The Effects of Taurine Depletion on Rat Heart Electrophysiology

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September, 1989
Taurine is an amino acid found in high concentration (20-30 mM) in mammalian heart. Treatment of rats with the transport antagonist guanidinoethyl sulfonate (GES) depletes cardiac taurine (>70%). Electrocardiograms were recorded weekly in restrained unanaesthetized rats. GES treatment caused a selective prolongation of the QT interval which was correlated with the degree of myocardial taurine depletion ($r^2=0.92$, $p < 0.001$). Ventricular muscle action potential durations (APD$_{95}$) from taurine-depleted hearts were significantly prolonged compared to control (88 $^+/-$ 8 ms vs. 66 $^+/-$ 9 ms; $p < 0.001$). Taurine supplements given to depleted rats reversed the taurine depletion and the QT prolongation; no effect was seen in controls. No action potential differences between control and "reversed" rats were seen. Superfusion of GES or taurine at concentrations of 0.2-10 mM had no effect on action potential characteristics of control or taurine-depleted hearts. When hamsters were GES-treated no cardiac biochemical or QT changes were seen.
La taurine est un acide aminé dont la concentration dans le tissu cardiaque des mammifères est élevée (20-30 mM). L'administration de sulfonate de guanidinethyle (GES) chez le rat épuise les réserves de taurine cardiaque (plus de 70%). Des électrocardiogrammes ont été enregistrés, hebdomadairement, chez des rats non-anesthésiés et sous contention. Le traitement avec GES a produit un allongement spécifique de l'intervalle Q-T correspondant au taux d'épuisement de la taurine myocardique ($r^2 = 0.92$, $p < 0.001$). Les potentiels d'action enregistrés ($DPA_{95}$) dans le muscle ventriculaire des rats traités ont été significativement prolongés en comparaison avec les rats déficients en témoins ($88 \pm 8$ ms vs. $66 \pm 9$ ms, $p < 0.001$). Chez les rats déficients en taurine, un supplément de cet acide aminé a corrigé le déficit et l'allongement du Q-T. Par ailleurs, aucun changement ne fut noté chez les rats témoins. Les potentiels d'action mesurés chez les rats dont les déficits en taurine furent corrigés ont été semblables aux potentiels enregistrés dans le groupe témoin. La surfusion de GES ou de taurine à des concentrations de 0.2 à 10 mM n'ont pas modifié les potentiels d'action mesurés chez les rats témoins ou déficients en taurine. Des hamsters traités avec GES n'ont présenté aucune de ces modifications biochimiques ou électrocardiographique.
Dedication

"Man's mind, once stretched by a new idea, never regains its original dimensions."

Oliver Wendell Holmes
Acknowledgements

This thesis, being of the document type, is divided into two main parts. The first part is comprised of a published paper of which I performed all the electrophysiological experiments. This paper was written by Dr. N. Lake in consultation with M. de Roode and Dr. S. Nattel. The biochemical data which appears in this paper were from assays kindly performed by Luisa De Marte. The second part of this thesis consists of unpublished results concerning the effects of taurine depletion on hamsters. In this case the electrophysiological experiments and the biochemistry were carried out by M. de Roode.

I wish also to acknowledge the assistance of Dr. S. Nattel and the use of his excellent electrophysiology laboratory and technician, without which none of this would have been possible. Photography was provided by R. Thompson. I would like to acknowledge the encouragement and inspiration given to me by Drs. F. Du Beau, R. Mosqueda-Garcia and M. Talajic. Dr. Du Beau is also responsible for translating the abstract into french. Last, but not least, I would like to thank Dr. N. Lake for her help and never ending patience in dealing with me.

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- ECG

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Table 5 QT intervals for hamsters in Study 1.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Δ</td>
<td>change (delta)</td>
</tr>
<tr>
<td>AFCR</td>
<td>American Federation for Clinical Research</td>
</tr>
<tr>
<td>Ag- AgCl</td>
<td>silver-silver chloride</td>
</tr>
<tr>
<td>AMP</td>
<td>amplitude</td>
</tr>
<tr>
<td>ANF</td>
<td>atrial natriuretic factor</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ANS</td>
<td>autonomic nervous system</td>
</tr>
<tr>
<td>APD&lt;sub&gt;75,95&lt;/sub&gt;</td>
<td>action potential duration to 75, 95% repolarization</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATP'ase</td>
<td>adenosine triphosphatase</td>
</tr>
<tr>
<td>A/D</td>
<td>analog/digital</td>
</tr>
<tr>
<td>B</td>
<td>Beta</td>
</tr>
<tr>
<td>B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>pyridoxine</td>
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<tr>
<td>C</td>
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</tr>
<tr>
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<td>copyright</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;++&lt;/sup&gt;</td>
<td>calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;-&lt;/sup&gt;</td>
<td>chloride ion</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>CSAD</td>
<td>cysteine sulfinic acid decarboxylase</td>
</tr>
<tr>
<td>°C</td>
<td>degrees centigrade/Celsius</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>Abbr</td>
<td>Term</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>DPA95</td>
<td>duration potentiel d'action 95%</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma amino butyric acid</td>
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<tr>
<td>GES</td>
<td>guanidinoethyl sulfonate</td>
</tr>
<tr>
<td>H</td>
<td>hydrogen</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>HCO₃</td>
<td>bicarbonate</td>
</tr>
<tr>
<td>I&lt;sub&gt;Ca&lt;/sub&gt;</td>
<td>inward calcium current</td>
</tr>
<tr>
<td>I&lt;sub&gt;EO&lt;/sub&gt;</td>
<td>early outward current</td>
</tr>
<tr>
<td>I&lt;sub&gt;K&lt;/sub&gt;</td>
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</tr>
<tr>
<td>I&lt;sub&gt;Na&lt;/sub&gt;</td>
<td>sodium current</td>
</tr>
<tr>
<td>i.p.</td>
<td>intra peritoneal</td>
</tr>
<tr>
<td>K⁺</td>
<td>potassium ion</td>
</tr>
<tr>
<td>KCl</td>
<td>potassium chloride</td>
</tr>
<tr>
<td>Km</td>
<td>equilibrium constant</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>MB-CPK</td>
<td>myocardial creatine phosphokinase</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>magnesium ion</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
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<td>millilitre</td>
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<td>millimolar</td>
</tr>
<tr>
<td>mm/s</td>
<td>millimeters/second</td>
</tr>
<tr>
<td>MOhm</td>
<td>mega Ohm</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
</tbody>
</table>
msec  millisecond  
N  nitrogen  
N=  number  
Na⁺  sodium ion  
nmole  nanomoles  
NS  not significant  
NYHA  New York Heart Association  
o  oxygen  
p  probability  
pH  - logarithm of hydrogen ion concentration  
pKa  - logarithm of ionization constant (acid)  
pKb  - logarithm of ionization constant (base)  
PO₄  phosphate  
r²  coefficient of determination  
s  sulfur  
S.D.  standard deviation  
TAU  taurine  
umol  micromole  
Vmax  maximum velocity of transport  
V_max  maximum upstroke velocity  
vs.  versus  
wt.  weight
Chapter 1

GENERAL INTRODUCTION

Since the discovery of taurine in 1827, when it was first isolated from ox bile, much work has been done concerning the functions in life of this ubiquitous amino acid (1). Experiments by Choughuley and Lemmon (2) have shown that taurine might even have been present in primitive earth conditions and hence possibly before life itself. Yet the only conclusive function that is known for taurine is its role in the formation of bile acids.

What then is known about this compound? This thesis will attempt to partly address this question and report my findings as to some possible actions of taurine in the heart.

Chemistry of Taurine

Taurine (2-aminoethanesulfonic acid) is derived from the amino acids cysteine and methionine. It is generally characterized as a B-amino acid. Taurine has a molecular weight of 125 daltons, a molecular formula of C₇H₁₄O₃NS, a pKₐ of 1.5 and a pKₐ of 8.74 owing to its sulfonic acid and amino moieties (3). At physiological pH (~7.4) its major ionic form is as a zwitterion, which is a molecule whose net charge is neutral but bears both negative and positive groups. The degree of ionization of taurine is remarkably constant from pHs of 6.8-7.8 compared to some other of its
related compounds (4). It is highly soluble in hydrophilic but not hydrophobic solvents.

Diversity

Taurine has been found in protozoa, anthropods, reptiles and mammals but in very few plants (3). In mammals taurine is present in almost every tissue studied including blood and breast milk. In the latter it is found to be very concentrated. In the rat taurine concentrations are generally found to be higher in males than in females (3). Taurine concentrations are also higher in the neonate than in the adult with the exception of the retina (5), heart, muscle and spleen which all increase in concentration with age in the rat (3).

Synthesis and Metabolism

Taurine synthesis takes place mainly in the liver but some extrahepatic synthesis is also present. However, there are major differences in the hepatic ability of taurine synthesis between different genera of mammals (3,6,7). Synthesis proceeds first from the oxidation of cysteine to cysteinesulfinic acid and then via decarboxylation to hypotaurine with the vitamin cofactor $B_6$. Cysteine Sulfinic Acid Decarboxylase (CSAD), the enzyme that catalyzes this step, is the rate limiting step in the synthesis and its content in a tissue is generally related to the synthetic capacity of that tissue (8,9). Oxidation of hypotaurine to taurine does not seem to be a regulated step in the pathway.
regulated step in the pathway. As the liver is the main synthetic site of taurine, the extrahepatic tissues are therefore dependent on influx and efflux to maintain tissue levels of taurine. Apart from the very slight white blood cell metabolism of taurine with hypochlorous acid to form taurochloramine (10) there is no extrahepatic metabolism of taurine in mammals. Hepatic metabolism of taurine forms taurocholate, a bile salt used for fat absorption. Therefore whole body taurine level depends on hepatic synthesis and dietary intake, balanced against renal excretion and hepatic metabolism.

Transport

Tissues such as the heart because of limited capacity to synthesize taurine are dependent on the transport of this compound to maintain tissue levels (11). Huxtable et al. characterized this transport system as having a $K_m$ of 45uM and a $V_{max}$ of 32 nmole/g dry weight tissue. This transport is dependent on the presence of $Na^+$, $Ca^{++}$ and energy for proper functioning (11). Taurine transport is enhanced by B-adrenergic agonists such as epinephrine and isoproterenol. This transport system is not specific for taurine but rather transports all B-amino acids and gamma amino butyric acid (GABA). Under normal circumstances saturation is achieved at physiological blood concentration levels of taurine (0.5 mM) and its transport can be pharmacologically inhibited by other B-amino acids and
structural analogs of taurine such as hypotaurine. More salient is the compound guanidinoethyl sulfonate (GES), a metabolically resistant analogue, which was used extensively to deplete taurine for the purpose of this thesis (4,11,12).

Possible Functions

Although taurine is implicated to act on many organ systems, this thesis will primarily focus on the interaction between taurine and the heart. The findings of previous studies have divided the functions of taurine in the heart into 4 main effects, they are: hormonal, metabolic, osmotic and ionic interactions.

Hormonal, Metabolic and Osmotic

Taurine through a low affinity membrane protein is believed to interact with the insulin receptor and have agonististic properties at that site (13,14,15,16). Infused taurine has an insulin-like effect as it can potentiate hypoglycemia when given together with insulin. In hamster studies it has been shown that the administration of a drinking water solution of 1.25% taurine increased the release of the hormone Atrial Natriuretic Factor (ANF) in the Cardiomyopathic hamster (17).
Atlas et al and Thurston et al have shown that there is an elevation of heart taurine concentration in hypernatremic and a fall in hyponatremic mice (18,19). This effect was specific to taurine and was not accompanied by changes in the level of any other amino acids including the B-amino acids which share the same transport system. It appears then that taurine is selectively concentrated in response to changes in osmotic pressure.

Apart from its possible influences with insulin, taurine appears to have other metabolic activities. Hearts which have been depleted of taurine exhibit an increased production of lactate and pyruvate as well as increased glycogen utilization. An elevation of the enzymatic activity of phosphofructokinase is suspected (20). Superfusion of rat hearts with taurine causes a transient decrease in the amount of cAMP which is associated with an increase in the inotropic state as measured by cardiac work (21).

Ionic

Na\(^+\) and K\(^+\)

By far the greatest amount of work that has been done regards the relationship between taurine and cardiac electrophysiology. The major ions that have been implicated are Na\(^+\), K\(^+\) and Ca\(^{++}\). Diacono and Dietrich
showed that superfusion of taurine caused a decrease in the fast inward Na\(^+\) current (I\(_{\text{Na}}\)) in the rat ventricular myocardium (22). They also found a decrease in the action potential duration (APD) and the action potential amplitude (AMP). These authors were not able to show that taurine could change the slow inward current (I\(_{\text{Ca}}\)) or the K\(^+\) current (I\(_{\text{K}}\)). However it should be noted that these investigators made their measurements with isotonic saline, which can cause technical problems. The difficulties in measuring I\(_{\text{Na}}\) are due to the speed and large magnitude of this current. These facts make reliable measurement of I\(_{\text{Na}}\) difficult and so and Diacono and Dietrich's results are not generally quoted in the literature. Nathan and Crass (23) studied the dog heart Purkinje fiber preparation and found that taurine superfusion did not alter the maximal upstroke velocity (V\(_{\text{max}}\)) which is an indirect measurement of I\(_{\text{Na}}\). They did however find a prolongation of the APD, with superfusion of taurine, which they ascribed to a change in either the K\(^+\) or Ca\(^{++}\) current. When taurine is given chronically to cardiomyopathic hamsters there is an increase in the already low level of Na\(^+\)/K\(^+\) ATP'ase activity (24). None of the above authors noted any change in resting membrane potential (which might be due to an increase in the pump current or to an alteration of intracellular ions) with the superfusion of taurine. Ca\(^{++}\)
Some effects of taurine are: the prevention of Ca\(^{++}\) overload in the cardiomyopathic hamster (24), positive inotropy (21), antiarrhythmic action against digitalis toxicity (33) and facilitation of Ca\(^{++}\) binding to the sarcolemma (13). Chelation of Ca\(^{++}\) with taurine has been shown to exist with the use of \(^{13}\)C nuclear magnetic resonance, but is responsible for binding less than 2% of intracellular Ca\(^{++}\) (25).

Ca\(^{++}\) and the Cardiomyopathic Hamster

The BIO 14.6 cardiomyopathic hamster represents an experimental model of some forms of heart disease (26). This animal characteristically has extreme overloads in its Ca\(^{++}\) levels associated with a necrotic myopathy, and irreversible loss of electrical and mechanical activity. The myopathy is similar to the one seen following chronic isoproterenol administration. The mechanism of this abnormal Ca\(^{++}\) overload seems to be associated with a poorly functioning sarcolemma which allows extensive Ca\(^{++}\) accumulation (24). These Ca\(^{++}\) levels can rise over the control level by 6 fold (27). The increased Ca\(^{++}\) triggers a dysfunction within the mitochondria that results in an uncoupling of the mitochondrial oxidative pathways. Azari, Brumbaugh and Huxtable (28) found that this Ca\(^{++}\) overload could partially be circumvented if cardiomyopathic hamsters were treated with a drinking water solution containing 1.25% taurine. This treatment significantly elevated the
heart taurine concentration and also reduced the morphological changes that are associated with the Ca\textsuperscript{++} overloading. Welty and McBroom (29) found that taurine supplementation not only reduced the severity of the spontaneous Ca\textsuperscript{++} overload but also decreased the Ca\textsuperscript{++} overload due to the administration of isoproterenol in both the BIO 14.6 and control (random bred) hamster. To further prove a role of taurine in the protection of the cardiomyopathic hamster Welty et al. administered the taurine depleting agent GES in the drinking water and showed that GES significantly lowered the taurine concentration in the myocardium (by approximately 45\%) and as a result there was an accentuation of the Ca\textsuperscript{++} overload (29). With significant depletion of the heart taurine concentration (about 38\% of control) no such effect on Ca\textsuperscript{++} was seen in control hamsters.

Ca\textsuperscript{++} and Inotropy

Inotropy refers to the efficiency of force generation of a muscular contraction. Positive inotropic agents increase the contractility of myocardial muscle. The inotropic state of cardiac muscle is dependent on many variables such as cAMP, external and internal Ca\textsuperscript{++} stores, preload, autonomic control, and the state of the contractile filaments (30). When heart muscle is superfused in a buffer containing a low concentration of Ca\textsuperscript{++} the negative inotropic state of the myocardium can be
reversed by the addition of taurine to the superfusate (31). Franconi et al. (32) found that in the superfused guinea pig heart there was a time-dependent spontaneous loss of taurine associated with a decrease in the force of contraction, when measured by the force displacement transducer. This decrease in the force of contraction could be reversed by the administration of high concentrations of taurine (10-100 mM) to the superfusate which caused an elevation of the myocardial taurine concentration. This reversal, of the inotropic state by taurine, was more pronounced in the presence of low external concentrations of Ca\(^{++}\) (1.5-1.8 mM) than in physiological concentrations. However studies by Mozaffari, Tan, Lucia and Schaffer (20) contradicted the findings of Franconi et al. Mozaffari et al. found that the taurine-depleted hearts of rats pretreated with the taurine uptake antagonist GES did not show decreased cardiac work capacity compared to control rats that were not taurine-depleted. It must be emphasized that their work does not rule out a change in myocardial contractility with taurine depletion. They also found that taurine depletion had no effect on the increase in cardiac work produced by insulin.

Antiarrhythmic

It has been shown (33) that taurine administration is beneficial in the prevention of the arrhythmias associated with both digoxin and epinephrine toxicity. These
arrhythmias are usually associated with abnormal automaticity which in the case of digoxin is an arrhythmia caused by elevated Ca\(^{++}\) levels due to a poisoned Na\(^{+}/K^{+}\) \textit{ATP'ase.} With this enzyme inhibited there is an elevation of intracellular Na\(^{+}\) and enhanced Na\(^{+}\) exchange in place of Ca\(^{++}\), this can cause a Ca\(^{++}\) overload (34). Taurine has also been shown to be beneficial in preventing arrhythmias in the guinea pig heart (35). Again in this case taurine's action might be due to its effect on reducing the Ca\(^{++}\) overload independent of its cause.

Facilitation of Ca\(^{++}\) binding to the Sarcolemma

It is believed that there are two taurine binding sites on the sarcolemma, a high and a low affinity one (13,14,15). The low affinity site is associated with enhancing the binding of Ca\(^{++}\) to the sarcolemma (13,36,37). The concentration at which taurine exerts its effect (Kd ~ 3.5-15 mM) is within the physiological range that is found within the rat heart and thus might have a significant physiological effect on Ca\(^{++}\) binding \textit{in vivo}. Ca\(^{++}\) binding to the sarcolemma has by previous investigators been shown to be highly correlated to myocardial contractility (38). Compared to the guinea pig the rat heart is more dependent on releasable internal stores of Ca\(^{++}\) than on Ca\(^{++}\) derived from the slow inward current to maintain its contractile activity. This is thought to be related to the observation that the rat has a shorter phase 2 duration of the action
potential plateau and thus less Ca\textsuperscript{++} entry (39). Studies by Isenberg (40) in sheep Purkinje fibers, using microinjection techniques, showed that there is an inverse relationship between the free internal concentration of Ca\textsuperscript{++} and the magnitude of the slow inward current \((I_{Ca})\). He postulated that the amount of Ca\textsuperscript{++} that flows in during the action potential is self-limiting because as it enters the cell it binds to the cytoplasmic surface of the sarcolemma where it transiently turns off the slow inward current. It might therefore be hypothesized that any preexisting alteration of the amount of Ca\textsuperscript{++} that is already bound to the sarcolemma would change the quantity of Ca\textsuperscript{++} influx needed to turn the slow inward current off. This point is important because this current is believed to be dominant in controlling the APD (39). Ca\textsuperscript{++} derived from the slow inward current \((I_{Ca})\) has not been shown to have an effect in either turning on or off the early outward current \((I_{EO})\) (41).

Beneficial in Ca\textsuperscript{++} Paradox

When heart tissue is superfused with a Ca\textsuperscript{++}-containing solution following a period of superfusion with a Ca\textsuperscript{++}-free solution, an elevation in the total tissue content of Ca\textsuperscript{++} and mechanical dysfunction are seen. This effect is termed the Ca\textsuperscript{++} paradox (42,43). This Ca\textsuperscript{++} overload causes massive ultrastructural damage and loss of mechanical function of the myocardium (44,45,46). Nayler et al. (47)
described the process of Ca++ accumulation during reperfusion as occurring in two parts, an early and a late phase of Ca++ entry. The first phase of Ca++ entry was related to the I_{Ca} and could be blocked by Ca++ channel blockers such as verapamil and nifedipine. The duration of this phase was described to last less than 120 seconds. The second or late phase of Ca++ entry occurred from the action of the Na+/Ca++ exchanger. Nayler et al. also showed that Na+ loading exacerbated the Ca++ overload.

When taurine is added to the reperfusate there is a substantial and significant decrease in the loss of mechanical function that normally occurs because of the Ca++ paradox (48). The above authors noted that taurine greatly reduced the late phase of Ca++ entry (Na+/Ca++ exchange). These authors did not examine effects of taurine on the early phase of Ca++ entry. Taurine is also of benefit in limiting the release of intracellular components such as myocardial creatine phosphokinase (MB-CPK), nucleotides and nucleosides following Ca++ paradox (48). Coupled with their morphorological observations this implies that taurine decreases the myocardial membrane damage which occurs with the Ca++ paradox. It is known in the newly-hatched chick that there is a transient decrease in the concentration of heart taurine from 2 to 9 days of age. This physiological change in heart taurine can be used to examine a change in the sensitivity of the myocardium to the Ca++ paradox. Takihara et al. (49) found
that 2 day old chicks with significantly higher levels of taurine had less loss of contractile force compared to either the 7 or 9 day old chicks, following the calcium paradox. Furthermore, 7 day old chicks receiving oral taurine supplementation had higher heart levels of taurine and suffered less loss of contractile force following calcium paradox than was found in non supplemented chicks. Supplemented chicks accumulated 37% less Ca\textsuperscript{2+} following the Ca\textsuperscript{2+} paradox protocol than did the non-supplemented chicks of the same age. Their and others' data (48) concerning the Ca\textsuperscript{2+} paradox, strongly indicate that taurine plays a role in modulating Ca\textsuperscript{2+} fluxes and/or its intracellular availability.

Conclusion: possible role of taurine in Ca\textsuperscript{2+} regulation

The above studies suggest that taurine modulates Ca\textsuperscript{2+} in heart cells. Its mechanism of action could be via its effect on altering the binding of Ca\textsuperscript{2+} to the sarcolemma, which in turn alters the amount of slow inward current (I_{Ca}) and early outward current (I_{E0}). It might also have some effect in altering the mobilization of Ca\textsuperscript{2+} among the various storage pools of Ca\textsuperscript{2+} such as the mitochondria, sarcoplasmic reticulum and Ca\textsuperscript{2+} binding proteins. Due to the possible relationship stated above between Ca\textsuperscript{2+} levels in the heart and the myocardial action potential, it would not be unreasonable to hypothesize that the depletion of
taurine may cause an alteration of some of the ionic fluxes in the heart.

Methods Of Study

Taurine Depletion

The function of taurine in the heart has previously been studied in two main ways: \textit{in vitro} and \textit{in vivo}. In the first method cells or cell type in question are isolated and placed in various concentrations of either taurine or one of its antagonists such as GES. It is thought that this method tests only the extracellular action of taurine, since the transport of extracellular taurine across the membrane is very slow. These experiments may have little to do with the intracellular action of taurine and therefore the results of these experiments should not be generalized. The second method is to alter the \textit{in vivo} level of tissue taurine by either increasing it or depleting it. Unfortunately the \textit{in vivo} administration of taurine has little effect in elevating tissue levels since the body merely compensates for the increased load of taurine by enhancing renal excretion (50). When faced with the problem of depletion two methods may be considered. The first is to place an animal on a taurine-free diet. However this only works in species that have low amounts of the taurine synthetic enzyme, CSAD, and therefore little synthetic capability. Furthermore this method is expensive and time-consuming since it may take months to years for an animal to deplete (51) due to the
high content of taurine in all tissues of the body and the slow turnover of taurine in the face of depletion (50). The second option open for experimental manipulation of in vivo taurine levels is the use of the taurine analog, GES. This method is easy, relatively inexpensive and even works on species that have significant amounts of the enzyme CSAD. A decrease in the effectiveness of taurine depletion occurs in those species, such as the guinea pig that have higher amounts of the GES hydrolyzing enzymes, amidinotransferase and amidinohydrolase (52). Huxtable et al. (11) have shown that tissue levels of taurine can be decreased with the systemic administration of the taurine analog, GES, either as an intraperitoneal injection or given in the rat's drinking water for a period of days. These authors noted that in a matter of weeks tissue levels of taurine could be depleted to as little as 25% of control in almost all tissues tested. However a major drawback of this technique is that an accumulation of GES occurs in place of the lost taurine. The effect of intracellular GES on cell physiology is not known but it is postulated to have some interactions with creatinine (20).

ECG

As well as being a pump the heart is also an electrical device and this electrical activity drives the mechanics of the heart. " The electrocardiogram (ECG) is a graphic description of the electrical activity of the heart
recorded from the body surface by a group of electrodes positioned to reflect activity from a variety of spatial perspectives " (53). The ECG has been used for over 100 years as a tool to non-invasively examine the heart. Its use can be virtually diagnostic for many heart diseases and conditions such as cardiac hypertrophy, atrial and ventricular arrhythmias, ischemic heart disease, myocardial infarction and pericarditis, to name but a few. A typical ECG contains a structured pattern of waves and intervals which repeat themselves at more or less fixed periods. The waves on the ECG represent cellular depolarization or repolarization, while the intervals are the times between the waves (see Figure 2). This massed electrical activity is generated at the cellular level and is related to cellular action potentials. There are three waves on the ECG; they are the P, QRS and the T. They are related to the depolarization of the atria, ventricles and then the repolarization of the ventricles respectively. Also present are three intervals; the PR, QT and RR. The PR interval is related to the time that it takes for an electrical impulse to travel from the atria to the ventricles. It is therefore related to the sum of intra-atrial plus atrio-ventricular conduction time. Since the QT interval starts at the beginning of the depolarization of the ventricles (QRS) and ends with the repolarization of the ventricles (T) it is therefore related to the APD in the ventricular tissue. The RR interval is simply the time
between successive QRS complexes and is therefore inversely related to the heart rate. Lombard has measured values for the ECG intervals in the rat to be about 180 msec for the RR and 66 msec for the QT interval (54). The advantage of characterizing and measuring these intervals and waves is that specific information about the heart can be determined from this non-invasive technique. For instance information about the Sinus Node can be determined from the time between successive P waves (PP interval) and the speed of intraventricular conduction can be assessed by the duration of the QRS complex. Although the rat is not a species commonly studied with the ECG, there have been several relevant studies performed on the rat. The ECG of the rat has waves similar to those of humans, and has proved to be useful for purposes of experimentation (55).

The duration of the QT interval is considered to reflect the duration of the ventricular action potential (56,57). The QT interval is prolonged by manoeuvres which prolong APD and is shortened by manoeuvres that shorten APD (58). In the mechanism for the generation of the T wave, proposed by Noble and colleagues (59), the wave of depolarization travels from the base to the apex of the heart, but repolarization proceeds from the apex to the base. Therefore the difference in APD between the apex and base of the heart is proportional to the distance between the two areas (59). Due to its smaller heart size this difference in APD should be much less in the rat compared
to humans. Watanabe et al. (60) have shown in fact that there is very little difference between the apex and base APDs in the rat heart. Due to these small regional differences in APDs a reliable and accurate estimation of the rat ventricular APD can be made with a sample of rat myocardium.

The ECG was used in my studies because it is non-invasive, can give specific information about many different areas of the heart and is commonly used as a screening device. Furthermore not only can the ECG be used to detect disturbances intrinsic to the heart but also to detect any extrinsic (i.e. secondary) disturbances such as endocrine, vascular and electrolyte, which potentially might be caused by taurine depletion.

For the purposes of this study it was necessary to repeat measurements at regular intervals on the same experimental animals, so that sequential changes could be studied, as well as limiting the number of animals that would be used for the purposes of the study. In order to do this it was essential to perform the ECGs on unanaesthetized animals. The use of anaesthetic agents not only confounds the interpretation of the ECG but also increases the risk of morbidity and mortality secondary to the experimental procedure itself, and therefore its use was avoided. With these goals in mind I attempted to see
if there were any electrophysiological changes associated with GES-induced taurine depletion in the rat and hamster heart.
EFFECTS OF TAURINE DEPLETION ON RAT CARDIAC ELECTROPHYSIOLOGY: IN VIVO AND IN VITRO STUDIES

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Abstract

Electrocardiograms were monitored in unanaesthetized rats during treatment with drinking water containing guanidinoethyl sulfonate (GES), an inhibitor of taurine transport, which depleted cardiac taurine content. Treatment led to a selective prolongation of the QT interval which was highly correlated with the degree of taurine depletion ($r^2=0.92$, $p < 0.001$). Compared to controls, the duration of ventricular muscle action potentials was significantly increased in GES-treated rats, and this accounted for the prolongation of QT intervals. Oral taurine supplements reversed GES-induced cardiac taurine depletion and the associated increased duration of action potentials and QT intervals. In vitro superfusion with 0.2-10 mM GES or taurine had no effect on action potentials of control or GES-treated rats. These data indicate that intracellular taurine may play a role in regulating myocardial APD, particularly during repolarization.

Introduction

Taurine is a naturally occurring sulfur-containing amino acid which is present in high concentration in mammalian hearts. In the rat, cardiac taurine levels are about 25-30 umol/g wet weight and constitute over 60% of the free amino acid pool. These levels appear to be
regulated mainly by transport processes (influx and efflux), while local metabolism is less important (61). The structural requirements for transport of taurine into the heart have been defined, and a technique has been described to specifically deplete rat cardiac taurine content in vivo to about 25% of control value by the use of a transport antagonist, guanidinoethyl sulfonate (GES) (11). The authors reporting the effects of GES noted no gross cardiovascular changes (11).

Exogenously administered taurine has positive inotropic effects, antagonizes the effects of low calcium or calcium blockers (35,36), and retards lesion development in calcium overload cardiomyopathy (62). Furthermore, taurine is effective against arrhythmias caused by hypokalemia, epinephrine, or digitalis toxicity (33,35). The mechanisms of these changes are not understood, but an interaction between taurine and sarcolemmal calcium binding has been suggested (36,37). Plasma taurine levels are usually in the range of 50 μM and rarely exceed 500 μM.

Findings from some of the above studies must be interpreted with caution since the concentrations of exogenous taurine used were in the range of 3.5 to 160 mM.

Taurine-free diets are ineffective in depleting myocardial taurine stores of adult rats, since changes in liver biosynthesis and renal excretion of taurine can
compensate for the absence of dietary taurine intake (50, 61). We therefore decided to investigate the role of taurine in the heart by evaluating cardiac electrophysiologic changes in rats during GES-induced taurine depletion and subsequent taurine repletion. Changes in the electrocardiogram (ECG) in vivo were related to alterations in myocardial taurine and GES content, as well as to alterations in the characteristics of action potentials studied in vitro.

Methods

GES was synthesized (8), purified by 3 crystallizations from water, and analyzed for taurine (63) and GES (64) concentration. Only GES of greater than 99% purity, with less than 0.1% taurine, was utilized. Animals were treated by the addition of GES to their drinking water at a concentration of 1.0 or 1.6%. Male rats of the Sprague Dawley strain with initial weights of 150–175 g were maintained on normal rat chow (Ralston Purina). For electrophysiologic experiments, 29 control and 18 GES-treated animals were utilized, while biochemical analysis was carried out on tissues from 21 additional control and 24 GES-treated animals.

Three separate studies were done using the specific methodology designated below.
1. Effects of chronic 1.6% treatment on rat ECGs, action potentials, and myocardial biochemistry. ECG activity was recorded weekly in chronic unanaesthetized rats. Nine animals were treated by the addition of 1.6% GES to their drinking water while 6 control rats received ordinary water. After 5 weeks, the treated animals were divided into two groups: the "continued" (4 rats) remained on GES treatment, while the "reversed" group (5 rats) had 1.2% taurine added to their drinking water for the next 3 weeks in addition to the GES, after which they received taurine alone. After 20 weeks of ECG monitoring, the rats were decapitated and their hearts rapidly removed for biochemical determination of taurine, GES, and DNA content, and for analysis of action potentials characteristics.

2. Effects of GES and taurine superfusion on rat cardiac action potentials in vitro. Action potential characteristics were measured in ventricular tissue from 23 additional untreated control animals under control conditions and then after superfusion with selected concentrations of GES or taurine (see below).

3. Time course of changes in ECG and myocardial taurine. Nine rats were placed on 1% GES and their ECGs were monitored once weekly for 8 weeks. In addition, 21 control rats and 24 rats treated with 1% GES were sacrificed at
various intervals to determine the time course of changes in cardiac weight, taurine, DNA, and GES content, during 1 to 8 weeks of treatment.

**ECG recordings in chronic animals:** Over a period of 2-3 weeks, rats were placed periodically in a plastic restrainer (Harvard type) to which they rapidly became adapted. They were shaved to permit the placement of disk electrodes (Grass Instruments). Their ECGs were subsequently recorded for 5-10 minutes, one to three times per week during the study. Recordings of the six standard ECG leads were made on a Mingograf Model 81 Recorder (Siemens) using paper speeds of 250 or 500 mm/s. This allowed a precision of measurement of $+/\_ 4$ or $+/\_ 2$ ms respectively, and ten measurements were averaged to give the result for each animal for each recording session. Group statistics were evaluated using the one way ANOVA with Scheffe contrasts for multiple comparisons and unpaired t-tests when only two means were compared. Group averages are expressed in this manuscript as the mean $+/\_ standard deviation.

**In vitro experiments:** Rats were decapitated and the heart rapidly removed and placed in oxygenated Tyrode's solution of the following composition (in mM): $Na^+$, 141; $HCO_3^-$, 22; $K^+$, 4; $PO_4^{2-}$, 0.9; $Mg^{++}$, 0.5; $Ca^{++}$, 2.0; $Cl^-$ 127; and glucose, 11. The pH was 7.4 after aeration with 95% $O_2$/ 5% $CO_2$. 
Electrophysiology: Small portions of the left ventricle containing the papillary muscles were excised and superfused with oxygenated Tyrode's solution in a tissue bath maintained at 37 ± 0.2°C. Preparations were stimulated with twice diastolic threshold current at a basic cycle length of 150 ms, chosen to simulate the average spontaneous cardiac cycle length in vivo. Cells were impaled with glass microelectrodes (10-30 MOhm) filled with 3.0 M KCl or 3.0 M K acetate connected via an Ag-AgCl junction to a microelectrode amplifier (WPI KS-700) and a storage oscilloscope (Tektronix 5115). Transmembrane potential was digitized using a Tecmar A/D converter and analyzed using an IBM PC and a custom made program (Bascom Consultants, Montreal) which gave the following parameters: resting membrane potential, action potential amplitude, duration to 75%, and 95% repolarization (APD75 and APD95 respectively), and maximal upstroke velocity during phase 0 (Vmax).

Ventricular tissue from 23 additional untreated rats (weight 229 ± 82 g) was used to evaluate the effects of taurine or GES, which were added to the superfusion fluid at a concentration of 0.2, 2.0 or 10 mM. In the first protocol, continuous impalements of single cells were maintained during control and drug infusion periods of at least one hour each, and the action potential
characteristics monitored at five minute intervals. Maintaining prolonged impalement of rat ventricular myocardial cells is difficult because of their contractile activity and small size, so a second protocol was devised in which multiple impalements were made during control, drug treatment, and washout periods.

Biochemistry: Small portions (50-80 mg) of right and left ventricular tissue were weighed, homogenized in 80% ethanol, and placed on ice for at least 2 hours. After centrifugation, the pellet was used for estimation of DNA content (65), while the supernatant was 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 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 (63) and GES (64). Amino acids other than taurine are retained by the column (66). All samples were assayed in triplicate and compared to concurrent standards.

Results

Effects of chronic 1.6% GES treatment. The animals remained healthy throughout the study and showed no gross alterations in behavior. They had initial body weights of
150-175 g and final weights of 516 +/- 132 g after 22 weeks of study, with no significant differences among the groups. During this time the RR interval, QRS duration, and PR interval showed no changes. The QT intervals of untreated animals (Figure# 1) increased from 60 +/- 4 ms to 71 +/- 2 ms over the initial 3 week period as the animals increased in weight to 339 +/- 9 g. The QT interval remained stable thereafter for 17 weeks of monitoring. GES treatment resulted in significant prolongation of the QT interval to 85 +/- 9 ms after 5 weeks (p < 0.001). In the "continued group" (GES-treated to the end of the study) the QT interval continued to increase slowly, reaching a maximum of 100 +/- 9 ms after 15 weeks of GES exposure. Among "reversed" rats the addition of taurine to the GES-drinking water for 3 weeks resulted in a small but significant reduction in QT interval (at week 8 it was 80 +/- 6 ms for "reversed" as compared to 91 +/- 2 ms for "continued", p = 0.012). The GES was then discontinued, and one week later the QT interval had decreased to 75 +/- 2 ms (not different from control rats). Figure# 2 shows the types of ECG changes produced by GES treatment, and their reversal after termination of GES.

In vitro studies: Table# 1 shows the characteristics of the action potentials recorded from ventricular cells for the three groups of rats after 22 weeks of study. An average of 10 cells per control rat and 5-6 cells per
"continued" or "reversed" rat were studied (see also Figure# 3). There were no significant differences in the resting membrane potential or the action potential amplitude, as compared to control or "reversed" groups; however, the duration to 75% or 95% repolarization was significantly prolonged ($p < 0.001$) and the upstroke velocity ($V_{\text{max}}$) was slightly reduced ($p < 0.05$). In a separate group of 14 weeks GES-treated rats, action potential duration values were similarly prolonged but the $V_{\text{max}}$ was greater than that of untreated animals (Table# II). The "reversed" group showed no differences from control. Table# I also shows the results of biochemical analysis of ventricular tissue from the three groups at the end of the study. Compared to control, the "continued" group had significantly reduced taurine levels and increased GES levels, with no changes in DNA content, while the "reversed" group showed no differences from control for DNA or taurine but had significantly reduced GES content.

**Effects of acute GES and taurine exposure in vitro:** In superfusion studies, exogenous taurine or GES had no effect at 0.2, 2.0 or 10 mM concentration on the action potential characteristics of cells from 23 untreated animals (Table# II). Ten mM taurine was also without effect on cells from four GES-treated rats (separate from Table# I rats) whose action potential durations were prolonged. Table# II shows the results from experiments in which a
number of cells impaled during GES or taurine superfusion and then after washout with control Tyrode's. Similar results were obtained in a limited number of experiments in which single cell impalements were maintained under control conditions, GES or taurine superfusion, and washout of GES or taurine.

Time course of effects of 1% GES treatment on rat ECGs and myocardial taurine and GES content: The effects of chronic treatment with 1% GES on QT intervals is shown in Figure 4. Treatment led to a gradual prolongation of QT interval over the 8 week period. The time course and extent of prolongation was similar to the previous study which utilized 1.6% GES (see Figure 1). For both studies, 8 weeks of GES treatment resulted in a mean QT interval of about 95 ms compared to 70 ms for untreated control rats. Body weight, heart weight, and ventricular DNA content were not affected by GES treatment. Figures 5 and 6 show the time courses of decrease in taurine and increase in GES content of ventricular tissue, with 1% GES treatment. In treated rats, taurine content declined steadily over the treatment period; in contrast, GES accumulation reached a plateau after 2 weeks of exposure to GES. In control animals the taurine and GES contents of ventricular tissue were stable over the eight week period with overall average values of 23.8 umol taurine/g and 0.40 umol GES/g wet weight. Figure 7 shows for treated animals the change,
taurine and GES, from these average control values. The change in QT interval (ΔQT) was obtained by subtracting the control mean from the treated mean for each observation week. Significant correlation ($r^2=0.92, p < 0.001$) was found between the change in QT interval and the change in taurine concentration, while the corresponding correlation between change in QT and change in GES content was substantially weaker ($r^2=0.49, p = 0.08, NS$).

**Discussion**

The physiologic role of taurine in the heart remains unclear although it has been the subject of many investigations. In the current study, we have demonstrated that taurine depletion in vivo produces reversible electrophysiologic changes. Treatment with the taurine transport inhibitor, GES, led to prolongation of the QT interval of the ECG (Figure 1, 2 and 4) accompanied by increases in the duration of ventricular muscle action potentials (Table I, II and Figure 3). There were no significant changes in other ECG intervals, and no evidence of ventricular ectopy. The increase in action potential duration of "continued" rats observed in vitro (22 ms, APD$_{95}$, Table I) was of the same order as the change in QT intervals observed in these animals in vivo (25 ms, Figure 1), and thus accounts for the QT changes observed. These effects could be reversed by administering taurine and discontinuing GES (see Table I, Figure 1, 2).
Little difference was observed between the effects of treatment with 1% GES or 1.6% GES. After 8 weeks of either treatment, the prolongation of QT intervals was almost identical (Figure 1 and 4), and the ventricular content of taurine or GES observed after 8 weeks of 1% GES (Figure 5 and 6) was very similar to that of the rats treated for 20 weeks with 1.6% GES (Table I). This implies that maximal effects have been achieved with 1% GES treatment. The importance of differences in \( \dot{V}_{\text{max}} \) between groups is difficult to evaluate in view of variability between cells and the absence of changes in ventricular conduction (as reflected by QRS duration) \textit{in vivo}. Since the effects of GES treatment on \( \dot{V}_{\text{max}} \) were small, of low statistical significance, and in the opposite directions for the two groups examined (Tables I and III), it is perhaps likely that GES has no substantial effect on \( \dot{V}_{\text{max}} \).

\textit{In vitro} superfusion with taurine concentrations in the range 0.2-10 mM were without effect on the electrophysiologic characteristics of rat ventricular cells (Table II). Effects have been observed by others (26,27) but these investigators studied higher concentrations and/or other animal species. Since \textit{in vitro} superfusion for 1-2 hours with GES-containing solutions (0.2-10 mM), also had no effect on ventricular action potentials (Table II), it seems unlikely that changes in extracellular GES or taurine \textit{per se} produced the cardiac electrophysiologic changes that we observed.
The major effect of GES treatment appears to be depletion of tissue taurine levels by antagonism of taurine uptake. The gradual decline of myocardial taurine content is shown in Figure 5. In adult animals, changes in other amino acids do not occur with up to 7 weeks of GES treatment (11,67). Changes in other amino acids have been observed when GES is given during development (5), but these may secondary effects. Rats treated with GES for 20 weeks had prolonged ventricular action potentials and their hearts contained significantly less taurine (72% reduction) and more GES than those from untreated control rats (Table I). The "reversed" group whose action potential characteristics at this point were not different from control values, had taurine and DNA contents not different from control. Their reduced GES levels may be a result of their treatment with taurine, or an artifact of the small sample size. The lack of any difference among the groups in DNA content indicates that treatment did not cause any cell death.

It is conceivable that the accumulation of GES by ventricular tissue during \textit{in vivo} GES treatment (Table I, Figure 6) produces part of the effects that we observed. Although GES is an endogenous compound (64), treatment with GES raises these levels much above control. However the time course of QT interval prolongation corresponds more closely to that of taurine depletion than that of GES.
accumulation (Compare Figure 4, 5 and 6). In addition, as shown in Figure 7, during the 8 week study the changes in QT interval were highly correlated with changes in taurine content ($r^2 = 0.92$, $p < 0.001$), but not significantly with changes in GES content ($r^2 = 0.49$, $p < 0.08$). This is consistent with an important relationship between QT, ventricular repolarization, and cardiac taurine levels, rather than GES content. A recent study by Mozaffari et al. (20) has also indicated similar effects on rat cardiac metabolism of taurine depletion by treatment with either GES or B-alanine (another taurine transport antagonist) although GES accumulates in tissue while B-alanine does not.

Our studies suggest that prolongation of the QT interval, due to increased duration of the action potential, results from the depletion of taurine in the heart. These changes could arise from alterations in any number of ionic currents flowing during the action potential plateau. Several investigators have reported that taurine alters sarcolemmal calcium binding (36,37) and thus taurine could be involved in the regulation of intracellular calcium content. Changes in intracellular calcium can alter ionic permeabilities to potassium (68) or sodium (69), and can influence electrogenic transport mechanisms (70). Delays in repolarization could result
from changes in any of these calcium-dependent membrane properties.

There is evidence that depressed cardiac taurine levels are produced by myocardial ischemia and hypoxia or agents which produce infarction (97). Our study suggests that certain electrophysiologic abnormalities, such as the prolonged QT intervals associated with myocardial infarction (80), might be attributed, in part, to loss of myocardial taurine. It is possible that defects or alterations in taurine transport mechanisms are also involved in congenital long QT syndrome or drug induced QT prolongation. In normal animals, taurine levels of the heart are high, are closely regulated via transport mechanisms rather than metabolism, and resist manipulation by dietary means (50,61). The present study indicates that intracellular taurine may influence myocardial repolarization. The possibility that regulation of the action potential by taurine is associated with control of other aspects of myocardial function (such as contractile activity) remains to be evaluated in future studies.

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Chapter 3

The Effect of GES Treatment on the Hamster

Introduction

Taurine, a sulfur containing amino acid, is found in a multitude of animal species and in high concentrations in almost every tissue of the body (3). In the hamster, taurine has been shown to be particularly beneficial in the experimental therapy of the cardiomyopathic hamster (Bio 14.6) (24,28,29,71). Oral supplementation with taurine increases the heart taurine level (29), and has the effect of preventing the overload of Ca++ that occurs with age in this strain of hamster (24,72,73). This strain of hamster has proved to be a useful tool in the understanding of human disease (26). In other species extracellular taurine has been shown to be a positive inotrope and can antagonize the effect of low Ca++ solutions and Ca++ channel blockers (74,75,76). Its intracellular cytosolic concentration has been directly related to the contractile strength of heart tissue in the guinea pig (32) and is believed to be protective against Ca++ overload in the Ca++ paradox with the newborn chick (49). In the dog and guinea pig, taurine has been shown to be antiarrhythmic against hypokalemia and digitalis toxicity (33,35). Furthermore, taurine loss has been implicated to be a possible factor involved in post infarction arrhythmia (23,77). Tissue taurine levels have
been experimentally altered by the oral use of the taurine transport antagonist guanidinoethyl sulfonate (GES) (11) and B-alanine (20). In the rat heart, taurine concentrations have fallen by as much as 75% with the use of GES (11). Previous researchers have found a comparable fall in the taurine level of the hamster heart (29).

Based on these results and our findings in the rat, we speculate that there might be an effect on the electrophysiology of the hamster heart when it is depleted of taurine. A QT or APD prolongation induced by taurine depletion in the hamster would show that the changes seen in the rat were not specific to that genus.

**Methods**

**General**

Two different studies were performed on two different groups of hamsters (Golden Syrian strain, Charles River, St. Constant, Que.). The first study consisted of 8 control and 8 experimental animals of initial weight 156 ± 13g. The experimental group received 10 weeks of 1% GES treatment and thereafter received 1.6% GES in the drinking water together with 1% GES in their food. For the purposes of taking the electrocardiograms (ECGs), hamsters were first acclimatized to a plexiglas restraining device (78) for a period of 7 days and then the ECG was recorded with the use of this restrainer. ECGs and weights were
monitored at 0, 3, 6, 8, 13 and 15 weeks, with the Siemens Mingograf Model 81 ECG recorder. Paper speeds of 250 and 500 mm/sec allowed an accuracy of measurement of +/− 4 and +/− 2 msec respectively. At week 8 two hamsters from the control and experimental group were sacrificed to determine the heart levels of taurine and GES. The second study comprised of 6 hamsters of initial weight 145 +/− 12g, that were given 1% GES in their food from 0-9 weeks of the study and 3% B-alanine in their drinking water from weeks 6 to 9. Weights were monitored at weekly intervals while ECG's were taken at 0, 5 and 9 weeks of the study. Data is expressed as mean +/− standard deviation.

GES and Taurine

Taurine was purchased from ICN Chemicals, Montreal, Quebec. The taurine transport antagonist GES was synthesized according to the methods of Huxtable et al. (11). In brief GES was made from taurine, methyl-isothiopseudourea and ammonium hydroxide. Purity was tested using the GES (64) and taurine (63) assay procedures. Only GES of better than 99% with less than 0.1% contamination with taurine was considered to be adequate for experimental use. The hamsters were given GES in their drinking water as a 1.0% (60 mM) or 1.6% (90 mM) solution. GES was added to the food at a concentration of 1%. Care was taken to ensure that the GES was finely crushed and mixed with the food.
Biochemical Assays For GES and Taurine

Following sacrifice of the hamster, 50-100 mg pieces of the right and left ventricle were excised and weighed, placed in 85% ethanol for homogenization and then on ice for two hours. After this time the sample was centrifuged and the supernatant removed and dried under a nitrogen gas stream. The residue was then resuspended in 0.525 ml of double distilled water. A 0.5 ml aliquot was passed down a dual bed ion exchange column which contained 0.8 x 2 cm of Dowex 50, H+ form layered over 2 cm of Dowex 1, Cl⁻ form, Biorad. Taurine and GES were eluted (66) with 3 ml of distilled water and an aliquot was colorimetrically assayed for taurine (63) using the ninhydrin reagent and for GES (64) using biacetyl. These assays were performed in triplicate and the results compared to standards run in parallel. Heart biochemical data was expressed as umol/g wet weight of tissue.

Results

No hamster deaths occurred, however, the Study #1 hamsters lost a great deal of weight (over 50 grams compared to controls). Analysis of the ECGs from the hamsters in Study #1 and Study #2 showed that there were no significant changes in any of the ECG variables. Table# III shows the QT intervals of the experimental and control hamsters for Study #1. As can be seen there was no significant change in the QT interval between the control
and experimental group for the duration of the study. However there was a small increase in the QT interval in both groups over time which was not statistically significant. From Table# IV it can be seen that there was a slight and significant depletion of taurine in the hearts of the hamsters, however the degree of depletion (23%) was not of the amount that was seen with the rat (77%) for the same duration of treatment (see Table# II). Table# V shows the QT intervals for the hamsters in Study #2. From this table it can be seen that there is again a small increase in the QT interval for the experimental hamsters however it was not of the magnitude that was seen in the rat studies. Biochemical studies were not performed on these animals because of the lack of any changes in the ECG. There were no weight losses seen in this group of animals compared to the animals in Study #1 which lost weight when given GES in their drinking water, as seen with Figure# 8.

Discussion

The results presented here indicate that GES treatment has only a small effect on heart taurine levels in the hamster compared to the rat. Taurine depletion in the hamster apparently had no electrophysiological effect. However it is speculated that perhaps the taurine levels were not adequately reduced. According to the results presented in our paper concerning rats, the relationship between QT interval and heart taurine concentration is one in which there is approximately a 1 msec prolongation in
the QT interval for each umol/g wet weight of taurine that is lost. From the results of Table IV we would then predict a QT interval prolongation of about 4-5 msec. Such a small amount is beyond the resolution of our technique to detect. A possible reason for our inability to deplete the hamster could be its higher capability to synthesize taurine in its liver compared to the rat; however the synthetic capabilities of the hamster have not been examined. This would make the hamster more difficult to taurine deplete. As is typical of most desert rodents, the hamster does not ingest very much water and so as a result of this gets a smaller dose of GES that is added to the drinking water, and therefore receives an inadequate dose of GES to antagonize taurine uptake.

The experimental group in Study #1 lost a significant amount of weight (Figure 8). The rat studies of Baba et al. (79) showed that food deprivation caused an increase in the heart content of taurine. There is a possibility that the opposition to taurine depletion induced by weight loss, resulted in a failure of GES to deplete myocardial taurine stores.

It is known that in species such as the guinea pig that the presence of the enzymes amidinotransferase or amidinohydrolase can cause GES to be broken down to make taurine (52). This process would negate the taurine
depleting effect of GES and could possibly raise the tissue taurine levels. All or some of these factors might have contributed to our experimental failure to deplete the taurine in the hamster heart. However, according to McBroom and Welty (62), hamsters of the F1B strain were depleted to 62% of control with a 60 day administration of oral GES. A possible reason for this success might be due to a difference in strains. The F1B strain is the control breed for the cardiomyopathic strain (BIO 14.6) and as such it might share some of its membrane anomalies (24) and therefore some differences in the transport of taurine across the membrane might exist. The BIO 14.6 hamster is known to have a higher resting heart taurine level (40% more), and with GES treatment depletes to a relatively further extent than does the F1B strain (7% more). These points make it likely that the F1B strain might deplete more than the strain of hamsters that were used in our study. In Study #2 no change in the QT interval was found for the 9 weeks of the study (Table# V). The first 6 weeks of the study consisted of administration of 1% GES added to the food. These animals did not lose weight and therefore probably were eating the food and so getting the GES. However only a small insignificant increase in the QT interval was seen. Following the 6th week of experimentation a 3% drinking solution of B-alanine was added in an attempt to deplete the hamsters of their heart taurine. B-alanine has been shown to deplete heart taurine
in rats (20), so it was not unreasonable to suppose that the heart taurine from hamsters might also be depleted. As Table V shows there was no significant increase in the QT interval with the B-alanine treatment. Since we could not find a QT interval change no biochemical analysis of the hamster hearts was performed. Again the lack of any QT interval prolongation might be due to the heart taurine levels being unchanged because of the difference in hamster strains (F1B vs our random bred strain) in the heart. Therefore our results do not negate a genus dependent change in the QT interval, but rather suggest that lack of results are due to the inability to deplete the hamster heart of taurine.

**Conclusion**

This experiment did not successfully answer the question of a species specific effect on the heart QT interval when it is depleted of taurine. The taurine transport antagonist GES only had a small depleting effect on hamster heart stores of taurine. Possible explanations discussed include differences in strains (random bred vs. F1B strain) and the effect of weight loss. The other taurine transport antagonist, B-alanine, was also unsuccessful in prolonging the QT interval and so it is believed that the heart taurine contents of these hamsters also did not change sufficiently. Due to the lack of
biochemical analysis this hypothesis can not be substantiated.
Extrinsic vs. Intrinsic Effects

Extrinsic Effects

Increased or prolonged QT intervals are associated with a number of extrinsic causes such as: congenital, infectious, endocrine/metabolic, CNS disturbances, drug induced and vascular (80, 81). Many of the above causes are not associated with APD prolongation. The cause of the prolonged QT interval that was produced after the administration of GES might be due to a direct effect of the GES on the heart tissue itself or it might have resulted from a secondary factor such as one of those named above. From the above list, factors such as congenitally and infectious causes can be eliminated from our differential analysis list. Under the heading of endocrine/metabolic, various disturbances such as of electrolytes (both intra and extracellular), hypothermia and a thyroid disorder might be implicated.

Electrolytes

Electrolyte imbalances such as hypocalcemia, hypokalemia and hypomagnesemia are known to prolong the QT interval (82). However this induced state of prolongation would be expected to be reversed upon placing the heart
tissue in a tissue bath that contained normal ionic concentrations. In our experiments we found that the APD was prolonged when the tissue from treated rats was placed in a Tyrode solution containing physiological concentrations of magnesium, potassium and calcium. Therefore it is unlikely that the causative factor in the prolonged QT interval was due to an alteration of extracellular ions. It is possible that an alteration of the intracellular ionic concentration might have caused the prolongation. It is known that low concentrations of intracellular potassium can cause repolarization deficits and APD prolongation. However low intracellular K\(^+\) results in a depolarization of the resting membrane potential which we did not observe. Low intracellular levels of Ca\(^{++}\) have an effect on ionic currents and might have caused the prolonged QT interval (69,80). This effect would occur in the absence of any changes in the resting membrane potential. As well this effect would not be expected to be rapidly reversed upon superfusion in a tissue bath that contains normal extracellular ions due to the slow nature of the exchange between extracellular and intracellular Ca\(^{++}\) (47). In view of references cited previously implicating an interaction between taurine and Ca\(^{++}\), this hypothesis therefore is a reasonable one.
Thyroid State

Hypothyroidism is known to be a cause of QT interval prolongation (83). The rats in question however did not show any of the associated manifestations of hypothyroidism such as weight gain, decreased activity level, bradycardia, hair changes, diarrhea and growth defects. With the lack of these features in our rats, this mechanism of QT prolongation is therefore unlikely. Hypothermia is also known to be associated with prolonged QT intervals. This effect can be a primary one or related to hypothyroidism. When excised heart tissue is placed in a tissue bath, one would expect that the APD would no longer be prolonged since the temperature of the tissue bath is regulated at 37° C. Therefore hypothermia is not a likely mechanism.

CNS

Alterations of the autonomic innervation of the heart can cause bradycardia which is strongly associated with a prolonged QT interval. In the absence of changes in heart rate, as was seen in our experiments, it is unlikely that this was the causative factor in the prolongation of the QT interval. It has been shown that left stellate (cervicothoracic) ganglion stimulation in the dog causes a prolongation of the QT interval (84). The mechanism of this change is thought to involve increased dispersion in refractoriness. Since the ECG is the summed potential of the individual dipoles, any change (increase or decrease)
of these dipoles might be expected to cause an apparent lengthening of the QT interval without a lengthening of the APD. This effect could be caused by an alteration in the tone of the Autonomic Nervous System (ANS), an interaction between taurine and the ANS is plausible since there is a relationship between brain taurine and hypertension (85). As well taurine has been proposed as a neurotransmitter in the Central Nervous System (CNS) (86,87,88). Its depletion in the CNS might cause an alteration in the sympathetic tone accounting for the prolonged QT interval. This effect should be normalized upon taking the heart out and placing it in a tissue bath. However the action potential studies performed indicate that there was still a prolongation of the APD of an amount that could account for the QT interval prolongation. This evidence makes the theory of an alteration of the ANS unlikely as a cause for the prolonged QT interval.

Vascular

Vascular compromise causing ischemia is associated with QT lengthening (89) and ventricular APD prolongation (90). If taurine depletion somehow causes ischemia then QT interval prolongation might result. Ischemia is usually of abrupt onset, may or may not be reversible, associated with myocardial infarction, ECG disturbances such as arrhythmias and can be associated with sudden death (91,92,93,94). None of these effects were seen with the experimental group
of rats and furthermore the QT changes occurred gradually over a period of about 8 weeks and never spontaneously reversed. The positive correlation with taurine loss and QT interval increase make this mechanism even less plausible as an explanation.

Drug Induced

There are many drugs that are known to prolong the QT interval. From my ECG studies it is possible that perhaps the lengthening of the interval over 8 weeks was due to an extracellular action of GES on the myocardial membrane or on its extracellular facing proteins. If this were true then this would mean that the biochemical changes that were observed in the cells were merely an effect of the treatment and not the causative factor associated with the ECG changes. It might also be possible that while the in vitro experiment was being performed that there was enough release of endogenous GES from the myocardial cells to sustain an elevated extracellular GES concentration. Two arguments contest the proposal that the extracellular GES level was the causative factor. The first is that the studies of Huxtable et al. (11) show that the blood concentration of GES reaches a maximum by about the first week of treatment and thereafter it is maintained at its serum plateau concentration of about 0.5 mM. This plateau negates the theory that the QT interval prolongation was associated with plasma concentration changes in GES. A
caveat exists in this argument in that the GES/taurine ratio continues to increase as taurine levels fall (11). If GES exerts its effects through antagonizing the actions of taurine then one would expect that the magnitude of the effects of GES be related to this ratio. Therefore the in vivo effects that were seen might have been related to GES/taurine blood levels.

The second argument against an extracellular effect of GES is from the in vitro superfusion studies. In my studies high concentrations (up to 10 mM) of GES were used and no action potential changes were noted. This high concentration is many fold higher than the normal plasma concentration associated with treatment and would therefore be expected to produce a prolongation of the APD. Since no APD changes were seen it is likely that GES has little or no extracellular effect.

Intrinsic Effect

GES Accumulation vs. Taurine Depletion

By eliminating secondary factors that might cause APD changes and QT prolongation we are left with the hypothesis that GES treatment has a direct effect on the myocardium. This direct effect may be due to either the depletion of taurine or the accumulation of GES in the heart or both. The strong correlation between taurine depletion and QT
changes and the lack of significant correlation between GES accumulation and any QT change, strongly suggest that it is the depletion of taurine which is responsible for the QT interval changes. However the possibility of a delayed effect following GES accumulation that results in the QT interval prolongation cannot be ruled out by these experiments. Experiments by Mozaffari et al (20), using GES or B-alanine to deplete myocardial stores of taurine, established that there was a decrease in cardiac work in taurine-depleted hearts. This deficit was present in both the GES and B-alanine treated rats. However, since B-alanine is metabolized it does not accumulate in the myocardium (95) and therefore can not be directly responsible for the observed effects. Because of the inter-relationship between cardiac work and cardiac electrophysiology their research may be relevant to mine. It also gives greater support to the conclusion that the important factor responsible for our observed electrophysiological effects was depletion of taurine and not accumulation of GES.

Possible Mechanisms

What then is the mechanism of action potential prolongation caused by taurine depletion? Chovan et al. (36) and others have shown (37) that taurine can increase the amount of Ca$^{++}$ that is bound to the sarcolemma. The
sarcolemma-bound Ca++ pool is strongly related to such effects as triggered Ca++ release for electrical/mechanical coupling (29,96), regulating ionic gating mechanisms (68,69) and maintaining structural integrity (46). We postulate that intracellular taurine through its action on facilitating Ca++ binding to the sarcolemma could have the following effects: positive inotropy, antiarrhythmic action, regulation of APD, and modulation of Ca++ pools. This proposed mode of action suggests the following. The depletion of intracellular taurine would be expected to result in a state in which sarcolemma pool of Ca++ is decreased which would result in a decrease in contractility, a predisposition to arrhythmias, altered APD, an increase in the lability of the membrane and intracellular abnormalities in Ca++ levels. Some of these effects have been previously described by other researchers, consistent with the proposed mode of taurine action (17,24,31,32,35,68,71,74).

The model proposed here involves the action of taurine on Ca++ binding to the sarcolemma. Ca++ binding alters surface field charges and thereby ionic gating. Isenberg (40) in the sheep, and Josephson (39) in the rat, have shown that Ca++ has the ability of turning off the slow inward current (I_{Ca}). Due to the rapid heart rate that is encountered in the rat heart, sometimes in excess of 350 beats/ min, only a short APD is permitted. This demands a
mechanism by which membrane currents can be rapidly turned on and off. The \( Ca^{++} \) current is believed to be the predominant factor in keeping the APD short (39). The effect of taurine depletion would be to delay the turning off of the \( I_{Ca} \) and therefore maintain this depolarizing current and prolong the APD.

Future Experiments

In order to establish a mechanism for the consequences of taurine depletion on the rat heart certain experiments should be performed. Studies should be performed in which taurine is depleted with the use of B-alanine, this might help to rule out any effect produced by accumulation of GES in the myocardium. If hearts of taurine-depleted animals have lower levels of \( Ca^{++} \) bound to the sarcolemma then a \( Ca^{++} \) assay should be done to establish the total cellular \( Ca^{++} \) content. Intracellular organelles, such as the sarcoplasmic reticulum, could be separated with centrifugation and their \( Ca^{++} \) content could also be measured. By using dyes which fluoresce in the presence of \( Ca^{++} \) an indication of the cytoplasmic level of that ion can be made (97). It would be predicted that during taurine deficiency the total \( Ca^{++} \) level would be slightly lower as would the \( Ca^{++} \) bound to the sarcolemma. Patch clamp studies could also be performed on isolated \( Ca^{++} \) channels in an attempt to see if the channel itself is altered or rather if the kinetics (i.e. voltage dependence) of the
channel have been altered by a modified charge field. This type of study should be performed in the presence and in the absence of taurine. And finally, a voltage clamp study could be done on isolated cells or cellular aggregates to see if there is an alteration of the slow inward current in heart cells of taurine-depleted rats.

Significance of Findings

Long QT syndromes in man have been associated with such causes as dietary deficiencies and fad diets (98). Following myocardial infarction there is a 50% loss of taurine (99). If the loss of taurine is substantial it may be responsible for sufficient APD prolongation, in ischemic zones of the myocardium, which may contribute to fatal reentrant arrhythmias (100,101). It is known that there is a spatial variation of QT intervals in individuals following an acute myocardial infarction (102). Patients with highly prolonged QT intervals following myocardial infarction have been shown to have an increased mortality than those who have lesser degrees of prolongation (103).

Cats fed a diet low in taurine have developed dilated cardiomyopathies (104) and diminished left ventricular performance, which is reversed when the animals are taurine supplemented. Heart failure patients given taurine supplementation have been demonstrated to achieve better
scores on the NYHA evaluation for congestive heart failure (105,106). Studies cited throughout this thesis involving taurine indicate an importance of taurine in the myocardium not only in species such as the rat, cardiomyopathic hamster, and guinea pig, but more clinically relevant also in humans. From the above it is possible that the loss of intracellular taurine not only can be a cause of fatal arrhythmias but also may decrease ventricular performance following its loss after a myocardial infarction. A better understanding of the physiological significance of taurine would lead to better therapies for heart ailments and a more thorough understanding of factors which govern the processes regulating the action potential in the heart.
Summary of Findings

1. ** Treatment with 1.6% or 1.0% GES produces similar effects on QT interval.

2. Treatment causes taurine depletion in the heart.

3. ** GES treatment causes prolongation of the QT interval.

4. ** The increases in APD can account for the QT prolongation.

5. ** The time course of prolongation of the QT interval was not correlated with that of GES accumulation.

6. ** Exogenously applied taurine and GES were without effect on ventricular muscle action potentials from control and treated hearts.

7. ** GES treatment in hamsters did not deplete myocardial taurine to the same degree as rats nor did it significantly prolong the QT interval.

** Denotes original work
Figure #1

- - - - Continued
\(\Delta - \Delta\) Reversed
\(\circ - \circ\) Control

QT Interval (ms)

Time (weeks)

-2 0 2 4 6 8 10 12 14 16 18 20
Figure 3

Reversed

Continued

Control

A

B

100 ms

0 mV

-80
Figure #5

- Control
- Treated

Taurine (umol/g wet wt.)

Time (weeks)
Figure #8

- ▲ ▲ control
- ● ● treated

Weight (grams)

Time (weeks)

0 3 6 8 10 11 12 13 14 15

0 75 100 125 150 175 200
<table>
<thead>
<tr>
<th>ACTION POTENTIAL CHARACTERISTICS AND BIOCHEMISTRY OF RAT VENTRICAL CELLS AFTER 20 WEEKS OF 1.6% GES TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
</tr>
<tr>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Number of cells tested</td>
</tr>
<tr>
<td>Resting potential (mV)</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
</tr>
<tr>
<td>APD75 (ms)</td>
</tr>
<tr>
<td>APD95 (ms)</td>
</tr>
<tr>
<td>Vmax (V/s)</td>
</tr>
<tr>
<td>Samples tested</td>
</tr>
<tr>
<td>Taurine (umol/g wet wt)</td>
</tr>
<tr>
<td>GES (umol/g wet wt)</td>
</tr>
<tr>
<td>DNA (mg/g wet wt)</td>
</tr>
</tbody>
</table>

Data shown are mean +/- S.D. Significant differences from control by ANOVA are indicated by * p < .05, ** p < .01, *** p < .001. Groups and abbreviations as defined in Methods. For biochemistry, 2 samples per animal were independently analysed in triplicate, from 10 control, 4 "continued", and 4 "reversed" rats.
TABLE II

Action Potential Characteristics During Superfusion with GES- or Taurine-containing Solutions.

<table>
<thead>
<tr>
<th>Solution</th>
<th>N*</th>
<th>AP025</th>
<th>AP045</th>
<th>Vmax</th>
<th>MAP</th>
<th>APA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93</td>
<td>27 +/- 5</td>
<td>54 +/- 7</td>
<td>248 +/- 60</td>
<td>-83 +/- 4</td>
<td>105 +/- 5</td>
</tr>
<tr>
<td>Taurine, 0.2 mM</td>
<td>61</td>
<td>20 +/- 6</td>
<td>55 +/- 8</td>
<td>239 +/- 45</td>
<td>-82 +/- 3</td>
<td>104 +/- 5</td>
</tr>
<tr>
<td>Control</td>
<td>97</td>
<td>30 +/- 9</td>
<td>62 +/- 10</td>
<td>235 +/- 59</td>
<td>-82 +/- 3</td>
<td>103 +/- 6</td>
</tr>
<tr>
<td>GES, 0.2 mM</td>
<td>47</td>
<td>27 +/- 7</td>
<td>58 +/- 8</td>
<td>247 +/- 62</td>
<td>-81 +/- 3</td>
<td>102 +/- 4</td>
</tr>
<tr>
<td>Control</td>
<td>162</td>
<td>29 +/- 8</td>
<td>54 +/- 9</td>
<td>235 +/- 60</td>
<td>-81 +/- 4</td>
<td>105 +/- 5</td>
</tr>
<tr>
<td>Taurine, 2 mM</td>
<td>99</td>
<td>28 +/- 8</td>
<td>55 +/- 7</td>
<td>224 +/- 51</td>
<td>-81 +/- 3</td>
<td>105 +/- 5</td>
</tr>
<tr>
<td>Control</td>
<td>111</td>
<td>30 +/- 7</td>
<td>60 +/- 9</td>
<td>264 +/- 64</td>
<td>-82 +/- 3</td>
<td>104 +/- 6</td>
</tr>
<tr>
<td>GES, 2 mM</td>
<td>70</td>
<td>31 +/- 4</td>
<td>59 +/- 6</td>
<td>269 +/- 69</td>
<td>-83 +/- 4</td>
<td>102 +/- 6</td>
</tr>
<tr>
<td>Control</td>
<td>171</td>
<td>30 +/- 10</td>
<td>68 +/- 12</td>
<td>257 +/- 67</td>
<td>-83 +/- 3</td>
<td>103 +/- 6</td>
</tr>
<tr>
<td>Taurine, 10 mM</td>
<td>77</td>
<td>30 +/- 7</td>
<td>66 +/- 8</td>
<td>270 +/- 62</td>
<td>-83 +/- 3</td>
<td>106 +/- 6</td>
</tr>
<tr>
<td>Control</td>
<td>151</td>
<td>37 +/- 12</td>
<td>74 +/- 13</td>
<td>245 +/- 78</td>
<td>-83 +/- 3</td>
<td>106 +/- 6</td>
</tr>
<tr>
<td>GES, 10 mM</td>
<td>91</td>
<td>38 +/- 11</td>
<td>76 +/- 11</td>
<td>224 +/- 74</td>
<td>-83 +/- 3</td>
<td>106 +/- 6</td>
</tr>
</tbody>
</table>

Results obtained using hearts from untreated rats:

Results from hearts of rats treated 14 weeks with GES:

<table>
<thead>
<tr>
<th>Solution</th>
<th>N*</th>
<th>AP025</th>
<th>AP045</th>
<th>Vmax</th>
<th>MAP</th>
<th>APA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125</td>
<td>47 +/- 13</td>
<td>83 +/- 17</td>
<td>314 +/- 153</td>
<td>-81 +/- 3</td>
<td>102 +/- 6</td>
</tr>
<tr>
<td>Taurine, 10 mM</td>
<td>45</td>
<td>50 +/- 9</td>
<td>84 +/- 11</td>
<td>338 +/- 167</td>
<td>-81 +/- 3</td>
<td>103 +/- 6</td>
</tr>
</tbody>
</table>

N* = number of impalements under each condition. MAP = membrane action potential at the onset of phase 0, in mV; APA = total action potential amplitude in mV. Other terms defined in Methods. Control values were obtained prior to taurine or GES exposure, and then 30 minutes after washout. One hour of GES or taurine superfusion was interposed with impalements of approximately 10 cells obtained under each condition from each preparation.
TABLE III

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 13</th>
<th>Week 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86 +/- 4</td>
<td>85 +/- 5</td>
<td>86 +/- 3</td>
<td>86 +/- 3</td>
<td>94 +/- 7</td>
<td>92 +/- 8</td>
</tr>
<tr>
<td>Experimental</td>
<td>87 +/- 3</td>
<td>86 +/- 8</td>
<td>86 +/- 8</td>
<td>83 +/- 5</td>
<td>90 +/- 7</td>
<td>89 +/- 8</td>
</tr>
</tbody>
</table>

QT intervals (msec) for Control and GES-treated hamsters. Experimental animals were treated with 1% GES in the drinking water for the first 10 weeks, and thereafter received 1.6% GES in the water together with 1% GES in the food. For the first 8 weeks of study 8 animals in each group were tested and after week 8 this was decreased to 6 animals per group. There were no significant differences in the QT intervals within or between the groups.

TABLE IV

<table>
<thead>
<tr>
<th></th>
<th>Taurine (umol/g wet wt)</th>
<th>GES (umol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.2 +/- 2.67</td>
<td>0.24 +/- 0.21</td>
</tr>
<tr>
<td>Experimental</td>
<td>17.8 +/- 4.1 ***</td>
<td>3.76 +/- 1.29 ***</td>
</tr>
</tbody>
</table>

Heart biochemistry for hamsters treated with GES for 15 weeks, as described above. 6 hamsters were used to determine the taurine and GES levels for the two groups. Significant differences were found *** p < 0.001 for taurine and GES levels between the experimental and control groups. Biochemical assays were performed in triplicate as described in Methods.
Table V

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 5</th>
<th>Week 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>86 +/- 3.9</td>
<td>85 +/- 3.0</td>
<td>90 +/- 5.0</td>
</tr>
<tr>
<td>Experimental</td>
<td>84 +/- 5.8</td>
<td>92 +/- 3.8</td>
<td>89 +/- 3.3</td>
</tr>
<tr>
<td>Not Sig.</td>
<td>Not Sig.</td>
<td>Not Sig.</td>
<td>Not Sig.</td>
</tr>
</tbody>
</table>

Study# 2 QT intervals for experimental and a weight matched control group. Data is expressed as mean +/- S.D. Six hamsters were used for the experimental group while 8 were used for the control. Experimental hamsters were given 1% GES in their food from 0-9 weeks and from the sixth week until the ninth were given in addition to the GES, 3% B-alanine in their water. No significant QT changes were observed in this study, nor were any weight differences.
Legends used for Figures 1-8

Figure 1  Effects of GES and taurine on the QT interval of the rat. Data shown are group means +/- SD. Open circles: untreated weight-matched controls (N = 6). Filled circles: rats treated with 1.6% GES (N = 9 to week 5). After week 5, 4 rats continued on 1.6% GES, while 5 rats allocated to the "reversed" group (filled triangles) received 1.2% taurine in addition to the GES. Their GES was discontinued after the week 8 recording session. GES treatment significantly prolonged the QT intervals; this effect was reversed by treatment with taurine and discontinuing GES.

Figure 2  Effects of GES and taurine on the rat ECG. ECG from the same animal: A, at week 0; B, after 5 weeks of 1.6% GES treatment; C, after 6 subsequent weeks of "reversal" treatment with taurine. RR interval showed no changes. GES prolonged the QT interval (B) and taurine reversed this increase (C). Time scale marker below C (100 ms) applies to all tracings.

Figure 3  Intracellular action potentials from rat ventricular muscle cells from one animal in each of the 3 treatment groups as defined in methods. Superimposition of action potentials from control and continued (A) and control and reversed (B). Treatment with GES is associated with slower repolarization and this effect is reversible.
Group data appears in Table I.

**Figure #4** Effects of 1% GES treatment on the QT interval of the rat ECG. Data shown are group mean $\pm$ S.D. Open circles: control, closed circles: treated. The abscissa is the same for Figures #4-7 and is week of observation or treatment. Significant treatment effects were observed after 1 week for QT interval.

**Figure #5** Effects of 1% GES treatment on ventricular taurine content. Data shown are group mean $\pm$ S.D. Open circles: control, closed circles: treated. The abscissa is the same for Figures #4-7 and is week of observation or treatment. Significant treatment effects were observed after 1 week for GES content.

**Figure #6** Effects of 1% GES treatment on ventricular GES content. Data shown are group mean $\pm$ S.D. Open circles: control, closed circles: treated. The abscissa is the same for Figures #2-5 and is week of observation or treatment. Significant treatment effects were observed after 1 week for GES content.

**Figure #7** This figure shows the difference ($\triangle$) between control and treatment values for QT interval (ms) and taurine or GES content (umol/g wet wt.) over time. Data shown are group mean $\pm$ S.D. The abscissa is the same for
Figures #4-7 and is week of observation or treatment. A QT is highly correlated with A taurine but not with A GES.

Figure# 8 Study# 1 hamster weights in grams (mean +/- S.D.) plotted against observation or treatment week. Open circles: control, closed circles: treated hamsters. There were eight hamsters used in each group up to the 8th week and thereafter six animals per group were used for the determination of the graphed data. Significant differences between the hamster weights were seen after week 3 (p < 0.05) and significance increased until week 12 (p < 0.001), following this the p value remained at p < 0.001.
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