRICLES

	Taurine (µmol/g wet wt)		GES (µmol/g wet wt)		DNA (mg/g wet wt)	
Control	20.23 ± 0.62	(25)	0.08 ± 0.02	(16)	0.467 ± 0.024	(16)
GES-treated	4.99 ± 0.24	(29)****	14.62 ± 0.47	(18)****	0.504 ± 0.040	(18)
GES-taurine	18.34 ± 1.36	(6)	6.33 ± 0.52	(6)****	0.509 ± 0.023	(6)

PAPILLARY MUSCLES

	Taurine (µmol/g wet wt)		DNA (mg/g wet wt)	
Control	19.26 ± 1.09	(28)	0.433 ± 0.040	(26)
GES-treated	6.00 ± 0.36	(32)****	0.492 ± 0.030	(28)
GES-taurine	20.30 ± 3.30	(10)	0.540 ± 0.059	(7)

Values are means ± SEM. Number of samples is shown in brackets. Level of significance of t-tests against control are indicated by **** $p<0.001$.

Table 2 B. Effects of perfusion on papillary muscle DNA and taurine content

	Taurine (µmol/g wet wt)		DNA (mg/g wet wt)	
Control				
Freshly dissected	22.69 ± 2.04	(8)	0.464 ± 0.064	(9)
Perfused	15.15 ± 0.50	(11)[a]	0.438 ± 0.066	(10)
GES-treated				
Freshly dissected	7.20 ± 0.42	(14)[c]	0.464 ± 0.039	(11)
Perfused	5.26 ± 0.45	(16)[b,c]	0.536 ± 0.035	(14)

Values are means ± SEM. Number of samples is shown in brackets. Perfused refers to those muscles assayed after being used for the *in vitro* studies. Muscles which were only briefly exposed to the tissue bath were excluded (although their values are included in part A).

a $p< 0.01$ versus freshly dissected control group

b $p< 0.01$ versus freshly dissected GES-treated group

c $p< 0.001$ versus appropriate control group

Table 3A. Papillary Muscle Mechanics of Control versus GES-treated Rats: Isometric Contractions

	N	TT (g/mm^2)	dT/dt ($g/mm^2/s$)	TPT (ms)	RT 1/2 (ms)	DC (ms)
1.3 mM Ca^{2+}						
Control	12	8.9 ± 0.4	92 ± 5	127 ± 4	126 ± 5	448 ± 15
GES-treated	14	6.8 ± 0.5***	71 ± 6*	131 ± 3	134 ± 6	509 ± 19*
2.5 mM Ca^{2+}						
Control	12	9.4 ± 0.5	100 ± 6	126 ± 3	130 ± 5	462 ± 10
GES-treated	13	7.1 ± 0.5***	76 ± 7*	127 ± 3	134 ± 6	498 ± 15
6.3 mM Ca^{2+}						
Control	12	9.5 ± 0.5	107 ± 6	124 ± 3	140 ± 7	485 ± 19
GES-treated	13	7.3 ± 0.5***	80 ± 6*	129 ± 4	144 ± 4	510 ± 23

GES-treated refers to the rats treated with GES for 6 weeks, while control refers to untreated rats housed for 6 weeks in adjacent cages. Values are means ± S.E.M., TT is total tension, dT/dt is maximum rate of tension development, TPT is time to attain peak total tension, RT 1/2 is time to attain 1/2 tension decline, DC is duration of complete contraction.

*$p<0.05$ ***$p<0.005$

Table 4A. Papillary Muscle Mechanics of Control versus GES-treated Rats: Isotonic Contractions

	N	Vs (Lmax/s)	TPS (ms)	RL 1/2 (ms)	DC (ms)	ΔL (L/Lmax)
1.3 mM Ca^{2+}						
Control	12	1.56 ± 0.09	147 ± 4	57 ± 3	369 ± 10	0.14 ± 0.01
GES-treated	14	1.79 ± 0.11	150 ± 3	68 ± 2***	399 ± 8*	0.16 ± 0.01
2.5 mM Ca^{2+}						
Control	12	1.77 ± 0.10	142 ± 3	56 ± 1	365 ± 8	0.15 ± 0.01
GES-treated	13	1.95 ± 0.14	148 ± 3	71 ± 2****	389 ± 10	0.17 ± 0.01
6.3 mM Ca^{2+}						
Control	12	1.83 ± 0.10	143 ± 3	66 ± 2	370 ± 10	0.16 ± 0.01
GES-treated	13	2.03 ± 0.15	148 ± 3	81 ± 2****	400 ± 9*	0.17 ± 0.01

GES-treated refers to the rats treated with GES for 6 weeks, while control refers to untreated rats housed for 6 weeks in adjacent cages. Values are means ± S.E.M., Vs is maximum shortening velocity - preload at Lmax, TPS is time to attain peak shortening, RL 1/2 is time to attain 1/2 shortening decline, DC is duration of complete contraction, ΔL is fractional change of papillary muscle length at peak shortening.

*$p<0.05$ ***$p<0.005$ ****$p<0.001$

Table 3B. Papillary Muscle Mechanics of Control versus GES/Taurine-treated Rats: Isometric Contractions

	N	TT (g/mm^2)	dT/dt ($g/mm^2/s$)	TPT (ms)	RT 1/2 (ms)	DC (ms)
1.3 mM Ca^{2+}						
Control-11	4	7.1 ±.0.6	72 ± 5[a]	136 ± 8	112 ± 6	430 ± 48
GES-taurine	5	8.2 ± 0.3	78 ± 5[a]	144 ± 6[a]	134 ± 8	499 ± 24
2.5 mM Ca^{2+}						
Control-11	4	7.2 ± 0.7	73 ± 6[a]	132 ± 7	127 ± 18	507 ± 84
GES-taurine	5	8.5 ± 0.4	81 ± 5[a]	141 ± 2[a]	141 ± 6	510 ± 19
6.3 mM Ca^{2+}						
Control-11	4	7.3 ± 0.7	75 ± 6[a]	128 ± 6	123 ± 10	505 ± 78
GES-taurine	5	8.3 ± 0.2	81 ± 4[a]	138 ± 3[a]	148 ± 8	512 ± 21

GES-taurine refers to the animals treated with GES for 6 weeks, after which time the animals were given taurine and GES for an additional 11 weeks. Control-11 refers to age-matched untreated rats housed in adjacent cages. Values are means ± S.E.M., TT is total tension, dT/dt is maximum rate of tension development, TPT is time to attain peak total tension, RT 1/2 is time to attain 1/2 tension decline, DC is duration of complete contraction.

a $p<0.05$ versus 'younger' control group (Table 3A)

Table 4B. Papillary Muscle Mechanics of Control GES/Taurine-treated Rats: Isotonic Contractions

	N	Vs (Lmax/s)	TPS (ms)	RL 1/2 (ms)	DC (ms)	ΔL (L/Lmax)
1.3 mM Ca^{2+}						
Control-11	4	1.61 ± 0.18	148 ± 9	60 ± 5	382 ± 21	0.14 ± 0.02
GES-taurine	5	1.61 ± 0.15	157 ± 6	67 ± 4[b]	372 ± 15	0.15 ± 0.01
2.5 mM Ca^{2+}						
Control-11	4	2.12 ± 0.21	143 ± 8	65 ± 4[b]	387 ± 19	0.17 ± 0.01
GES-taurine	5	1.67 ± 0.15	158 ± 4	72 ± 4[b]	380 ± 14	0.15 ± 0.01
6.3 mM Ca^{2+}						
Control-11	4	2.13 ± 0.23	142 ± 8	71 ± 5[a]	373 ± 28	0.17 ± 0.01
GES-taurine	5	1.64 ± 0.15	156 ± 8	76 ± 3[b]	390 ± 18	0.15 ± 0.01

GES-taurine refers to the animals treated with GES for 6 weeks, after which time the animals were given taurine and GES for an additional 11 weeks. Control- 11 refers to age-matched untreated rats housed in adjacent cages. Values are means ± S.E.M., Vs is maximum shortening velocity - preload at Lmax, TPS is time to attain peak shortening, RL 1/2 is time to attain 1/2 shortening decline, DC is duration of complete contraction, ΔL is fractional change of papillary muscle length at peak shortening.

a $p<0.05$ versus 'younger' control group (Table 4A)

b $p<0.005$ versus 'younger' control group (Table 4A)

Table 5. Papillary Muscle Mechanics of Control and GES-treated Rats: 3 Minute Rest Interval

	N	TT (g/mm^2)	dT/dt ($g/mm^2/s$)	TPT (ms)	RT 1/2 (ms)	DC (ms)
1.3 mM Ca^{2+}						
Control	12	9.7 ± 0.5	103 ± 6	131 ± 1	133 ± 6	465 ± 17
GES-treated	14	7.6± 0.5***	84 ± 7*	131 ± 3	143 ± 6	519 ± 19*
2.5 mM Ca^{2+}						
Control	12	9.8 ± 0.5	104 ± 5	130 ± 3	126 ± 12	471 ± 11
Ges-treated	13	7.5± 0.5***	82 ± 7*	130 ± 3	138 ± 7	509 ± 21*

Values are means ± S.E.M., TT is total tension, dT/dt is maximum rate of tension development, TPT is time to attain peak total tension, RT 1/2 is time to attain 1/2 tension decline, DC is duration of contraction.

*p<.05 ***p<.005

Table 6. Papillary Muscle Mechanics of Control and GES-treated Rats: Paired Stimuli

		200 ms Interval		300ms Interval	
	N	TT (g/mm^2)	dT/dt ($g/mm^2/s$)	TT (g/mm^2)	dT/dt ($g/mm^2/s$)
1.3 mM Ca^{2+}					
Control	12	9.6 ± 0.5	101 ± 5	9.0 ± 0.4	94 ± 6
GES-treated	14	7.3 ± 0.4****	77 ± 5***	6.8 ± 0.5***	72 ± 6*
2.5 mM Ca^{2+}					
Control	12	9.8 ± 0.4	99 ± 5	9.1 ± 0.5	92 ± 5
Ges-treated	13	7.3 ± 0.5***	77 ± 7*	7.1 ± 0.5**	72 ± 6*

Values are means ± S.E.M., TT is total tension, dT/dt is maximum rate of tension development, *p<.05 ** p<0.01 ****p<.001

Table 7. Papillary Muscle Mechanics of Control and GES-treated rats: Basal Stimulation and Paired Stimuli

		TT (g/mm^2)		% TT	TT (g/mm^2)	% TT
	N	Basal Rate	200 ms Interval	% Basal tension	300 ms Interval	% Basal tension
1.3 mM Ca^{2+}						
Control	12	8.9 ± 0.4	9.6 ± 0.4	108	9.0 ± 0.4	101
GES-treated	14	6.8 ± 0.5***	7.3 ± 0.4****	107	6.8 ± 0.5***	100
2.5 mM Ca^{2+}						
Control	12	9.4 ± 0.5	9.5 ± 0.4	101	9.1 ± 0.5	97
GES-treated	13	7.1 ± 0.5***	7.3 ± 0.5***	103	7.1 ± 0.5**	100

Values are means ± SEM, TT is total tension, Basal Stimulation refers to 0.1 Hz stimulus rate, paired stimuli are superimposed on 0.1 Hz stimulus frequency
**p<.01 ***p<.005 ****p<.001

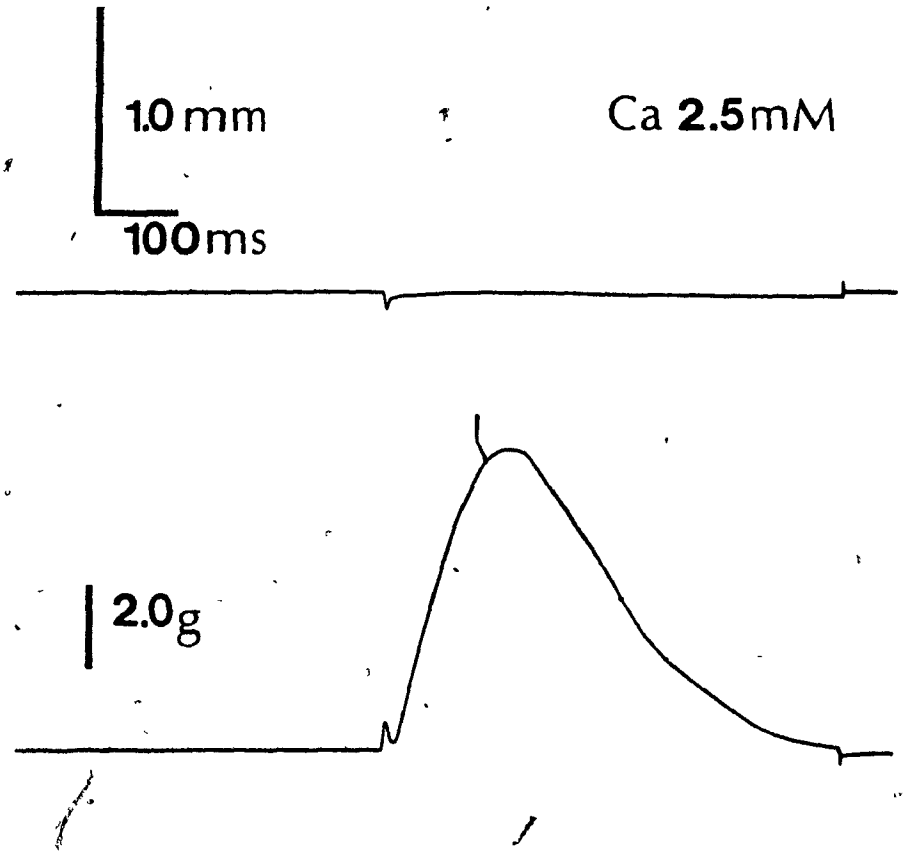

Figure 4. A representative isometric twitch. Pen recorder traces of the output from the length and force transducer. The upper trace shows the change in muscle length, the lower trace shows the force generated by the muscle.

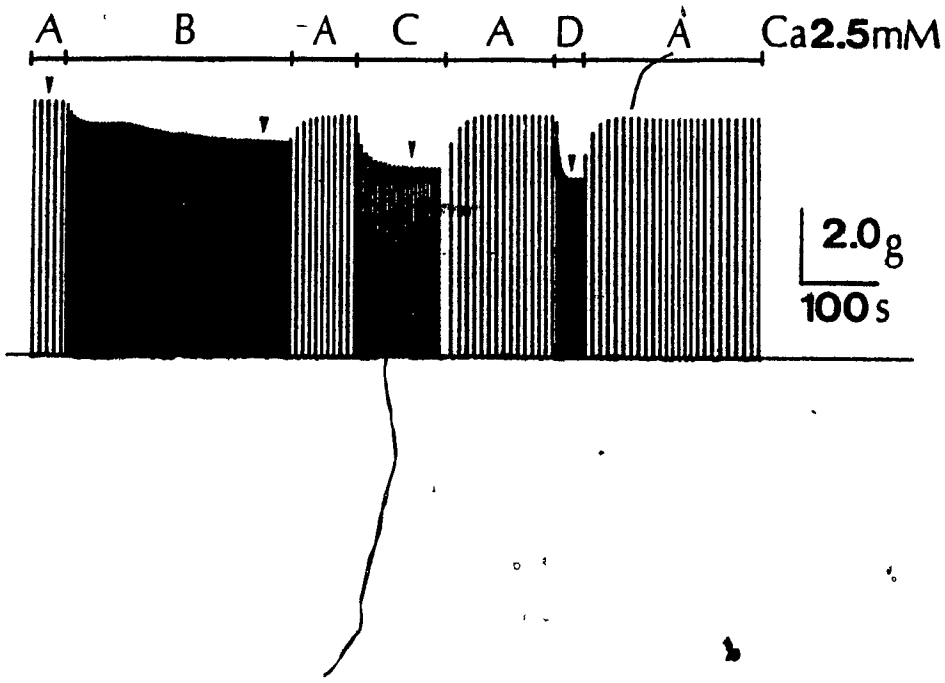

Figure 5 The force-frequency relationship. Representative force-frequency traces of isometrically contracting papillary muscle. A,B,C and D indicate the stimulation frequency of 0.1, 0.2, 0.4, and 0.6 Hz respectively. Arrows indicate points at which tension measurements would be made, after stability was achieved. The negative staircase effect is apparent.

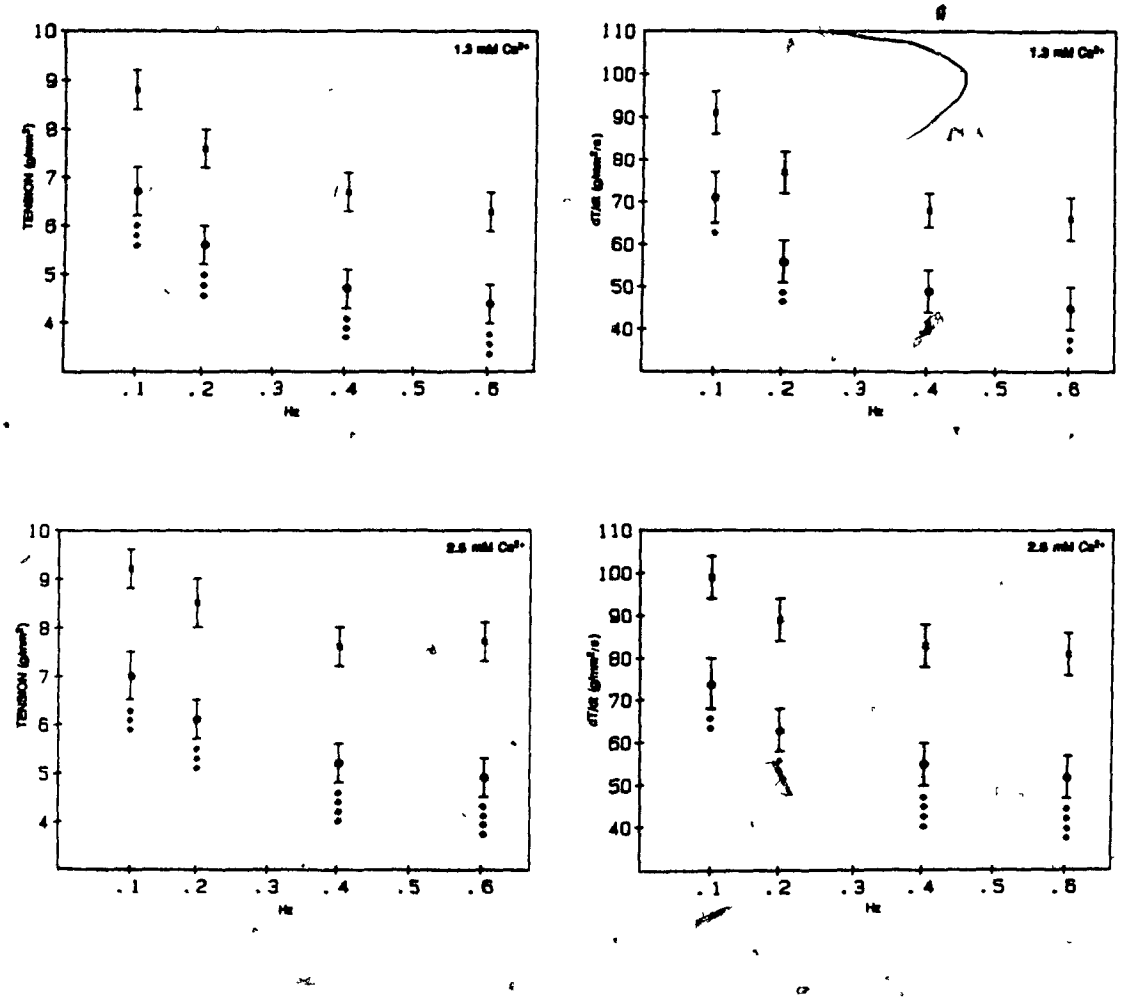

Figure 6. The force-frequency relationship of papillary muscles at two calcium concentrations. Data points are means ± SEM for 12 control papillary muscles (X) and 14 taurine-depleted papillary muscles (•). Left hand panels show values for total tension, while right hand panels illustrate dT/dt; the maximum rate of tension development, as a function of stimulation frequency. The significance of unpaired t-tests is indicated by

*p<0.05 **p<0.01 ***p<0.005 ****p<0.001

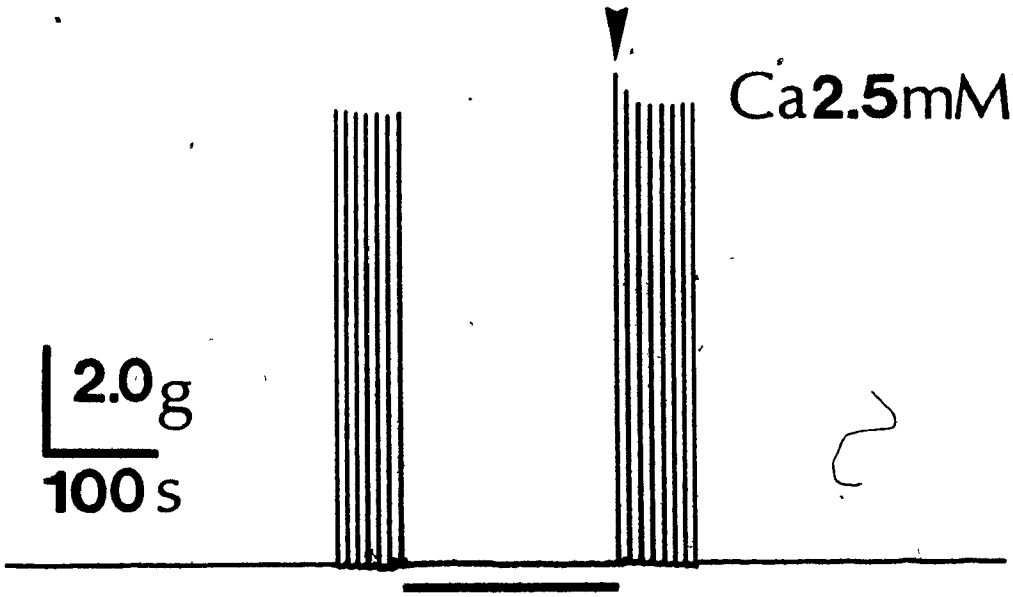

Figure 7. The mechanical restitution response. Representative trace of the potentiating effect of a 3-minute rest interval on isometrically contracting papillary muscle. The horizontal bar indicates the stimulus-free period, the arrow points to the potentiated contraction.

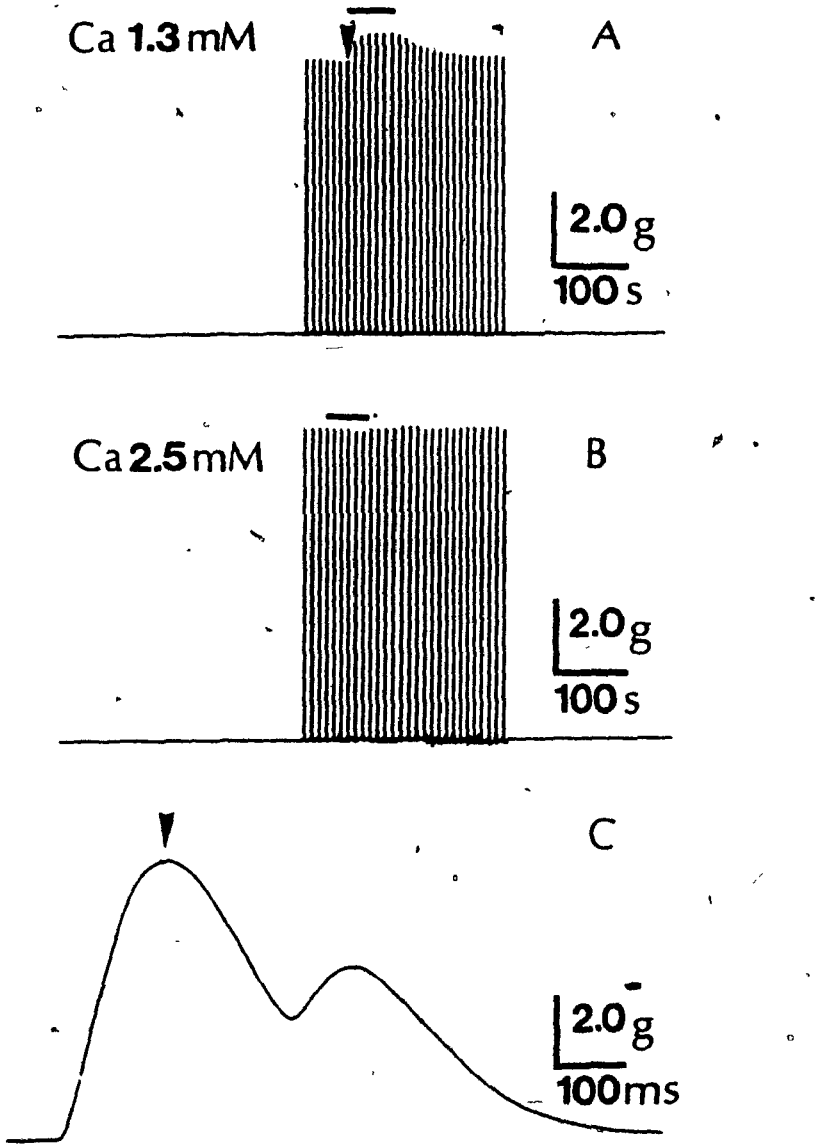

Figure 8. Contractile responses to paired stimuli. Representative traces of isometric contractions at two different calcium concentrations in the bath. The horizontal bars above parts A and B indicate the application of paired stimulation (300 ms interval). The arrow in part A indicates the response to the first paired stimulus. The arrow in part C shows, under the same conditions as A, but higher sweep speed, the increased peak total tension which was measured when the response to paired stimuli had stabilized. Potentiation was most apparent in low calcium .

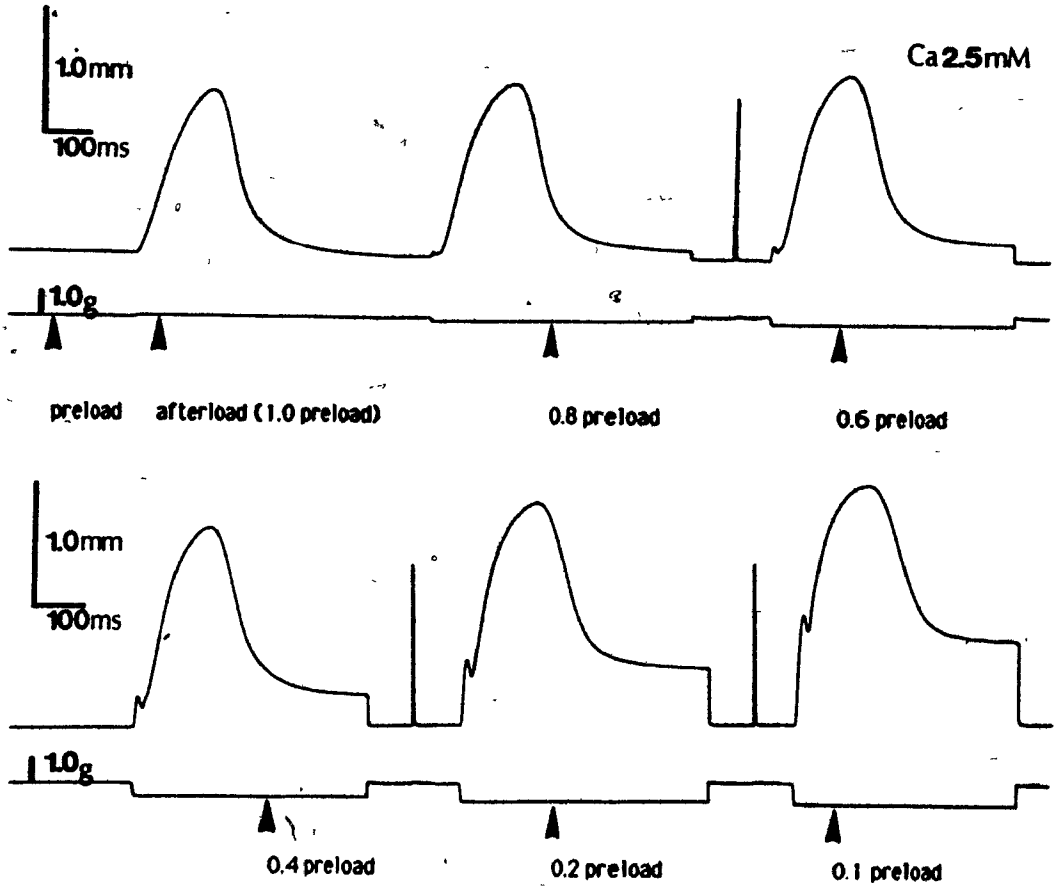

Figure 9. Isotonic and unloaded contractions. The upper trace shows the shortening profile of the contracting muscle, the lower trace monitors the tension on the muscle before and during contraction. The velocity-tension relationship could be determined from this type of experiment (see Fig. 10.).

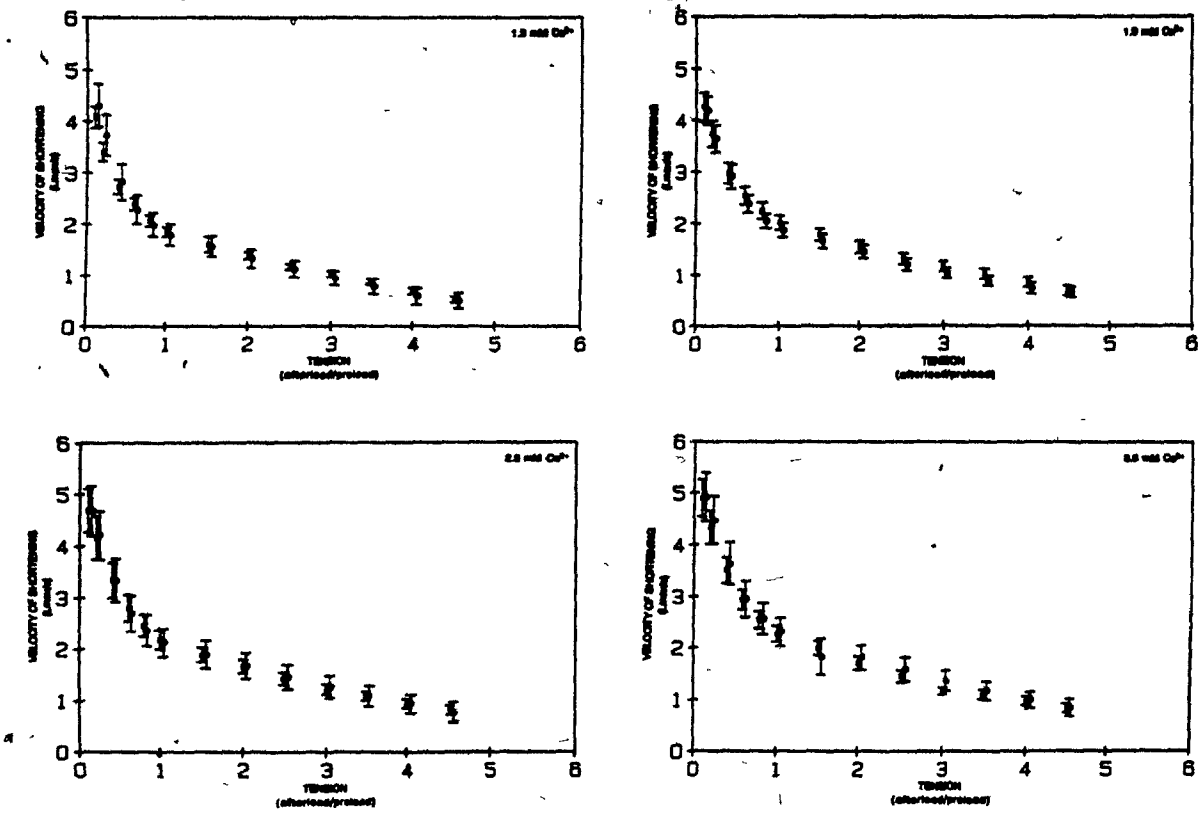

Figure 10 Velocity-tension curves for papillary muscle at four calcium concentrations. Data points are means ± SEM for 12 control (X) and 8 taurine-depleted (•) papillary muscles. The •'s have been displaced by 0.04 units to the right on the tension axis for clarity. At each calcium concentration there were no significant differences between the values or the extrapolated Vmax for the two experimental groups.

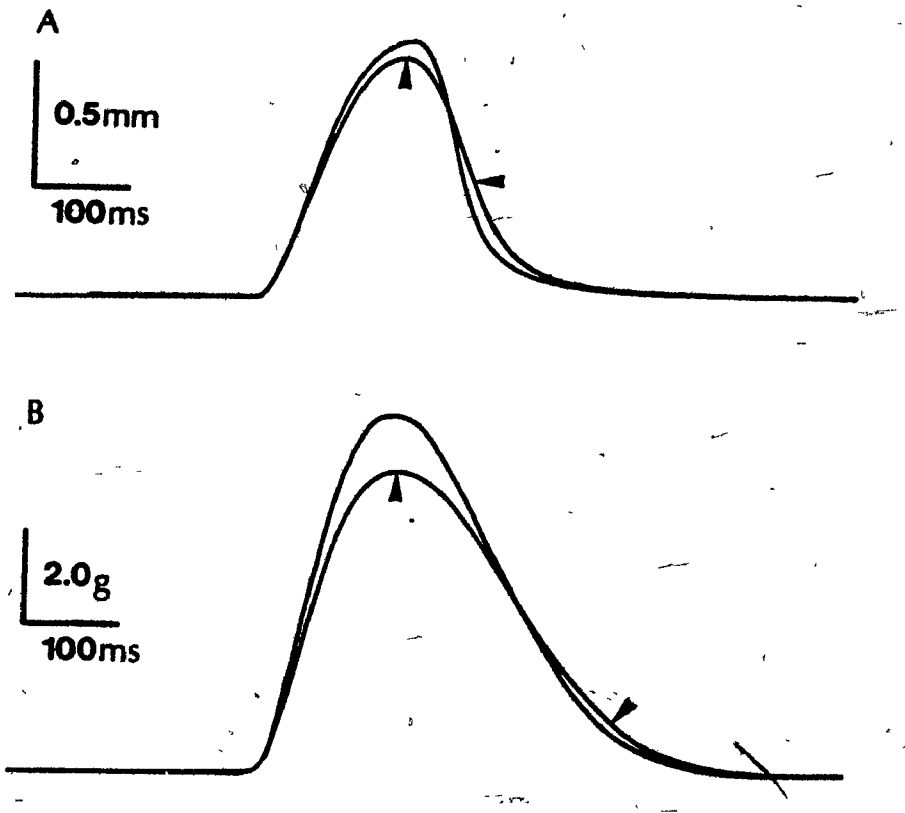

Figure 11. Isotonic and Isometric Contractions. Superimposed traces of contractions of taurine-depleted (indicated by arrows) and control papillary muscles. Part A displays isotonic contraction, part B - isometric.

DISCUSSION

General Findings

In isometric contractions the total tension developed by the muscle is indicative of the number of activated actin-myosin sites, which is modulated by the concentration of free cytosolic calcium. Phosphorylation of the contractile proteins increases the kinetics of contraction, but has little effect on the maximum force developed (Winegrad, 1984).

In the present study it was found that during isometric contractions taurine-depleted muscles generated significantly lower tensions and lower maximal rates of tension development than controls. This suggests that cytosolic calcium in taurine-depleted muscles did not rise to levels as high as controls. Also the twitch duration tended to be prolonged in taurine-depleted muscles, suggesting that the processes that govern sarcoplasmic calcium removal were less efficient. Varying extracellular calcium concentrations did not alter these differences, suggesting that an abnormality in intracellular calcium uptake and release (most likely by the SR) may be present.

Observed relative deficiencies in the tension generated and the maximal rate of tension development of taurine-depleted muscles compared to control were maintained at four different stimulation frequencies; both groups of muscles displayed a similar negative force staircase phenomenon at rates above 0.1 Hz.

While with increasing stimulation frequency rat papillary muscles usually display a negative force frequency relationship (Figure 5), an inverse phenomenon is observed when the stimulation is interrupted for a short period of time; the first stimulus following the rest interval results in a potentiated contractile response the so called 'mechanical restitution' response (Figure 7).

In preliminary tests we found that a three minute rest interval resulted in the greatest mechanical restitution in control papillary muscles. This time interval was used to study the contractile characteristics of taurine-depleted muscles. While the rest period was

followed by potentiation of contraction, taurine-depleted muscles retained depressed contractile responses relative to control; the muscles generated significantly lower total tensions and rates of maximal tension development, and the contractions tended to be prolonged (Table 5).

As mentioned earlier, paired-pulse stimulation results in contractile potentiation. This potentiated response to the subsequent stimulus is demonstrated in Figure 8; the arrow points to the first paired stimulus. Only with the second paired stimulus does potentiation occur (Figure 8A). Potentiation by paired stimulation is observed most readily at low calcium concentrations (Table 7) presumably because at higher calcium concentrations the calcium buffering sites and calcium uptake processes are operating at near saturation. It is possible that in low extracellular calcium, the rise in total tension to a new tension plateau during paired stimulation represents a higher steady state of intracellular calcium recirculation. At higher extracellular calcium concentrations there is no significant increase in the total tension during paired stimulation (Table 7); the tension plateau reached by the muscles is similar for the basal frequency and paired stimulation (Figure 8B). This implies that at 2.5 mM calcium and 0.1Hz stimulation (basal), the muscle is operating at a near optimal steady state of intracellular calcium recirculation. Pairing stimuli under these conditions or at higher calcium concentrations may not be able to augment calcium uptake into (and release from) the SR.

Both taurine-depleted and control muscles showed an equal degree of potentiation to paired stimulation, thus the relative (baseline) differences between the two groups remained the same and significant. Taurine-depleted muscles consistently generated lower total tensions and maximal rates of tension development. These differences were independent of the extracellular calcium concentration or the paired pulse interval (Table 7).

In order to evaluate the kinetics of unloaded contractions, Vmax and isotonic contractions were studied. There were no differences in Vmax between the taurine-depleted and the control muscles at the four calcium concentrations tested. This finding suggests that the turnover rate of individual actin-myosin crossbridges (which is positively correlated with

myosin ATPase activity) or the rate of rise of free sarcoplasmic calcium (immediately preceding the contraction) is not influenced by taurine depletion. The shortening velocity and the time to attain peak shortening also remained unaltered in the taurine-depleted muscles, but the kinetics of relaxation were different compared to the control. Relaxation time to decline to half shortening length, and the duration of contraction were prolonged. Since the relaxation phase is governed by the rate of removal of free calcium from the sarcoplasm, these findings support the concept that taurine depletion is associated with lower kinetics of SR calcium uptake.

A weakness of the present study arises due to the required pharmacologic induction of taurine depletion; as taurine depletes, GES accumulates in the tissue (Table 2A). Taurine repletion, while maintaining high intracellular GES content, was attempted using GES-taurine protocol. In a subset of taurine-depleted animals intracellular taurine was 90% repleted while GES levels remained high (Table 2B). With taurine repletion the QT intervals shortened back to control values (Figure 3), and the contractile indices were not different from age-matched controls (Tables 3B and 4B). However, when comparisons were made to the 'younger' control group (ie. Tables 3B, 4B GES-taurine cf. Tables 3A, 4A) although the total tension attained control levels (of the younger controls), other contractile parameters differed from these controls. But since the 'older' controls also differed from the 'younger' controls on these very parameters, we concluded that there may be age-dependent effects which are confounding our interpretation of repletion and recovery. Age-dependent contractile differences in rat myocardium are well documented (Froechlich et al., 1978; Lakatta and Yin, 1982; Wei et al., 1984), and in our study taurine-repleted animals were 11 weeks older than 'younger' controls. However, since the present experiments had a small sample size (Tables 3B and 4B) our results need to be confirmed and clarified in future studies.

The Role of Calcium in Muscle Contraction

Between contractions the contractile unit of cardiac muscle, the myofibril, is maintained in a relaxed state by the thin filament inhibitory proteins, troponin and tropomyosin. During contraction the myofilaments become activated when calcium ions bind to the troponin I subunit thereby releasing the inhibitory proteins' action. The calcium responsible for activating the contractile proteins is released from stores in the SR by membrane depolarization and translocated across the sarcolemma via the second inward current (Isi) during the plateau of the AP (Morad and Goldman, 1973; Fabiato and Fabiato, 1975). Thus the performance of the heart as a pump and its contractile characteristics during the muscle shortening, is, to a large extent, dependent on the concentration of calcium in the cytoplasm following depolarization.

Conversely, the rate of relaxation of mammalian cardiac muscle is directly related to the rate of cytosolic calcium decrease from systolic concentrations (10^{-5}M) to diastolic concentrations (10^{-7}M). The rate of calcium removal from the cytoplasm depends on three factors: (I) the uptake of calcium into subcellular compartments (primarily the SR), (II) the extrusion of calcium across the sarcolemma, and (III) the phosphorylation state of the contractile proteins, since phosphorylation of troponin I decreases troponin affinity for calcium, enhancing muscle relaxation (Winegrad, 1984). Although the three processes operate simultaneously, in the rat heart reuptake is the single most important factor controlling the beat-to-beat calcium homeostasis.

The force of myocardial contraction is directly dependent upon the level of free cytosolic calcium present during the contraction, while the durations of isometric and isotonic contractions are, to a large degree, dependent on the rate of removal of calcium from the cytoplasm. In this study there were three major contractile changes observed in taurine-depleted muscles: (I) lower total tension and lower maximal rate of tension increase, (II) the durations of both isometric and isotonic twitches tended to be prolonged, and (III) the relaxation phase of isotonic contractions was prolonged. Alterations of extracellular calcium concentration had little or no effect on the magnitude of the

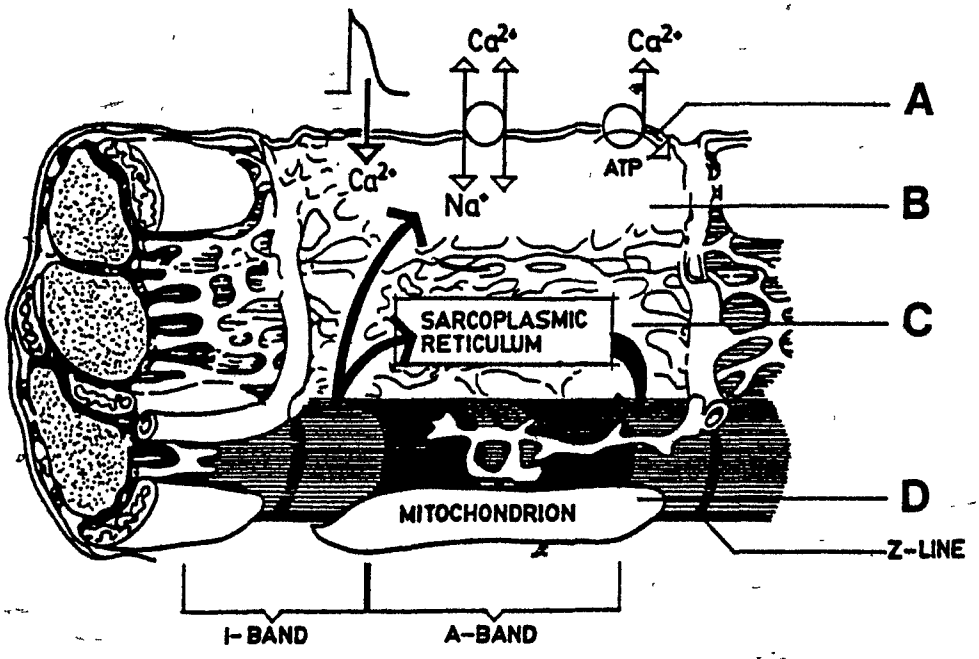

Figure 12. Calcium and the Cardiac Cell. A model of the cardiac cell after Katz (1977). A, B, C and D refer to the possible sites of intracellular calcium buffering: A - the "inner leaflet" of the sarcolemma, B - the sarcoplasmic binding compounds, C - the sarcoplasmic reticulum (SR), D - the mitochondrion. Heavy black lines indicate calcium circulation between the SR, the sarcoplasm and the myofibrils. Three modes of calcium movement across the sarcolemma are indicated: (a) that through specific calcium channels during the action potential (Isi), (b) the Na/Ca exchanger and (c) the Ca-ATPase pump.

contractile changes from control. Each of the altered contractile parameters is likely, however, to reflect intracellular calcium fluxes. Hence, although no direct measurements of myocardial calcium content or fluxes were done in this study, the data suggests that taurine deficiency alters intracellular calcium handling by the myocardium.

In order to further interpret the data, the processes which alter intracellular calcium oscillations and the possible role of taurine at each of these levels must be analyzed. These inter-related processes can, for clarity, be categorized into three groups: (I) trans-sarcolemmal currents and calcium fluxes, (II) 'passive' intracellular calcium buffering, and (III) calcium uptake, storage, and release by intracellular compartments (mostly the SR).

Sarcolemmal Modulation of Calcium Fluxes

Ventricular contractions are usually initiated by sarcolemmal depolarizations. The ventricular AP of the rat can be divided into three phases: depolarization, brief plateau, and repolarization. The depolarizing upstroke of the AP is attributed mainly to the fast inward sodium current (tetrodotoxin (TTX) sensitive) (Mitchell et al., 1984). This large sodium conductance is brief because of rapid voltage inactivation of the channels. Concurrently with the voltage inactivation of Na-TTX channels, a transient outward potassium current (4-aminopyridine sensitive) occurs that gives rise to a rapid repolarizing deflection (Mitchell et al., 1984). The Isi is thought to be mediated mostly by calcium ions passing through specific channels (Isenberg and Klockner, 1980) and to be responsible for the genesis of the plateau phase (Isenberg and Klockner, 1980).

The brief plateau phase in the rat stands in sharp contrast to the prolonged plateau encountered in other mammalian species, such as the guinea pig (Coraboeuf et al., 1968). This short plateau of the rat AP may result from two specific electrophysiological phenomena: high intracellular free calcium - calcium current inactivation (intracellular calcium inactivating Isi) (Josephson et al., 1984a), and an early onset

outward current (Josephson et al., 1984b).

Finally, the rat AP has a pronounced slow phase of repolarization which is dependent on the balance between outward conductance (most likely due to potassium) and a depolarizing exchange current. The depolarizing Na/Ca exchange mechanism is in turn dependent on intracellular and extracellular sodium and calcium concentrations (Schouten and terKeurs, 1985). It is a low affinity, high capacity system that operates electrogenically, exchanging three sodium ions for one calcium ion (Pitts, 1979). During the slow repolarization phase of the AP this Na/Ca exchange mechanism is involved in extruding calcium ions across the sarcolemma, thus prolonging the repolarization process.

In addition to the Na/Ca exchanger and the calcium channel (Isi), heart sarcolemma contains a specific calcium transporter: calcium-adenosine triphosphatase (Ca-ATPase) (Caroni and Carafoli, 1980). This ATPase is a high affinity, low capacity system, which exports calcium with essentially the same efficiency throughout the entire functional cycle of heart cells (Caroni and Carafoli, 1980).

Thus the sarcolemma, containing both low and high affinity calcium export systems, contributes both to the precise intracellular regulation of calcium in the sub-micromolar range, and to the transport of bulk amounts of it. The sarcolemmal membrane also serves as a regulator of calcium influx, which, in case of rat, triggers contraction via its effects on the SR (Fabiato, 1983; Bers, 1985).

Rat hearts depleted of taurine show a prolonged duration of the ventricular AP (Lake et al., 1987), but the mechanism is as yet unknown. One possibility is that changes in intracellular calcium homeostasis, as suggested by the present observations on contractility, may lead to a prolonged slow repolarization phase (see below), but further studies are required to explore this hypothesis.

Passive Intracellular Calcium Buffering

The very low concentration of ionized calcium within heart cells, and the tight regulation of intracellular calcium fluxes are a consequence, in large part, of high affinity binding of calcium with specific proteins (Fabiato, 1983). These proteins can be divided into two functional classes, depending whether they are integral components of membranes, or soluble in the cytoplasm. The relative contribution of these two classes of proteins to homeostasis of calcium is still, however, an unresolved question.

There are two classes of soluble cytosolic compounds capable of binding calcium: specific binding proteins, and low molecular weight compounds. They are both able to chelate calcium as well as other divalent cations (Carafoli, 1985). Calmodulin, present in micromolar concentrations in the cell, and troponin C, are the two specific calcium-binding proteins. Their primary role, however, may not be to provide cytosolic buffering for calcium, but rather to mediate signal transduction. Low molecular weight compounds such as adenine, nicotinamide adenine nucleotides, inorganic phosphate, citrate and other tri- and dicarboxylates contribute significantly to the buffering of cytosolic calcium, keeping its ionic activity lower than its total concentration (Carafoli, 1985). Future experiments are required to assess how taurine may influence calcium binding by the various cytosolic constituents, as no studies, to my knowledge, have yet addressed this question.

While osmotic limitations restrict the quantity of soluble calcium-binding compounds, calcium complexing proteins integral to membranes are not so limited. In fact, sarcolemmal and sarcoplasmic reticular membranes are able to bind calcium in large amounts (Fabiato, 1983). Fabiato (1983) argues that despite their large capacity for binding, the available sarcolemmal binding sites may not be the major contributors to physiological buffering of free ionic calcium because of their low affinity.

However, because many studies have suggested a role for taurine in regulating the membrane affinity for calcium, intracellular calcium

buffering via the sarcolemmal membrane needs to be re-assessed in the presence of physiologic concentrations of intracellular taurine. In the late seventies Kulakowski et al. (1978) demonstrated the existence of high and low affinity taurine receptors on the sarcolemma. Chovan et al. (1979; 1980) found that taurine enhanced calcium binding to the membrane and postulated that low affinity sarcolemmal taurine receptors are involved in the regulation of calcium binding to the sarcolemma. More recently, Sebring and Huxtable (1985) reported that taurine increased high affinity calcium binding to sarcolemmal vesicles in buffers mimicking intracellular conditions (low Na, high K), while it had no effect on calcium binding in buffers mimicking extracellular conditions. It has been demonstrated that the sarcolemmal membrane is asymmetrical in nature, and that there are significant differences in calcium binding to its respective sides (Nayler, 1982). Although Sebring and Huxtable did not determine the 'sidedness' of their vesicles their findings suggest that if taurine modulates calcium binding, the process occurs on the intracellular side of the membrane. This hypothesis is consistent with the fact that high taurine concentration (in the 'modulating' range) is found only in the sarcoplasm, not in the extracellular fluid. In the future, in order to quantitate the effects of taurine on calcium binding to the inner leaflet of the sarcolemma, the 'inside-out' and the 'right-side-out' sarcolemmal vesicles should be pre-separated (Mas-Oliva et al., 1980) before the binding studies.

Although presently it is not known how taurine may alter calcium binding to the sarcolemma, recent studies, which evaluated taurine binding to the membrane, suggest that taurine may interact with the membrane phospholipids (Sebring and Huxtable, 1985). It is possible that this taurine-phospholipid interaction on the inner aspect of the sarcolemma changes the membrane binding dynamics for calcium.

Intracellular Sites of Calcium Uptake and Storage

Within heart cells, calcium transporting systems are found in the mitochondria and the SR. There are three basic calcium transport modes found in these organelles: calcium ATPase, Na/Ca exchanger, and electrophoretic uniporter (Carafoli, 1985). Assuming, arbitrarily, a Km of 1 uM as the dividing line between the high and the low affinity uptake systems, only the ATPase transport systems fall into the high affinity class. There are two different ATPase calcium transporters: one in the sarcolemma (mentioned above), and another one localized in the SR. The two transporters have a similar value for Km (about 0.5 uM Ca), but Vmax for the SR enzyme is about 40 to 60 times greater than the sarcolemmal one (Carafoli, 1985). Na/Ca exchangers are found in both the sarcolemmal and the mitochondrial membranes, but the Vmax of the mitochondrial exchanger is much lower that that of the sarcolemmal (Carafoli, 1985). The electrophoretic calcium uniporter is found only in the mitochondria. It is a low affinity uptake system that was found to have a low Vmax for calcium transport, when tested in a buffer containing physiologic concentrations of magnesium (Carafoli, 1985).

The existence of low and high affinity transport systems for the regulation of the intracellular calcium concentration is in keeping with the different demands of the signalling function of calcium. During the contraction and the relaxation cycle of the myofibrils, rapid and precise modulation of calcium in a very low ionic concentration range is required. However, since cardiac function is dependent on many variables (ie. load, volume, nutrient/oxygen delivery etc.), in some instances larger amounts of calcium may have to be transferred from compartments when its ionic concentration has increased to the micromolar range and above. Under such conditions, low affinity transport systems for calcium may be utilized. Presently, the exact range in which the regulation of calcium occurs, linked to the contraction/relaxation cycle, is not well defined, since there is no precise information on the value of sarcoplasmic calcium ion activity during tension development. Calculated values indicate that sarcoplasmic calcium oscillates below the micromolar range during contraction/relaxation cycles, with the exception of peak

activation – when this value may be exceeded (Fabiato, 1983). Thus the systems regulating cardiac calcium must operate efficiently at concentrations below one micromolar. Since the high affinity, large-capacity uptake system for calcium is found predominantly in the SR, this organelle is generally accepted as the structure on which heart cells depend for the fine regulation of sarcoplasmic calcium.

The reported results of studies that have evaluated the effects of taurine on calcium binding to, and calcium uptake by the SR, as well as the effects of taurine on ATPase kinetics, are not in agreement; however they have been done on different species and tissues, and have utilized different methods. In one study, which assessed the effect of taurine on SR vesicles isolated from rat skeletal muscle, it was found that millimolar concentrations of taurine increased (by about 25%) the rate of calcium uptake as well as the total sequestering capacity of the vesicle (Huxtable and Bressler, 1973). But in another study, 20 mM taurine had no effect on calcium binding to SR or on Ca-Mg-activated ATPase activity in partially purified SR vesicles from rat hearts (Chubb and Huxtable, 1978). Contradictory results have also been reported for taurine effects on guinea pig SR (Dolara et al., 1976; Remtulla et al., 1978).

Although some investigators still argue in favour of a mitochondrial contribution to calcium homeostasis and tension control in cardiac muscle (Fry and Miller, 1985), other studies indicate that mitochondria within normal heart cells do not store large amounts of rapidly exchangeable calcium (Barnard, 1981; Somlyo et al., 1982). Most of the evidence indicates that mitochondria can play a role only in long term calcium homeostasis in the heart (see discussion in Carafoli, 1985). It would be instructive to evaluate the role of taurine in mitochondrial calcium metabolism in the heart. This would be particularly interesting in congestive heart failure, when myocardial taurine levels are known to double (Huxtable and Bressler, 1974).

Although the regulation of calcium fluxes within heart cells has been studied for years, relatively little is known about the role of taurine. The effects of taurine deficiency in the heart can be studied by the use of the taurine-depletion paradigm. Rat hearts depleted of taurine show electrophysiological (Lake et al., 1987), and contractile

changes, but the mechanism is still not established. Exogenous taurine has been demonstrated to influence calcium binding to the sarcolemma, and, in some studies, calcium exchange by SR. No studies are available on sarcolemmal currents in hearts depleted of taurine, or on the effects of taurine in mitochondrial metabolism of calcium. Although the effects of taurine deficiency offer only an indirect way of looking at taurine action, this approach has opened a new avenue of investigation of the physiological role of this compound in the heart. The present contractile studies, which utilized this approach, indicate that taurine depletion may be associated with calcium-processing deficiency of the SR.

Interpretation of Major Findings in Light of Calcium Homeostasis

The most recent hypothesis of ECC mechanism in the rat (terKeurs, 1987) states that following the upstroke of the AP, calcium entry into cardiac cells (via Isi) triggers calcium release from the SR. Calcium released from the SR then activates the contractile machinery, and, during the relaxation phase of contraction, is partially sequestered into the SR together with some calcium that entered the cell during the AP (Fabiato, 1983). Since calcium influx across the sarcolemma must, in the steady state, balance calcium efflux - the remaining calcium leaves the cell through the sarcolemma, partially in exchange for sodium (Na/Ca exchanger), and partially via the calcium pump (sarcolemmal calcium ATPase).

In the early eighties, investigators who studied the kinetics of the Isi concluded that there is an inverse relationship between the magnitude of this current and its duration (Marban and Tsien, 1981). In the following year, elaborating on this finding, a model for calcium dependent calcium channel inactivation was proposed (Standen and Stanfield, 1982). Two years later this hypothesis was tested in rat ventricular cells and Isi was shown to be inactivated by intracellular calcium (Josephson et al., 1984a). This hypothesis was further tested in combined contractile and electrophysiological studies (Schouten and terKeurs, 1985). In these studies the 'post-extrasystolic potentiation' protocol was utilized in order to obtain contractile potentiation; the

baseline stimulation of a contracting muscle was followed by a short burst of stimuli, then the stimulation was interrupted for a given time interval, after which a single isometric twitch, with its accompanying AP, was recorded. The force of contraction was found to be increased, the duration of the plateau of the AP was shortened, while the repolarization phase and the overall duration of the AP was prolonged. The proposed mechanism was such that greater calcium release following the brief stimulus interruption gave rise to more vigorous contraction, and resulted in earlier inactivation of the Isi (hence the shortening of the plateau of AP). At the same time, the higher level of sarcoplasmic calcium present during the relaxation phase of contraction served as additional substrate for the Na/Ca exchanger, thus prolonging the repolarization process and AP duration. Other studies that utilized different contractile protocols (ie. paired pulse paradigm) showed findings that were consistent with this model of calcium fluxes (Schouten, 1984).

Taurine depletion in the rat heart is associated with specific electrophysiological (Lake et al., 1987) and contractile changes which can be interpreted in the context of the above ECC hypothesis. This interpretation may provide some insights into the physiological role of myocardial taurine.

In the present contractile studies it was found that total tension and the maximal rate of tension development were significantly lower than control in taurine-depleted muscles. These differences were independent of calcium concentration in the bath or the rate of stimulation. Since these tension parameters are dependent on the level of sarcoplasmic calcium during contraction, these findings suggest that in taurine-depleted muscles the release of calcium from SR may be depressed. With less calcium release from SR, a smaller fraction of myofibrils would be activated, hence lower total tensions and decreased maximal rates of tension development would be expected during isometric contractions. The similar time to peak tension of isometric contractions of taurine-depleted and control muscles, suggests that the actual amount of released calcium is depressed.

One way to depress SR calcium release might be by decreasing calcium

uptake into this organelle. In taurine-depleted muscles the durations of isotonic contractions were increased due to a prolonged relaxation phase (Table 4). This finding suggests that calcium uptake into the SR was slower, since relaxation is dependent, to a large degree, upon the rate of removal of calcium from the sarcoplasm. A decrease in SR calcium uptake in taurine depleted hearts is compatible with the work of Huxtable and Bressler (1973). It is thus possible that taurine depletion results in a deficiency of calcium transport and storage in the SR and that this led to the prolongation of twitch duration found in the taurine depleted rat hearts. Since force frequency studies in taurine depleted myocardium gave rise to a similar negative staircase response compared to control, abnormalities in calcium transport, if present, do not appear to be stimulation rate dependent.

In addition to our contractile findings, earlier electrophysiological studies conducted in our laboratory (Lake et al., 1987) are consistent with the interpretation that taurine depletion is associated with lowered calcium uptake and release by the SR. Slower repolarization led to significant prolongation of the AP duration observed in the absence of upstroke changes in rat papillary muscles depleted of taurine (Lake et al., 1987). If taurine-depleted muscles have slower calcium uptake into the SR, more calcium may be recirculating through the sarcolemma. Hence, with each beat, delayed repolarization may be reflective of relatively higher activity of the sarcolemmal Na/Ca exchange mechanism.

There is an alternative explanation for the above findings. Taurine depletion may decrease calcium release by the SR indirectly. It is possible that myocardial taurine plays a role in calcium storage through, perhaps, an effect on calcium binding. Taurine enhancement of sarcolemmal (Huxtable and Sebring, 1986), and SR (Dolara et al., 1976) calcium binding has been shown. Some support for this 'altered SR calcium storage' hypothesis comes from the studies which showed that when stimulation was interrupted for three minutes (the time required in controls for maximal potentiation of contraction), taurine depleted muscles still generated significantly lower total tensions than controls. Thus, even with a long rest interval, the amount of calcium released by

the SR appears to be lower in taurine-depleted muscles, possibly because the releasable pool is smaller.

Thus from this study it would appear that the high physiological concentrations of myocardial taurine may be important in intracellular calcium homeostasis. The contractile data indicate that taurine-depleted muscles have depressed contractility. The specific contractile changes suggest that calcium handling by the SR may be altered in taurine-depleted muscles such that calcium release by the SR is decreased during muscle contractions. The depressed calcium release by the SR may be a consequence of decreased calcium uptake and storage by this organelle in taurine-depleted muscles. Decreased kinetics of calcium uptake by the SR and, consequently, relatively higher activity of the Na/Ca exchanger, could account for both the contractile and the electrophysiological changes observed in taurine-depleted muscles.

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