INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.
AZARI, JAMSHID

THE MECHANISM OF TAURINE UPTAKE AND ITS ALTERATION IN CARDIOMYOPATHY

The University of Arizona

Ph.D. 1979

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106 18 Bedford Row, London WC1R 4EJ, England
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs ✓
2. Colored illustrations
3. Photographs with dark background
4. Illustrations are poor copy
5. Print shows through as there is text on both sides of page
6. Indistinct, broken or small print on several pages throughout
7. Tightly bound copy with print lost in spine
8. Computer printout pages with indistinct print
9. Page(s) lacking when material received, and not available from school or author
10. Page(s) seem to be missing in numbering only as text follows
11. Poor carbon copy
12. Not original copy, several pages with blurred type
13. Appendix pages are poor copy
14. Original copy with light type
15. Curling and wrinkled pages
16. Other
THE MECHANISM OF TAURINE UPTAKE AND ITS ALTERATION IN CARDIOMYOPATHY

by

Jamshid Azari

A Dissertation Submitted to the Faculty of the Department of Pharmacology through the COMMITTEE ON PHARMACOLOGY (GRADUATE) In Partial Fulfillment of the Requirements For the Degree of DOCTOR OF PHILOSOPHY In the Graduate College THE UNIVERSITY OF ARIZONA

1979
I hereby recommend that this dissertation prepared under my direction
by Jamshid Azari
entitled THE MECHANISM OF TAURINE UPTAKE AND ITS ALTERATION IN
CARDIOMYOPATHY
be accepted as fulfilling the dissertation requirement for the Degree
of Doctor of Philosophy.

Dissertation/Director  Date

As members of the Final Examination Committee, we certify that we have
read this dissertation and agree that it may be presented for final
defense.

Date

Date

Date

Date

Final approval and acceptance of this dissertation is contingent on the
candidate's adequate performance and defense thereof at the final oral
examination.
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: [Signature]
ACKNOWLEDGMENTS

This work is dedicated to my wife Gretchen for her patience and understanding, to my daughter Leila who has added a new dimension to my life, to my friend and colleague Douglas Eisenstein for his valuable friendship, and to my dissertation director Dr. Ryan J. Huxtable for his guidance and support during this study.

I would like to thank the members of my graduate advisory committee: Drs. Eldon Braun, Thomas Burks, William Dantzler, and John Palmer, for their critical advice and constructive suggestions.
# TABLE OF CONTENTS

| LIST OF TABLES | vii |
| LIST OF ILLUSTRATIONS | ix |
| ABSTRACT | x |

## CHAPTER

1. INTRODUCTION ........................................ 1
   - General Information on Taurine ................. 1
   - Tissue Taurine Content ......................... 5
     - Taurine Metabolism ......................... 5
     - Taurine Biosynthesis ...................... 6
     - Taurine Transport ......................... 10
       - Modification of Cardiac Taurine Concentration .................. 12
   - Physiological Role of Taurine ............... 13
     - Role of Taurine in CNS .................. 13
     - Cardiovascular Role of Taurine .......... 18
     - Miscellaneous Role of Taurine .......... 25
   - Blockade of Taurine Transport ............. 29
   - Amrinone .................................. 30
   - Cardiomyopathy and Taurine ............... 31
   - Rationale and Objectives ................ 35

2. METHODS ........................................... 39
   - Animals .................................. 39
   - Heart Perfusion .......................... 39
   - Reserpine Treatment ...................... 40
   - Ionophore Study .......................... 40
   - Verapamil and Glucagon Studies .......... 40
   - Recording of Heart Activity ............. 41
   - Influx Rate of Radiolabeled Compounds .... 41
   - Liquid Scintillation Counting .......... 41
   - Study of Taurine Analogs ................. 42
   - Membrane Preparation ..................... 42
   - Calcium Binding Study .................... 42
   - Protein Determination .................... 43
   - Effect of Amrinone on Rate of Taurine Influx .......... 43
   - Effect of Amrinone on Contractility and Heart Rate .......... 44
   - Influx Rate of Radiolabeled Calcium .... 44
   - Efflux of 45Calcium ....................... 44
TABLE OF CONTENTS--Continued

<table>
<thead>
<tr>
<th>Tissue Preparation for Cyclic AMP Determination</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclic AMP Determination</td>
<td>45</td>
</tr>
<tr>
<td>Spectral Analysis</td>
<td>46</td>
</tr>
<tr>
<td>Taurine Analysis</td>
<td>46</td>
</tr>
<tr>
<td>Receptor Binding Assay</td>
<td>47</td>
</tr>
<tr>
<td>Rate of Taurine Influx in Hamsters</td>
<td>48</td>
</tr>
<tr>
<td>Dietary Taurine Treatment</td>
<td>48</td>
</tr>
<tr>
<td>Sample Preparation for Histology</td>
<td>48</td>
</tr>
<tr>
<td>Lesion Evaluation</td>
<td>49</td>
</tr>
<tr>
<td>Materials</td>
<td>49</td>
</tr>
<tr>
<td>Calculations</td>
<td>49</td>
</tr>
</tbody>
</table>

3. RESULTS ................................................. 50

Mechanism of the Adrenergic Stimulation of Taurine Influx in the Heart .................................................. 50
Effect of Glucagon on Taurine Influx .......................... 50
Effect of Ionophores on Taurine Influx ........................... 51
Involvement of Calcium and Inotropy in Taurine Influx ................................................................. 52
Specificity of the Taurine Transporting System in the Heart ................................................................. 54
Transport of β-Guanidoethyl Sulfonate .......................... 56
Effect of Isoproterenol on β-Guanidoethyl Sulfonate Influx ................................................................. 60
Positive Inotropic Effect of Taurine and β-Guanidoethyl Sulfonate ............................................................. 60
Effect of Taurine on Sarcolemmal Calcium Binding .................. 63
Effect of Amrinone on Cyclic AMP Levels ........................... 63
Effect of Amrinone on Contractility and Heart Rate ............... 66
Effect of Amrinone on Rate of Taurine Influx ....................... 69
Calcium Efflux from Amrinone Treated Hearts ...................... 69
Localization of Calcium Overload .................................. 71
Regional Concentration of Taurine ................................. 74
Effect of Advancing Cardiomyopathy on Cation Concentrations .......... 74
β-Receptor Binding in the Heart .................................... 78
Effect of Taurine on Cation Concentrations .......................... 80
Effect of Taurine on Controlling Cardiac Lesions ..................... 82
Effect of Taurine on Cation Concentrations in Advanced Stages of Cardiomyopathy ........................................ 82
Rate of Taurine Influx ............................................ 89
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>DISCUSSION</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Taurine in Heart</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Amrinone</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>Cardiomyopathy and Taurine</td>
<td>108</td>
</tr>
<tr>
<td>5.</td>
<td>SUMMARY</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>LIST OF REFERENCES</td>
<td>116</td>
</tr>
</tbody>
</table>
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Effect of Pacing on Taurine Influx</td>
<td>36</td>
</tr>
<tr>
<td>2. Effect of Various Agents on the Rate of Taurine Influx</td>
<td>37</td>
</tr>
<tr>
<td>3. Effect of Glucagon on Taurine Influx Rate in the Heart</td>
<td>51</td>
</tr>
<tr>
<td>4. Effect of Ionophores on Taurine Influx</td>
<td>53</td>
</tr>
<tr>
<td>5. Effect of Verapamil, Isoproterenol, and Verapamil Plus Isoproterenol on Taurine Influx</td>
<td>55</td>
</tr>
<tr>
<td>6. Effect of Co-Perfused Analogos on Taurine Influx in the Heart</td>
<td>58</td>
</tr>
<tr>
<td>7. Effect of Isoproterenol on β-Guanidoethyl Sulfonate Influx</td>
<td>61</td>
</tr>
<tr>
<td>8. Effect of Taurine on Calcium Binding to Sarcolemma</td>
<td>65</td>
</tr>
<tr>
<td>9. Effect of Amrinone on Cyclic AMP Levels</td>
<td>67</td>
</tr>
<tr>
<td>10. Effect of Amrinone on Contractility and Heart Rate</td>
<td>68</td>
</tr>
<tr>
<td>11. Effect of Amrinone on Rate of Taurine Influx in Rat Hearts</td>
<td>70</td>
</tr>
<tr>
<td>12. Effect of Amrinone on Rate of Taurine Influx in Guinea Pig Hearts</td>
<td>70</td>
</tr>
<tr>
<td>13. Calcium Efflux from Amrinone Treated Guinea Pig Hearts</td>
<td>73</td>
</tr>
<tr>
<td>14. Effect of Amrinone on Residual Calcium in Guinea Pig Hearts</td>
<td>73</td>
</tr>
<tr>
<td>15. Regional Cation Concentrations in Hearts from 30-40 Day Old Hamsters</td>
<td>75</td>
</tr>
<tr>
<td>16. Regional Concentration of Taurine in 30-40 Day Old Hamsters</td>
<td>76</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>17.</td>
<td>Regional Cation Concentrations in Hearts from 60-70 Day Old Hamsters</td>
</tr>
<tr>
<td>18.</td>
<td>β-Adrenergic Receptor Binding in Hearts from 90 Day Old Hamsters</td>
</tr>
<tr>
<td>19.</td>
<td>Effect of Taurine on Cation Concentrations</td>
</tr>
<tr>
<td>20.</td>
<td>Effect of Taurine on Calcium and Taurine Concentration in the Heart</td>
</tr>
<tr>
<td>21.</td>
<td>Effect of Taurine on Controlling Cardiac Lesions</td>
</tr>
<tr>
<td>22.</td>
<td>Effect of Taurine on Cation Concentrations in Advanced Stages of Cardiomyopathy</td>
</tr>
<tr>
<td>23.</td>
<td>Rate of Taurine Influx</td>
</tr>
</tbody>
</table>
## LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Pathway of Taurine Biosynthesis</td>
<td>8</td>
</tr>
<tr>
<td>2. Hypotaurine Biosynthesis via Cysteamine</td>
<td>9</td>
</tr>
<tr>
<td>3. Taurine Analogs</td>
<td>57</td>
</tr>
<tr>
<td>4. Guanidoethyl sulfonate Lineweaver-Burk Plot</td>
<td>59</td>
</tr>
<tr>
<td>5. Inotropic Effect of Taurine and Guanidoethyl sulfonate (GES) in Rat Hearts</td>
<td>62</td>
</tr>
<tr>
<td>6. Effect of Taurine on Calcium Binding to Sarcolemma</td>
<td>64</td>
</tr>
<tr>
<td>7. Calcium Efflux from Amrinone-Treated Guinea Pig Hearts</td>
<td>72</td>
</tr>
<tr>
<td>8. The Heart from 60-70 Day Old Cardiomyopathic Hamster without Taurine</td>
<td>84</td>
</tr>
<tr>
<td>9. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with Grade of 0 Assigned to the Severity of Necrosis in a Single Blind Study</td>
<td>85</td>
</tr>
<tr>
<td>10. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with Grade of 1+ Assigned to the Severity of Necrosis in a Single Blind Study</td>
<td>86</td>
</tr>
<tr>
<td>11. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with a Grade of 2+ Assigned to the Severity of Necrosis in a Single Blind Study</td>
<td>87</td>
</tr>
<tr>
<td>12. Sodium Calcium Exchange Mechanism</td>
<td>100</td>
</tr>
</tbody>
</table>
ABSTRACT

This work elucidates a number of points about taurine including: the mechanism of isoproterenol stimulated transport of taurine into the heart, the structure activity relationship at the transport site, taurine's role in modulating calcium in the heart, and whether taurine is capable of controlling calcium overload in the cardiomyopathic hamsters. It establishes a possible mechanism for positive inotropy produced by amrinone, and amrinone's effect on taurine transport.

Taurine influx is mediated by a high affinity, saturable transport system. This transport is responsible for maintaining a constant concentration of taurine which has been shown to be difficult to change under various experimental conditions. However, isoproterenol has been shown to stimulate the influx rate of taurine in a dose dependent process in the rat heart. Since isoproterenol stimulation leads to manifold changes in the heart, it is not clear which of these changes are responsible for the increase in the influx rate of taurine. The following experiments were carried out to attain my goals.

Glucagon, which increases cyclic AMP levels in rat heart by stimulating adenylate cyclase, increased contractility and the rate of taurine influx. Thus rate of taurine influx is not unique to the β-adrenergic stimulation of the heart.

A number of ionophores capable of inducing passage of specific ions were tested for their effect on taurine influx. The ionophores tested (valinomycin for K⁺ ion, A23187 for Ca²⁺, and monensin for Na⁺)
had no significant effect on the rate of taurine influx, indicating influx of taurine is independent of ionic fluxes.

Verapamil blocks calcium influx to the heart. In rat hearts verapamil decreased contractile force without significant effect on taurine influx. Co-perfusion of rat hearts with isoproterenol and verapamil caused blockade of the calcium movement across the membrane concomitant with increased cyclic AMP levels. In hearts co-perfused with verapamil, taurine influx increased to the same level attained with isoproterenol. Thus increased influx of taurine is the result of increased cyclic AMP alone.

A number of taurine analogs were tested to evaluate structure-activity requirements of the taurine transporting system in the heart. Structural requirements at the taurine transport site are precise and only those analogs that closely resemble taurine structurally are capable of inhibiting taurine transport. Both taurine and its analog, β-guanidoethyl sulfonate, in hearts perfused with Kreb-Henseleit buffer containing 0.5 mM calcium and 0.1 mM taurine or β-guanidoethyl sulfonate, produced a biphasic effect on contractility: an initial decrease followed by an increase. The biphasic effect may be due to taurine decreasing calcium binding to the sarcolemma.

Amrinone is a new cardiotonic agent. In guinea pig hearts, this drug had no effect on the levels of cyclic AMP. However, contractility increased by 29-33% when doses of 10-20 μg/ml were used. Amrinone stimulated the rate of radiolabeled calcium (45 Ca) binding and/or influx without affecting the efflux. Thus inotropy by amrinone is the result of regulation of calcium flux. Amrinone had no effect on the rate of
taurine influx in the rat hearts, but stimulated the influx in guinea pig hearts.

Cardiac lesions in Syrian hamsters that develop as a result of calcium overload are localized mainly in the left ventricles. This overload is not due to alterations in β-receptor density or affinity, and becomes more pronounced with advancing age. In 60-70 day old hamsters, there is a 14-fold increase in the calcium levels in the left ventricles, while other ions such as magnesium or iron, and taurine are not altered. Placing cardiomyopathic animals on 0.1 M solution of taurine, calcium overload decreased and cardiac necrosis was less severe.
CHAPTER 1

INTRODUCTION

In much of the investigative work cited in this section, investigators have used rather high concentrations of taurine to produce their effects. Such high concentrations, although within pharmacological limits, are not within acceptable physiological limits. It is rather difficult to draw any physiological conclusions from such studies.

General Information on Taurine

Taurine (2-aminoethanesulfonic acid) is a sulfur-containing beta-amino acid, which does not incorporate into proteins, but is found as a free amino acid in high concentrations in nerve, muscle, brain, heart, and other tissues (Jacobsen and Smith, 1968). It is considered a β-amino acid because of the position of the carbon atom to which the amino (-NH$_2$) group is attached. At physiological pH, taurine exists largely as a zwitterion.

Taurine was first reported in living material by Tiedmann and Gmelin (1827). They isolated a compound from ox bile which they named bile-asparagine. Twenty years passed before it was shown that the compound contained sulfur. Evidence of the presence of taurine in tissues from a number of invertebrates (Cloetta, 1855; Fredericq, 1878; Letellier, 1887) and vertebrates (Krukenberg, 1881; Limbricht, 1865; Valenciennes and Fremy, 1857; Verdeil, 1851) gradually accumulated over the next decades. Krukenberg (1881) and von Furth (1903) toward the
end of the nineteenth century, in an extensive survey, found that taurine had a wide distribution in animals. Succeeding reports by Astrup, Carlstrom, and Stage (1952), Awapara (1956), and Roberts et al. (1957) confirmed the abundance of taurine in animal tissues.

Taurine appears to have a limited distribution in plants. Taurine or taurine derivatives are found mainly in Rhodophyceae, a sub-class of algae of predominantly marine or sublittoral ecology (Ericson and Carlson, 1954; Lindberg, 1955). No taurine is found in fresh water algae (Ericson and Carlson, 1954; Fowden, 1951). There are only a few genera of fungi containing taurine (Fuerst and Wagner, 1957; Kelly and Weed, 1965). In higher plants, taurine has been identified in pollen from five dicotyledonous genera (Marquardt and Vogg, 1952). No other reports on the presence of taurine in plants have been reported. Karas et al. (1964), assuming an absence of taurine in plants, have suggested using taurine as a chromatographic reference in analysis of plant extracts.

In mammals, least is known about taurine concentrations in human tissues. Taurine concentrations are higher in the fetal human brain than in brain tissue from children and adults (Okumura, Otuski, and Kameyama, 1960). An analysis of different sections of the human brain by the same authors has shown the highest concentrations of taurine in the frontal and occipital lobes (Okumura et al., 1960). The levels of taurine in the brain specimens from patients with brain tumors, tuberous sclerosis (Okumura et al., 1960), Lowe's syndrome, or unspecified mental retardation (Zachmann, Tocci, and Nyhan, 1966) do not differ significantly from the levels in normal human brains. However, in those
disorders in which there is a coupling between the central nervous system and motor activity, there appears to be a change in taurine concentration. Huxtable, Azari, et al. (1979) have reported an increased level of taurine in all the regions they studied from the brains of two patients with Friedreich's ataxia. Furthermore, van Gelder, Sherwin, and Rasmussen (1972) have reported that biopsied sites of maximal seizure activity in epileptic patients are characterized by low concentrations of taurine and glutamic and aspartic acid.

Taurine content is also higher in fetal human livers than in adult livers (Ryan and Carver, 1966). Taurine levels in adult human liver are intermediate between the high levels in carnivorous animals such as dogs and cats, and the low or absent levels in herbivorous animals such as rabbits and guinea pigs (Jacobsen and Smith, 1968). The concentration of taurine from human milk is about 1.5-fold higher than the average plasma concentration of taurine in others (Armstrong and Yates, 1963). The first quantitative identification of taurine in human cerebrospinal fluid (CSF) was reported by Knauff (1958). Muting and Shivaram (1959), using the paper chromatographic method and spectrophotometry, have reported a concentration of 16-36 μmoles/liter of taurine in human CSF. The taurine concentration in cerebrospinal fluid of 18 mental defectives, including two patients with mongolism, showed no significant deviation from the normal range (Perry and Jones, 1961).

The concentration of taurine in plasma or serum from normal adults and from children after the first few weeks of life has been reported to range from 25 to 150 μmole/liter (Jacobsen and Smith, 1968). These high values, as well as the considerable range of the normal
taurine concentration, have led to suggestions that the spuriously high levels are due to lysis of taurine-rich leukocytes and platelets (Frame, 1958; Soupart, 1962). These authors, using a method in which the plasma is separated from formed elements of the blood by rapid dialysis (to prevent cell lysis) before being subjected to paper chromatography, have found taurine to be completely absent from plasma or present in concentrations not exceeding 20 μmole/liter. In patients with granulocytic leukemia, the leukocytes and platelets contain less than normal amounts of taurine (Rouser et al., 1962). The level of taurine in plasma does not appear to be influenced by sex (Ackermann and Kheim, 1964); pregnancy (Christensen et al., 1957); or treatment with cortisol (Zinneman, Johnson, and Seal, 1963), estrogen, or progesterone (Zinneman, Seal, and Doe, 1967).

Studies on the renal clearance of taurine have been carried out by a number of investigators (Tarver and Schmidt, 1942; Cusworth and Dent, 1960; Ling, 1957) and widely different values ranging from 0.2 to 20 ml/min have been reported. These variations in taurine clearance may result in part from uncertainty about the true levels of taurine in plasma. Taurine is the major ninhydrin-positive compound in the urine of newborn babies (Woolf and Norman, 1957; Jagenburg, 1959) and full-term infants excrete substantially more taurine than premature infants (Jagenburg, 1959; Berry, 1960). Information is not available on the clearance and reabsorption of taurine in the immediate postpartum period. No decisions can therefore be made as to whether the large excretion of taurine in that period reflects the concurrent
hypertaurinemia or the immaturity of the renal transport system, as suggested by Schreier (1962).

There are a number of reports indicating an increased excretion of taurine in urine. Exposure to radiation causes a generalized aminoaciduria in which hypertaurinuria may be a prominent feature (Cavalieri et al., 1960). Relative and, in some cases, absolute hypertaurinuria has been reported after such traumatic events as burns (Nardi, 1954), surgical procedures (Turner and Blum, 1964), febrile convulsions (Berry, 1960), and pneumonia or wound infections (Berry, 1960).

Some studies have shown a generalized aminoaciduria in cases of myotonia dystrophica (Blahd, Bloom, and Drell, 1955) and a significant increase in the excretion of taurine from the patients with muscular dystrophy has been reported by Hurley and Williams (1955). There is also an elevation in the urinary excretion of taurine with normal plasma levels in patients with Friedreich's ataxia (Lemieux et al., 1976).

**Tissue Taurine Content**

The content of taurine in many mammalian tissues is high compared to that of other amino acids (Jacobsen and Smith, 1968). Such concentrations may be brought about by metabolism, uptake, or a combination of such processes.

**Taurine Metabolism**

In slices from dog heart, the conversion of taurine to isethionic acid is the only metabolic process utilizing taurine as a substrate (Read and Welty, 1962). Because isethionic acid lacks an amino group, its detection by general methods for amino acid analysis (e.g.,
ninhydrin test, or coupling with fluorescamine and subsequent use of spectrofluorometry) is not possible. Read and Welty (1962) have quantified the level of isethionic acid in the dog heart by crystallization and subsequent weighing of the isethionic salt. Huxtable and Bressler (1972) have found that the conversion of taurine to isethionic acid occurs at a rate too slow to be an important regulatory mechanism. Peck and Awapara (1967) identified isethionic acid as a taurine metabolite in rat brain on the basis of paper chromatographic analysis. Using the chromatographic procedure, Huxtable and Bressler (1972) have reported conversion of taurine to a compound with isethionic acid properties in a number of tissues. The development of a sensitive analytical technique which involves extraction, partial purification and methylation with diazomethane, followed by gas liquid chromatography by Applegarth, Remtulla, and Williams (1977) has revealed the isethionic acid dilemma. With this sensitive technique, isethionic acid was not detected in rat or dog heart, and only 2 µg/g tissue was found in rat brain. These results provide evidence against catabolism being a regulatory mechanism in maintaining taurine concentration.

Taurine Biosynthesis

Taurine biosynthesis is a complex problem in that several routes have been established or postulated, the relative importance of which vary from species to species and organ to organ. None of the enzymes supposedly involved in the biosynthesis has been purified to homogeneity or has had appropriate biochemical studies done on it. The conversion of cysteine to taurine via cysteine sulfinic acid and
hypotaurine to taurine (Fig. 1) is the pathway which has been studied intensively. The action of the enzyme cysteine sulfinic acid decarboxylase has been the best studied step in taurine biosynthesis. However, the enzyme has not been well characterized nor has its substrate specificity been established.

In addition to the pathway described, there are putative pathways involving cysteic acid either directly from cysteine or via phosphoadenosine phosphotransferase. The conversion of cysteine, via cysteine sulfinic acid as intermediate, to cysteic acid, followed by decarboxylation to taurine is the direct pathway for taurine biosynthesis. This pathway, however, has not been detected in the heart (Yamaguchi et al., 1973). Dupré and DeMarco (1964) have demonstrated a pathway of taurine biosynthesis involving the intermediates cysteamine and hypotaurine to occur in the hearts of certain species (Fig. 2). This pathway is poorly understood, since the metabolic origin of cysteamine in the heart is not known. The hydrolysis of coenzyme A currently is the only pathway by which cysteine may be converted to cysteamine (Cavallini et al., 1976). Based on the high tissue concentration of taurine and very limited hydrolysis of coenzyme A (Novelli, Schmetz, and Kaplan, 1954), it is unlikely that the turnover of coenzyme A is a reasonable source for cysteamine (Awapara, 1976). Huxtable (1978b) has suggested that pantothenic acid, independent of its function in coenzyme A synthesis, acts as a carrier group for cysteine in the production of cysteamine. In contrast to the lack of information concerning the source(s) of cysteamine, the oxidation of cysteamine to hypotaurine has been well studied (Huxtable and Bressler, 1976). The
HS-CH₂CH-NH₂
CO₂H

CYSTEINE

↓

HO₂S·CH₂CH-NH₂
CO₂H

CYSTEINE SULFINIC ACID

HO₃S·CH₂CH-NH₂
CO₂H

CYSTEIC ACID

HO₃S·CH₂CH₂-NH₂

HYPOTAURINE

HO₂S·CH₂CH₂-NH₂

TAURINE

1 — Cysteine dioxygenase (E.C. 1.13.11.20)
2 — Cysteine sulfonic acid decarboxylase (E.C. 4.1.1.29)
3 — Hypotaurine dehydrogenase (E.C. 1.8.1.3)
4 — Cysteine sulfinate dehydrogenase (E.C. 1.8.1.--)

Fig. 1. Pathway of Taurine Biosynthesis
HS\cdot\text{CH}_2\text{CH}\cdot\text{NH}_2 \rightarrow \text{HS}\cdot\text{CH}_2\text{CH}_2\cdot\text{NH}_2 \rightarrow \text{HO}_2\text{S}\cdot\text{CH}_2\text{CH}_2\cdot\text{NH}_2

\text{CYSTEINE} \quad \text{CYSTEAMINE} \quad \text{HYPOTAUrine}

1 -- Cysteamine dioxygenase (E.C. 1.13.11.19)

Fig. 2. Hypotaurine Biosynthesis via Cysteamine
oxidative enzyme involved, cysteamine dioxygenase, has been found in liver, muscle, and heart (Huxtable and Bressler, 1976; Dupré and DeMarco, 1964; Cavallini et al., 1966) and has been partially purified and the enzyme kinetics studied (Cavallini, Scandura, and Dupré, 1971). Cavallini et al. (1976), using radiolabeled cysteine and phosphopantothenyl cysteine, have found in the heart the preferred substrate to be phosphopantothenyl cysteine, and it is converted to cysteamine, hypotaurine, and taurine, whereas no conversion of cysteine to these substrates was detected. The information concerning the last step in taurine biosynthesis, the conversion of hypotaurine to taurine in rat brain slices, requires NAD$^+$ (Oja, Karvonen, and Lähdesmäki, 1973) indicating an enzymatic process. However, there is no information available for the hypotaurine conversion in the heart. Most enzymatic steps involved in the biosynthesis of intracellularly required substrates operate on a feedback inhibition; that is, the end product will inhibit further biosynthesis. In the rat heart, with a very high concentration of taurine, it is a likely possibility that such enzymes, if they exist, are under inhibition, or their affinity for substrate is so low that they are of no consequence. Thus, processes other than biosynthesis may be involved in regulating taurine level in the rat heart.

Taurine Transport

Circulating taurine derived either from biosynthetically active tissues or dietary sources is taken up by tissues throughout the body (Awapara, 1957; Huxtable and Bressler, 1972). Intracellular concentrations are generally several-fold higher than extracellular
concentrations, suggesting the gradient is maintained by an active transport process. Taurine transport in Ehrlich tumor ascites cells is inhibited by 2,4-dinitrophenol (a metabolic inhibitor) and anoxia (Kromphardt, 1963). Taurine uptake has been studied in tumor cells because of the extremely high concentration gradients existing across the cell membrane (Christensen, Hess, and Riggs, 1954). Structurally related β-amino acids such as β-alanine and hypotaurine inhibit the transport of taurine in Ehrlich tumor cells, while the transport is unaffected by α-amino acids (Christensen, 1964). The β-alanine uptake, however, is not appreciably blocked in these cells by taurine; instead, it is inhibited by several other amino acids. Relying on such observations, Christensen and Liang (1965) have suggested the presence of different transport sites for α-amino and β-amino acids. The active and energy dependent transport of taurine in human platelets has been shown to be blocked by metabolic inhibitors such as dinitrophenol, cyanide, and P-chloromercuribenzoate (Gaut and Nauss, 1976). These compounds by uncoupling the mitochondrial electron transport from that of oxidative phosphorylation impair the cellular capacity for energy production which is required for active transport. It has been suggested that taurine is actively transported into milk ducts of lactating women (Armstrong and Yates, 1963). In a synaptosomal preparation from rat brain, the presence of a high affinity, temperature sensitive, and sodium dependent active transport mechanism for taurine has been reported (Hruska, Huxtable, and Yamamura, 1978). Such transport is inhibited by β-alanine and hypotaurine, while the homologs of taurine, aminomethane sulfonic acid and 3-aminopropane sulfonic acid, were
ineffective inhibitors. Similar competitive inhibition of taurine uptake by β-alanine and hypotaurine has been reported in rat brain slices (Lähdesmäki and Oja, 1973). Taurine uptake has also been observed in rat heart and kidney slices with no inhibition by β-alanine (Awapara and Berg, 1976). However, others have shown that in isolated perfused rat hearts, taurine uptake is inhibited by β-alanine (Chubb and Huxtable, 1978a).

In vivo experiments involving rats and rabbits in which $^{35}$S-taurine was injected into them indicated that the rate of taurine uptake varies from organ to organ, but generally can be divided into organs which take up taurine rapidly, and those with slow uptake (Awapara, 1957; Guinneboult et al., 1956; Kromphardt, 1963). Liver, pancreas, and kidney are examples of organs having rapid uptake, while skeletal muscle, heart, and brain are in the group with slow uptake. Injecting humans with $^{35}$S-taurine, Sturman et al. (1976) have determined pool sizes by kinetic analysis using plasma, urine, and feces radioactivities. Their results indicate two general pools for taurine. One pool was relatively small and turned over rapidly; the other was much larger, but turned over at a much slower rate. The result of pool size studies in humans is consistent with those in animals.

Modification of Cardiac Taurine Concentration

There have been a number of attempts to manipulate cardiac taurine concentrations. Rats and other animals have taurine concentrations that remain invariant under a range of experimental conditions. Rats fed a diet deficient in vitamin B-6 have maintained normal
concentrations of taurine in the heart, but show a severely reduced excretion of taurine (Sturman, 1973). Cysteine sulfonic acid decarboxylase, one of the enzymes responsible for synthesis of taurine, requires pyridoxal-5'-phosphate (vitamin B-6) as a cofactor (Hope, 1955; Rassin and Sturman, 1975). Furthermore, rats fasted for prolonged periods or rats whose diets were supplemented with excess taurine have not shown any changes in the level of their cardiac taurine (Awapara, 1956). However, there are significant elevations in the taurine level in affected ventricles of animals and man with congestive heart failure (Peterson, Read, and Welty, 1973; Huxtable and Bressler, 1974).

Huxtable and Bressler (1974) have shown that taurine concentration is elevated 100% in the affected ventricles of persons who died of congestive heart failure, the changes being specific to the heart. In contrast to the inability to alter taurine level in the normal rat heart, Chubb and Huxtable (1978a) have shown that in the isolated perfused rat hearts, isoproterenol, a β-adrenergic agonist, stimulates the rate of taurine influx by 20-30%.

Physiological Role of Taurine

In this section, introduction of the physiological role of taurine has been divided into its role in the central nervous system (CNS), cardiovascular (CV) system, and miscellaneous effects.

Role of Taurine in CNS

Taurine is a structural analog of the inhibitory neurotransmitter substance, γ-aminobutyric acid (GABA), differing from it in having 2 instead of 3 carbon atoms and in the nature of the acidic
moiety. The presence of taurine in high concentrations in excitable tissues such as brain, heart, and skeletal muscle (Jacobsen and Smith, 1968) has prompted the search for a possible neurotransmitter role for taurine.

Curtis and Watkins (1965), by applying taurine iontophoresically to the vicinity of a neuron, noted an inhibitory effect of taurine on the rate of neuronal firing, thus proposing an inhibitory neurotransmitter role for taurine. In the microiontophoretic application of taurine and GABA in the cerebellar cortex of the rat, both taurine and GABA produced a dose-dependent depression of spike frequency of cerebellar neurons (Frederickson et al., 1978). The same authors have also examined the inhibitory effect in cerebellar Purkinje cells, which were shown to be antagonized by bicuculline and picrotoxin. Since bicuculline and picrotoxin are antagonists of the inhibitory effect of GABA, Frederickson et al. (1978) have concluded that the action of taurine in the cerebellum is similar to that of GABA. Horri et al. (1971) have studied the effects of homotaurine upon the sympathetic ganglia of dogs and have reported that homotaurine was similar in its action to hexamethonium in producing a blockade of both the postganglionic electrical impulses and the peripheral vasoconstrictor response resulting from preganglionic electrical stimulation at the splanchnic nerve. Taurine also exerts a powerful inhibitory effect on the bioelectrical activity of chicken retina (Pasantes-Morales et al., 1973) and suppresses some epileptic convulsions in man and those in a variety of experimental models of epilepsy in animals (Izumi, Igisu, and Fukuda, 1974; Mutani et al., 1974; Derouaux, Puil, and Naguet, 1973; Thursby and Nevis, 1974).
There is no doubt that taurine, when injected iontophoretically, does exert an inhibiting effect on most neurons, and that this effect is blocked by bicuculline and strychnine (Haas and Hosli, 1973). However, to be classified as a neurotransmitter, taurine must fulfill a number of criteria. The criteria are that (1) taurine must be synthesized in presynaptic neural elements; (2) it must be present at presynaptic sites; (3) in response to presynaptic stimulation, it should be released into the synaptic cleft; (4) mechanisms should exist for the termination of its action; (5) its effects, when applied exogenously, should be identical to the action of the endogenous transmitter (Wermann, 1966).

Cystein sulfinic acid decarboxylase, a key enzyme in the taurine biosynthetic pathway, is present in synaptic nerve endings (Davison, 1956; Peck and Awapara, 1967; Agrawal, Davison, and Kaczmarek, 1971). Furthermore, the activity of this enzyme has been shown to be attenuated by the electrical stimulation of rat brain slices (Oja et al., 1973). Such attenuation leads to an increase in level of taurine, which may be involved in impulse transmission in the CNS.

Within synaptic vesicular fractions from rat brain, high concentrations of taurine have been reported (De Belleroche and Bradford, 1973). Pasantes-Morales et al. (1972) have shown presynaptic localization of taurine in the inner synaptic layer of retina from several species.

Davison and Kaczmarek (1971) preincubated rat brain cortex slices with $[^{35}S]$-taurine, then transferred these slices into a taurine-free medium. The release of $[^{35}S]$-taurine from these slices was
enhanced by electrical stimulation. Similar results were found in rat spinal cord slices (Collins, 1974) and cat cortex in vivo (Kaczmarek and Adey, 1974). The release of labelled taurine from isolated retina of chicken following light or electrical stimulation has been reported by Pasantes-Morales, Salcedo, and Lopez-Colome (1976).

Termination of the action of taurine following its release appears to involve an uptake process rather than metabolism. A saturable, high affinity uptake system has been found in cortical slices (Kaczmarek and Davison, 1972), in synaptosomes from rat brain homogenates (Hruska et al., 1976), and in retina from several species (Pasantes-Morales et al., 1972; Starr and Voden, 1972). Huxtable and Bressler (1976) have found that metabolism to isethionic acid is too slow to postulate metabolism as a mechanism for the termination of taurine activity at synaptic junctions.

A conflict exists when the question is asked whether these findings signify that taurine is a true neurotransmitter or neuromodulator. Davison and Kaczmarek (1971) and Kaczmarek and Adey (1974) have argued strongly in favor of a neurotransmitter role for taurine. Their argument is based on the findings described above. However, some of the same arguments as well as findings described below favor a neuromodulator role for taurine.

The evidence in favor of a neuromodulator role for taurine is as follows. In the superior cervical ganglion and cerebral cortex of the rat, taurine suppressed high potassium evoked release of acetylcholine and norepinephrine from both regions, while having no effect on uptake. Furthermore, taurine inhibited the release of norepinephrine
from crude synaptosomal fractions without affecting the uptake of norepinephrine. Considering such results, Muramatsu et al. (1977) have suggested that taurine acts as a neuromodulator of neuronal activity, possibly by stabilizing excitable membranes and by suppressing the release of the neurotransmitter at the synapses. Consistent with a membrane stabilizing effect, Huxtable and Bressler (1973) have found that taurine protected and maintained the integrity of skeletal muscle sarcoplasmic reticulum membranes in the presence of phospholipase C. The concentration of taurine is known to be low in areas of epileptic foci in the brain of man and in a number of experimental models (van Gelder et al., 1972; Koyama, 1972). Since the administration of taurine effectively suppresses seizure activity (van Gelder et al., 1972), taurine may be functioning as a modulator of membrane excitability, its absence being responsible for the hyper-reactivity of cells in epilepsy. However, brain taurine levels are normal in at least one genetic epilepsy (Huxtable and Laird, 1978). Furthermore, the uptake of taurine is not limited to neurons, but also occurs in glial cells (Ehinger, 1973). The neurotransmitter release is thought to be calcium dependent (Mulder and Snyder, 1974); however, Sieghart and Heckl (1976) found that calcium suppressed the potassium induced release of taurine from synaptosomal fractions of rat cerebral cortex. Finally, Lopez-Columbe, Erlij, and Pasantes-Morales (1976) have reported that the release of taurine from chick retina following light stimulation was calcium independent.

Although the neurotransmitter or neuromodulator role of taurine in CNS is far from being generally resolved, in cat retina decreased
taurine concentration has pathological consequences. Hayes (1976) has found the reduction of taurine in the retina of cats to be one-third to one-half the normal concentration precipitated retinal dysfunction. He has also found that taurine was a necessary dietary requirement in cats, since a taurine deficient diet resulted in retinal degeneration followed by blindness in cats.

Cardiovascular Role of Taurine

The concentration of taurine is highest in the heart. In addition, cardiac taurine level is very difficult to alter experimentally. However, in advanced pathological states, an alteration in taurine concentration has been found. Peterson et al. (1973) have demonstrated an increase in the taurine and methionine concentrations in the right ventricle of dog heart in which right-sided congestive heart failure was experimentally induced. In the non-hypertrophied left ventricle, no change in amino acid concentration occurred. In humans dying of chronic congestive heart failure, Huxtable and Bressler (1974) have shown an increase of 100% in the taurine concentration in the left ventricular muscle. Such changes were specific to the heart, since they could not find any change in taurine concentration in the aorta or skeletal muscle. The physiological role of taurine in the heart has not been studied as extensively as in the CNS. However, the high, relatively constant concentrations of taurine in the non-stressed heart and its considerable elevation in congestive heart failure have led investigators to seek a physiological function for taurine in the heart.
Read and Welty (1963) have shown that taurine administered intravenously to dogs is an effective antiarrhythmic agent for epinephrine induced premature ventricular contractions and for the arrhythmias of acute or chronic digitalis toxicity. Further work showed that taurine administered to intact dogs prevented the large myocardial efflux of potassium which accompanies epinephrine induced arrhythmias (Welty and Read, 1964). These authors have argued that inside the cell taurine, regardless of its source, could be oxidatively deaminated to the highly charged derivative, isethionic acid. The negatively charged isethionic acid could bind potassium and restrict the outward movement of potassium, a mechanism consistent with an antiarrhythmic role for taurine. Huxtable (1976) has presented arguments against the isethionic acid theory. Furthermore, Applegarth et al. (1977), using the sensitive technique described above, have shown that isethionic acid could not be detected in dog heart. There are conflicting reports as to the antiarrhythmic role of taurine in preventing digitalis induced arrhythmias. Read and Welty (1963) and Chazov et al. (1974) have reported that taurine exerted antiarrhythmic properties in guinea pig and dog hearts treated with strophanthin-K. ST segment changes, T-wave inversion, and conduction abnormalities induced by strophanthin-K were corrected by taurine. In addition, taurine aggravated arrhythmic abnormalities in hearts perfused with a potassium-free medium, suggesting antiarrhythmic role of taurine is potassium-dependent.

More recently, Hinton, Diaz-Souza, and Gillis (1975) repudiated the antiarrhythmic properties proposed for taurine after evaluating this compound in an established model of digitalis toxicity used for
screening antiarrhythmic drugs. Taurine administered over a wide dosage range was ineffective in preventing digitalis arrhythmias, and actually aggravated the arrhythmias. The discrepancies between the report by Read and Welty (1963) and Chazov et al. (1974) with those of Hinton et al. (1975) stems from the severity of arrhythmia used to test the antiarrhythmic properties of taurine. The arrhythmias used by Hinton et al. (1975) were more severe than those used by other authors. Furthermore, Read and Welty or Chazov did not use any control animals in their experiment to indicate the duration of arrhythmia. The antiarrhythmic effect they have observed by taurine could be simply the diminished effect of digitalis. Digitalis induces its positive inotropic effect by the inhibition of \( \text{Na}^+,\text{K}^+\text{-ATPase} \) pump in the heart (Akera, Ku, and Brody, 1976). Furthermore, the onset of arrhythmia by digitalis is a consequence of sodium pump inhibition. Based on reports as to the antiarrhythmic effect of taurine, Akera et al. (1976) tested the effect of taurine and its metabolite, isethionic acid, on \( \text{Na}^+,\text{K}^+\text{-ATPase} \) activity. In guinea pig hearts, taurine and isethionic acid up to 100 mM failed to affect \( \text{Na}^+,\text{K}^+\text{-ATPase} \) activity. They have suggested that the antiarrhythmic effect of taurine, if any, must be exerted on events after inhibition of \( \text{Na}^+,\text{K}^+\text{-ATPase} \) pump.

Dietrich and Diacono (1971) have studied the effect of taurine on isolated perfused guinea pig and rat hearts. Ouabain always exerts a negative inotropic effect in rat hearts and a positive inotropic effect in guinea pig hearts, both in normal and low calcium medium. Taurine exerts in both species an effect similar to that of ouabain. Furthermore, the effects of taurine and ouabain are additive.
Potentiation of the inotropic effect of strophanthin-K in guinea pig auricles by taurine has also been reported by Guidotti, Badiani, and Giotti (1971). In addition, Guidotti and Giotti (1969) have found that taurine antagonizes the negative inotropic effect of low calcium medium in the guinea pig. These results suggest that taurine may affect calcium fluxes. Dolara et al. (1973) have studied the effect of 8 mM taurine on calcium washout from isolated guinea pig hearts. Perfusing the hearts with a Tyrode solution containing 2.7 mM calcium for 15 minutes, in the presence or absence of 8 mM taurine followed by a period of washout with a calcium-free solution, Dolara et al. (1973) found taurine treated hearts showed a less marked decrease in contractile strength caused by a decreased loss of calcium from the heart. Kinetic analysis of the calcium washout indicated a three compartment system for calcium. Two of these compartments exchanged calcium rapidly, whereas the third compartment exchanged calcium very slowly. Taurine treatment decreased the initial concentration of free calcium in the three compartments, but increased the total amount of calcium that could be washed from these compartments. Dolara et al. (1973) incorporating such results suggest that taurine may interact with intracellular structures in such a way as to increase the intracellular binding. Dolara et al. (1976) have identified a calcium-taurine interaction in the sarcoplasmic membrane from guinea pig hearts. The total calcium binding to the sarcoplasmic reticulum (SR) was increased in the presence of taurine. They have proposed that the increased inotrophy, the potentiation of digitalis action, and the counteraction of the negative inotropic effect of the low calcium medium exerted by taurine are due to higher levels of
reversibly bound calcium by SR. Huxtable and Bressler (1973), using the millipore technique, demonstrated that taurine increases calcium binding to the SR of rat skeletal muscle. Iwata and Fujimoto (1976) have shown that 3 mM taurine potentiates the positive inotropic effect of ouabain in the guinea pig heart, and the potentiation is consistent with an increase in the level of calcium intracellularly. These authors could not find any potentiation at 0.5 mM taurine concentration, and the action of taurine on calcium loading is even greater at a low potassium medium. Diacono and Dietrich (1976), in an electrophysiological study of rat and guinea pig hearts, have concluded that the inotropic effects of taurine are dependent on some modification in the intracellular distribution of calcium, rather than on an increase in calcium influx during the electrical cell activity.

A number of investigators have studied the binding of calcium to the sarcoplasmic reticulum. No effects of taurine on calcium binding to cardiac SR prepared from Wistar or Okomoto hypertensive rats were found (Chubb and Huxtable, 1978b). Entman, Barnet, and Bressler (1977) have isolated SR from adult dog cardiac muscle. They found no effect of taurine on yield, calcium transport, ATPase, binding, or the release of calcium from SR. They have suggested that the role of taurine in cardiac muscle is not likely to be found in the regulation of SR. Furthermore, Remtulla, Katz, and Applegarth (1978) have studied the effect of taurine on the ATP-dependent calcium transport in guinea pig cardiac ventricle homogenates and in microsomal preparations enriched with SR. Taurine (5-50 mM) did not affect ATP-dependent calcium binding or uptake in either of their preparations, or alter the rate of decay
of calcium uptake activity. Tada, Kirchberger, and Katz (1976) have shown cyclic AMP-dependent protein kinase to stimulate calcium uptake in preparations of cardiac SR. Taurine did not affect the enhancement of calcium transport by cyclic AMP-dependent protein kinase either in the homogenate preparation or in the SR (Remtulla et al., 1978). These authors have suggested the possibility that taurine exerts its effects in the cardiac cell by altering the passive diffusion of ions or by affecting calcium release from sarcotubular, mitochondrial, or sarcoplasmic reticulum stores. Recently, Schaffer, Chovan, and Werkman (1978) have shown in rat heart that taurine caused a transient 2-fold decrease in cyclic AMP levels and this was accompanied by a slight (5%), but statistically significant, positive inotropic effect. They have suggested that the decreasing levels of cyclic AMP, while contractility is increasing, could be due to increased phosphodiesterase (enzyme responsible for the metabolism of cyclic AMP) activity. Chovan et al. (1979) have shown taurine to increase the calcium binding to the low affinity sites in both the high sodium-low potassium and low sodium-high potassium buffers to the sarcolemma prepared from rat hearts. However, their results disagree with those of Izumi, Butterworth, and Barbeau (1977). Taurine decreases calcium binding to the microsomes isolated from rat cerebral cortex, thus leaving the effect of taurine on calcium binding unclear.

The action potential of cardiac muscle appears to be due to three transients: (1) an early rapid increase in the inward sodium conductance responsible for the rapid upstroke of action potential; (2) a slowly rising sustained increase in the inward calcium current
responsible for the overshoot and plateau phase of the action potential; and (3) a slowly rising increase in the outward potassium conductance responsible for the repolarization phase of the action potential (Weidmann, 1974). Read and Welty (1976) have shown in guinea pig papillary muscle that 20 mM taurine affects all phases of the action potential. It lowers the resting potential, the overshoot, the slope of phase II and III and prolongs the duration of the action potential. They have interpreted the action of taurine on the action potential as an effect on the membrane's calcium, since they reproduced some of the effects of taurine by lowering the external calcium. This observation provides a basis for understanding the effects of taurine on cardiac action potential.

Finally, the effect of taurine on the vascular system has recently been examined by Nara, Yamori, and Lovenberg (1978). They have studied the effects of taurine on the blood pressure in Wistar Kyoto rats (WKY), spontaneous hypertensive rats (SHR) derived from Wistar Kyoto, and a stroke-prone substrain (SHR-SP). They found that the addition of taurine (3%) to the drinking water of rats during the period of 4-14 weeks of age had little effect on the blood pressure in normotensive WKY, and highly retarded the development of hypertension in SHR. In the SHR-SP, however, there was a slightly significant reduction in the development of hypertension. They have found that genetically hypertensive rats, particularly the SHR-SP substrain, have defects in taurine metabolism, and have suggested that this may be related to the severity of hypertension. There is also a report by
Yamaguchi et al. (1967) that taurine can lower blood pressure in patients with essential hypertension.

Miscellaneous Role of Taurine

This section describes numerous functions that taurine may have in invertebrate and vertebrate animals.

**Osmoregulation.** In a number of invertebrates, a constant cell volume in the media is maintained at different salinities by controlling intracellular concentrations of various ions and amino acids. Because of the high concentration in marine animals, Krogh (1939) originally pointed to a role for taurine in the regulation of osmotic pressure. Simpson, Allen, and Awapara (1959) reported a close correlation between intracellular concentrations of taurine and the salinity of the animal's habitat. The possible role of taurine in osmoregulation was further strengthened by the fact that taurine was absent from fresh water species. Transfer of aquatic animals to environments of different salinities resulted in changes in taurine concentration (Jeuniaux, Bricteux-Gregoire, and Florkin, 1962; Schoffeniels and Gilles, 1972). Lange (1963) has suggested that the role of taurine in regulating cellular isotonicity may be an important sparing effect on the use of protein forming amino acids in osmoregulation.

**Bile Acid Formation.** The only physiological function for taurine which is firmly established is its involvement in bile acid conjugation. In most fishes, reptiles, and amphibians, bile acids are conjugated exclusively with taurine and glycine (Jacobsen and Smith,
The conjugation ratio with taurine or glycine is species specific and is a function of diet (Doisy, Daniels, and Zimmerman, 1956; Haselwood, 1962). The ratio of bile conjugation with taurine is higher in carnivores compared to herbivores. Eighty per cent of rat bile is conjugated with taurine whereas rabbit bile is conjugated almost exclusively with glycine.

Bile acids are metabolites of cholesterol. Conjugation of these acids with taurine (and glycine) results in the elimination of cholesterol from the body. The absence of taurine or glycine in the dietary regimen leads to a decrease in the metabolism of cholesterol and eventually elevated cholesterol in the serum. This hypothesis is supported by the work of Portmann and Mann (1955). They experimentally induced hypercholesteremia and atherosclerosis in monkeys by placing them on a diet high in cholesterol and low in sulfur amino acids. The hypercholesteremia was easily reversed by adding methionine, cysteine, or taurine to the diet. Similar results were obtained in rats and mice with experimentally induced hypercholesteremia (Fillios and Mann, 1954; Herrmann, 1959). Similar experiments were conducted on hypercholesterolemic rabbits and man in an attempt to lower the serum cholesterol levels by increasing the bile salt conjugation (Truswell et al., 1965; Burns and Self, 1969). In these studies, dietary taurine decreased the glycine to taurine ratio, but had no effect in lowering the serum cholesterol levels. They have suggested that cholesterol metabolism is not affected by the manipulations of bile acid conjugation in man.
Hormonal Release. N-methyl-DL-aspartic acid (NMA) is a potent neuroexcitatory analog of glutamate that induces an acute burst of luteinizing hormone (LH) release when administered subcutaneously to weanling or adult male rats. Price et al. (1978) have reported that the simultaneous subcutaneous administration of GABA or taurine inhibits the release of LH, while the release of LH is not affected by the dopamine receptor blocker, chlorpromazine. They have suggested that GABA or taurine blocks the effect of NMA, not at the cholinergic excitatory receptors responsible for release of LH, but at some other focus, either on the same cell or on a cell postsynaptic to it. Collu, Charpenet, and Clermont (1978) have shown that the intraperitoneal or intraventricular administration of taurine to male rats does not modify pentobarbital-induced sleep or pituitary hormone release. However, a large increment in plasma growth hormone levels was induced by morphine administration which was completely blocked by the intraventricular administration of taurine. Kuriyama and Nakagawa (1976) have reported that the oral administration of taurine antagonizes the stress induced elevation of blood sugar by reducing epinephrine output from the adrenal gland. They have suggested that the regulatory mechanism most likely involves the inhibition of epinephrine release from adrenal chromaffin granules, possibly by increasing the affinity of calcium to the granules or by stabilizing the granular membranes, or both.

Behavioral Effect and Temperature Regulation. Sgaragli and Pavon (1972) have shown that the intracerebroventricular (ICV) injection of taurine in rats causes a complete disruption of temperature
regulation. Hruska et al. (1976) by the ICV injection of taurine in mice and by the measurement of rectal temperature have shown that taurine produces hypothermia. The hypothermia produced by taurine is completely abolished by the simultaneous injection of amphetamine. Since amphetamine-induced hyperthermia is mediated through the hypothalamus, Hruska et al. (1976) have suggested taurine-induced hypothermia is also mediated through the hypothalamus. The cholinomimetic drugs did not antagonize the hypothermia produced by taurine. Thut et al. (1976) have tested the effect of the taurine injection into the peritoneal cavity (IP) on the eating and drinking behavior of male mice. Taurine depressed conditioned responding for food and water. This ability to depress the number of responses for food and water was also observed when various homologs and analogs of taurine were administered. They have suggested that taurine when administered IP may enter the hypothalamus more readily than other brain areas and therefore may depress hypothalamic function more readily than other areas of the brain.

**Lesion Control.** Kainic acid, a rigid glutamate analog with potent excitatory effects on neurons, causes a degeneration of neurons when injected locally into the brain, presumably as a result of sustained excitation or depolarization (Olney, 1978). Injection of kainic acid into the neostriatum of rats causes biochemical, histological, and behavioral changes similar to those seen in Huntington's disease (Mason, Sandberg, and Fibiger, 1978). The extent of neuronal degeneration was assessed by measuring glutamic acid decarboxylase and choline...
acetyltransferase. Sandberg, Staines, and McGreer (1979) have examined the possible effects of chronic treatment with taurine on the neurotoxic action of kainic acid in rat neostriatum. Taurine, 70 mM in the drinking water of rats with neostriatal lesion, showed a protection of cholinergic neurons degeneration, caused by kainic acid.

**Blockade of Taurine Transport**

β-Alanine and hypotaurine have been reported to block taurine transport across myocardium and other organs (Chubb and Huxtable, 1978b; Hruska et al., 1978; Lähdesmäki and Oja, 1973). β-Alanine has potential usefulness as a transport inhibitor. However, it has physiological and pharmacological actions of its own, and there is a non-saturable component to its uptake by the heart (Huxtable, Laird, and Lippincott, in press). Hypotaurine, another inhibitor of taurine transport, is taken up by myocardial cells, where it is rapidly oxidized to taurine. Therefore, if the objective is to study the physiological effect of lowering intracellular taurine concentration, hypotaurine will be of very little usefulness. Close structural analogs of taurine, such as 1,2-dimethyl taurine and 2-methyl-cyclohexane sulfonic acid, show no affinity for the transport site (Huxtable and Chubb, 1977; Chubb and Huxtable, 1978c).

The role of guanidoethyl sulfonate, a close structural analog of taurine, at the taurine transport has not been established. This analog of taurine does not occur in mammalian heart (Guidotti and Costagli, 1970), and it has been claimed to have no pharmacological effect of its own in mammalian heart. A limited number of species belonging to the phyla annelida and sipuncula contain the guanido
derivatives of taurine and possess the enzymatic means of forming the corresponding phosphagens (phosphoguanidoethyl sulfonate). The phosphagens may play a significant role in the storage of energy in a few species of worms (Ennor and Morrison, 1958; Thoai and Robin, 1965; Thoai and Roche, 1960).

**Amrinone**

Amrinone, a recently synthesized cardiotonic drug, has been found to exert a strongly positive inotropic action in a variety of in vivo and in vitro preparations (Alousi and Farah, 1978). In the anesthetized dog, the intravenous infusion of amrinone at a rate of 10, 30, or 100 μg/km/min has been shown to increase the cardiac contractile force in a dose related response (Farah and Alousi, 1978). The drug in dogs does not affect the heart rate or systolic blood pressure. Amrinone given to twenty normal human volunteers intravenously produced a dose-related decrease in the heart rate; it corrected abnormal pre-ejection period, left ventricular ejection period, and duration of total electromechanical systole (DeGuzman, Munoz, and Palmer, 1978). Amrinone administered to eight patients with severe congestive heart failure due primarily to cardiomyopathy who were receiving full doses of digitalis brought about a positive inotropic effect (Benotti et al., 1978). In experimental animals, the amrinone effect is not blocked by propranolol, or modified by reserpine treatment (Farah and Alousi, 1978). In dogs, the drug works independently of β-adrenergic receptors and it does not have any significant effect on catecholamine metabolism, or in the Na⁺,K⁺-activated adenosine triphosphate level in the heart (Alousi
and Farah, 1978; Farah and Alousi, 1978). These authors have suggested that amrinone induces its positive inotropic effect by acting directly on the contractile machinery of the myocardium. Meisner, Van Breeman, and Palmer (1979) have reported that amrinone causes a dose dependent inhibition of norepinephrine or 80 mM K\(^+\)-induced contractions of rabbit aorta. Furthermore, amrinone does not seem to act as a Ca\(^{2+}\) influx inhibitor. Recently, Onuguluchi and Tanz (1979) have reported that amrinone in Langendorff perfused guinea pig hearts leads to a coronary vasodilation and increased myocardial oxygen consumption (MVO\(_2\)), consistent with its role as a cardiotonic agent.

**Cardiomyopathy and Taurine**

Extensive efforts have been made to find a specific biochemical abnormality which may be considered responsible for heart failure. In the failing heart, some investigators have demonstrated a defect in energy production (Argus et al., 1964; Lindenmayer, Sordahl, and Schwartz, 1968; Schwartz and Lee, 1962), while others have claimed an impairment of the mechanisms for energy storage and utilization (Fleckenstein, Döring, and Kammermeier, 1966; Furchgott and De Gubareff, 1958). These studies provide evidence for the traditional formulation that heart failure is a condition of multiple etiology consistent with the hypothesis of Olson (1956). Olson has suggested that there exist at least two molecular classes of heart failure; i.e., those in which the defect lies in the generation of adenosine triphosphate (ATP) and those in which the defect lies in the utilization of ATP. This hypothesis concerning abnormalities at the level of energy production
and utilization, however, has failed to explain observations indicating normal energy stores, mitochondrial function, and myofibrillar ATPase activities in certain types of failing hearts (Pool, 1974). There are also reports available in which normal energy metabolism in the failing heart has been indicated (Buckley and Tsuboi, 1961; Chidsey et al., 1966; Plaut and Gertler, 1959; Sobel et al., 1967).

Membrane systems such as mitochondria, sarcoplasmic reticulum, and sarcolemma are the sites of important enzymes such as adenylate cyclase, $\text{Na}^+,\text{K}^+$-ATPase, $\text{Mg}^{2+}$-ATPase, and $\text{Ca}^{2+}$-ATPase (McNamara, Sulakhe, and Dhalla, 1971; Basmussen, Goodman, and Tenenhouse, 1972; Schwartz, Lindenmayer, and Allen, 1972). These membranes, in particular, participate in raising and lowering the intracellular level of ionized calcium for initiating contraction and relaxation, respectively. A number of investigators have directed their attention to studying the participation of these membranes in the pathogenesis of heart failure (Dhalla et al., 1974; Lindenmayer et al., 1971; Nayler et al., 1971). Decreased ability of the segments of sarcoplasmic reticulum from different types of failing hearts to accumulate calcium has been interpreted to suggest a defect in the process of excitation-contraction coupling (Gertz et al., 1967; Harigaya and Schwartz, 1969; Muir et al., 1970; Schwartz et al., 1973; Dhalla, 1976). In this regard, it is assumed that decreased calcium uptake by sarcoplasmic reticulum would reduce calcium stores which are available for release upon excitation and this would result in poor contractions of the failing myocardium; further-decreased ability of the sarcoplasmic reticulum to remove calcium from the myoplasm would be associated with the prolongation of the relaxation
phase. Thus, changes in the sarcoplasmic reticulum membrane can account for the impairment of contractile properties of the failing heart.

There are also reports concerning the changes in the ability of mitochondria from failing hearts to transport calcium (Muir et al., 1970; Sordahl et al., 1973; Dhalla et al., 1975). Alteration in the sarcolemma has received relatively little attention. This has been mainly due to difficulties in isolating cardiac sarcolemma in a relatively pure form. Recently, Singh et al. (1975) have developed a technique to isolate pure sarcolemma from cardiomyopathic hamsters. Their study indicates that the sarcolemma fraction obtained from the failing hearts at advanced stages of myopathy exhibited no change in the basal adenylate cyclase activity. The activity of this enzyme, however, in the presence of catecholamines or NaF was lower in the failing heart sarcolemma than that in the control. The activities of Ca$^{2+}$-ATPase, Mg$^{2+}$-ATPase, and Na$^+$,K$^+$-ATPase in the failing heart sarcolemma were also less than the control values. In light of reports concerning the membrane stabilizing effect of taurine (Huxtable and Bressler, 1973), and its role in increasing the mitochondrial binding of calcium (Dolara et al., 1973), there have been no studies as to the role of taurine in controlling the development of cardiomyopathy, except the finding that taurine concentration increases in congestive heart failure (Huxtable and Bressler, 1974; Peterson et al., 1973).

Friedreich's ataxia (FA) is an autosomal recessively inherited spinocerebellar ataxia (Barbeau, 1978), appearing in childhood and progressing to invalidism or death in middle age at the latest. Neurological symptoms of the disease are a lack of coordination in the limbs,
the loss of knee reflexes, and skeletal deformities. However, the cardiac problems associated with the disease are those that ultimately cause death. The cardiomyopathy is usually hypertrophic in type and occasionally obstructive. It appears to be an integral part of FA and not a phenomenon secondary to the disease process (Barbeau, 1978). According to Sanchez-Casis, Coté, and Barbeau (1976), the one heart they autopsied showed severe and diffuse intercellular fibrosis, with areas of intracellular calcification. There are a number of studies indicating that alterations in cell calcium uptake and content are closely related to cardiac necrotic processes (Bajusz, 1969; Fleckenstein, 1971; Fleckenstein et al., 1975). The calcium overload may be caused by a number of mechanisms. Hartman and Booth (1960) have suggested that sympathetic overactivity secondary to lesion of the vagal nuclei is responsible for myocardial damage. In such a case, the cardiac muscle is abnormally sensitive to adrenergic stimulation which then causes an increased flux of calcium across the cell membrane. Another possible biochemical abnormality is an impairment of oxygen transportation or utilization (Malo et al., 1976). Finally, a generalized defect in the membrane systems such as mitochondria, sarcoplasmic reticulum, and sarcolemma could be responsible for calcium overload (Dhalla, 1976). Based on the histological findings of calcium deposits in the heart, as well as the increased urinary excretion of taurine (Lemieux et al., 1976) in FA patients, Huxtable (1978a) has suggested that taurine may be easily involved in the pathogenesis of the heart problems associated with FA. Huxtable and Bressler (1973) have suggested that the pathological effects of high cellular calcium
concentrations stem from the ability of the free calcium ion to cause energy depletion of the cell leading to calcified deposits.

Inbreeding of the Syrian golden hamster has resulted in strains of hamsters having inherited an autosomal recessively transmitted cardiomyopathy (Lossnitzer and Bajusz, 1973) aside from inherited susceptibility and resistance to carcinogens (Homburger, 1972). Among the strains available are Bio 14.6 and 82.62, which have a slow developing disorder (Bajusz, 1969). Animals of the Bio 14.6 strain develop genetically-induced cardiomyopathic lesions at approximately sixty days of age (Bajusz et al., 1969). The lesions are followed progressively by cardiac hypertrophy at some 120 days of age, compensated cardiac failure at about 200 days, and full-blown failure with the typical sequellae culminating in death at an age of approximately 300 days (Schwartz, Sordahl, et al., 1972). The cardiac function in these animals has been shown to be markedly depressed (Brink and Lochner, 1967, 1969; Lochner and Brink, 1970), a condition that is preceded by necrotizing myocardial lesions (Stauch and Lossnitzer, 1975). The lower activity of enzymes located in the sarcolemma has been shown by Singh et al. (1975) and the calcium overload has been confirmed by Bajusz (1969) and McBroom and Welty (1977).

**Rationale and Objectives**

β-Adrenergic stimulation of the heart leads to manifold changes. Such changes include increased heart rate, enhanced ionic fluxes, and increased cyclic AMP levels. Previous work in isolated perfused rat hearts (Table 1) has shown heart rate does not affect the rate of
Table 1. Effect of Pacing on Taurine Influx — From Huxtable and Chubb (1977, p. 409).

<table>
<thead>
<tr>
<th>Beats/min</th>
<th>Influx ( \text{nmole/g dry wt/min} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>14.16 ( \pm 0.08 )</td>
</tr>
<tr>
<td>350</td>
<td>14.27 ( \pm 0.72 )</td>
</tr>
<tr>
<td>450</td>
<td>13.56 ( \pm 0.73 )</td>
</tr>
</tbody>
</table>

Hearts were perfused with Krebs-Henseleit buffer containing \( 5 \times 10^{-5} \) M \( [^3H]\)-taurine. The sinoatrial node was ablated and the hearts were paced at rates indicated. Data are expressed as mean \( \pm \) SEM from four hearts in each group.

taurine influx. However, increasing cyclic AMP levels in the heart stimulates the rate of taurine influx (Table 2). With increased heart rate being ineffective, the contribution of enhanced ionic fluxes to stimulation in rate of taurine influx was examined. Increased levels of cyclic AMP in the heart stimulate the rate of calcium influx. Either the increased cyclic AMP levels promote the enhanced taurine influx, or the stimulated calcium influx is responsible for the increased taurine uptake. Therefore, these two possibilities were examined. Once the mechanism for stimulated taurine influx is established, the pharmacological approach would be to study the structural requirement at this transport site.

Amrinone is a new cardiotonic drug that induces positive inotropy in dogs without increasing cyclic AMP levels. It became of interest to test whether positive inotropy independent of cyclic AMP
Table 2. Effect of Various Agents on the Rate of Taurine Influx —
From Chubb and Huxtable (1978a, p, 369).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Influx (nmole/g dry weight/min)</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.01 ± 0.40</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>23.96 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>119 ± 4</td>
</tr>
<tr>
<td>Theophylline</td>
<td>22.67 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112 ± 1</td>
</tr>
<tr>
<td>Dibutyryl cAMP</td>
<td>25.21 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123 ± 8</td>
</tr>
</tbody>
</table>

<sup>a</sup> <i>p < 0.05</i>.

Perfusion concentrations employed were taurine, 1 x 10^-4 M; isoproterenol, 4 x 10^-7 M; theophylline, 1 x 10^-3 M; dibutyryl cAMP, 9.5 x 10^-4 M. Control rate for the dibutyryl cAMP experiment was 20.47 ± 0.24. Four animals were used in each experiment.

stimulation has any effect on rate of taurine influx. Since there was no information available as to inotropic effect and alteration in cyclic AMP levels by amrinone in animals other than dogs, such points needed to be clarified first. It has been suggested that amrinone's inotropic effect is the result of direct action at the myofilaments. However, my observations did not support such a suggestion. Since cardiotonic agents usually produce their inotropy through modification of calcium ion, the effect of amrinone on calcium kinetics was studied in guinea pig hearts. Guinea pigs were chosen because in isolated perfused rat hearts, amrinone did not produce a significant inotropic effect.

Attempts have been made to elucidate the physiological and pharmacological role of taurine in various tissues and animals. There
are reports implicating taurine as a responsible agent for modulating calcium levels in the heart. Such modulation could be either at the intracellular membranes or at the sarcolemma. With lack of effect by taurine on the isolated sarcoplasmic reticulum from the heart, sarcolemma offered another site at which the effect of taurine on calcium could be studied. In attempts to establish a physiological role for the taurine-calcium interaction the effect of taurine and its close structural analog on cardiac contractile force was examined.

Finally, the cardiomyopathic hamsters in which cardiomyopathy is due to calcium overload offered an ideal model for testing the physiological effect of taurine in the regulation of calcium overload. The objectives of this work are to determine: (1) which one of the changes in response to β-adrenergic receptor activation in rat hearts is responsible for the enhanced rate of taurine influx, (2) what structural requirements exist at the taurine transporting sites, (3) how amrinone produces its positive inotropic effect in the guinea pig hearts and what is the effect of amrinone on the rate of taurine influx, (4) whether taurine affects calcium binding to the sarcolemma and could such effect influence the contractility, and (5) whether taurine affects calcium overload in the cardiomyopathic hamsters and what are the consequences of calcium regulation by taurine on calcium dependent necrosis.
CHAPTER 2

METHODS

Animals

Animals used in this research were male Sprague-Dawley rats weighing 200-250 g, obtained from Charles River Co. (Wilmington, MA); male cardiomyopathic Syrian golden hamsters (CM) of the Bio 14.6 strain and random bred control hamsters (RB) weighing 65-140 g, obtained from Telaco (Bar Harbor, ME); and male Hartley guinea pigs weighing 275-325 g obtained from Hilltop Animal Laboratories (Chatsworth, CA).

Heart Perfusion

Depending on the experiment, different animals were anesthetized with pentobarbital sodium (5 mg/kg) (Abbott Laboratories, Chicago). The hearts were rapidly excised and placed in a beaker containing ice-chilled continuously oxygenated 0.9% saline. While in oxygenated saline, the aortas were cannulated with a blunt stainless cannula, then perfused by the Langendorff technique described below in a non-circulating system with a Krebs-Henseleit bicarbonate buffer (37°C) containing glucose, continuously gassed with 95% O₂ and 5% CO₂. The appropriate flow rate was maintained constant throughout the experiment. The Krebs-Henseleit (K-H) buffer contained the following salts (in mM concentrations): NaCl, 120; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25; and glucose, 5.5. The oxygenated buffer had a pH of 7.4.
Reserpine Treatment

Four to six hours prior to sacrifice rats were given reserpine. Rats received a single intraperitoneal (IP) injection of reserpine phosphate (5 mg/kg) (Ciba Laboratories, Summit, NJ) to ensure the depletion of catecholamines in the heart.

Ionophore Study

The reserpine-treated hearts from male rats were cannulated with an 18 gauge cannula and perfused retrogradely at a flow rate of 6 ml/min in a non-recirculating system with K-H buffer. After six minutes of perfusion, the buffer was switched to one containing $[^3]H$-taurine (25 μM, 0.05 μCi/ml) and, where appropriate, the ionophore under test. Ionophore perfusion was continued for 4.5 minutes followed by a five minute washout period. The stock solution of ionophores was prepared by dissolving them in 100% ethanol and the final desired concentration was obtained by diluting 50 microliters of stock to 50 milliliters of K-H buffer. All the control animals received the same amount of 100% ethanol in the perfusion buffer. A pilot study showed the same concentration of ethanol not to affect the contractile force or the heart rate.

Verapamil and Glucagon Studies

These studies performed as described above, except that the animals were not treated with reserpine, and the perfusion time was five minutes with verapamil ($3.4 \times 10^{-7}$M) and isoproterenol ($4 \times 10^{-7}$M) and four minutes in the case of glucagon (0.75 mg/ml).
Recording of Heart Activity

In the above experiments, and those with amrinone, the heart rate and the contractile forces were measured by a strain ring force transducer attached to the apex of the heart and recorded on an oscillographic recorder (Electronics for Medicine, IR-4, White Plains, NY).

Influx Rate of Radiolabeled Compounds

In experiments that involved the determination of rate of influx of the radiolabelled taurine, $^3\text{H}$-taurine or $^{45}\text{CaCl}_2$, the influx was determined in two ways: (1) measurement of the difference in radioactivity of the perfusate and effluent, and (2) measurement of $^3\text{H}$-taurine or $^{45}\text{Ca}$ remaining in the heart following washout.

Following washout, the hearts were homogenized in 8 volumes of 5% trichloroacetic acid using a Polytron homogenizer (Brinkmann), at setting #5, for 1.0 minutes and the homogenate was centrifuged at 40,000 $\times$ g for 20 minutes in a Sorvall RC2-B centrifuge. The radioactivity in the deproteinized supernatant was determined by liquid scintillation counting.

Liquid Scintillation Counting

Radioactivity was determined on a Beckman LSC 250 liquid scintillation counter. Data reported as cpm were counted at a constant efficiency and data were expressed as dpm after correction for efficiency by the channel ratio method.
Study of Taurine Analogs

The rat hearts were perfused at a flow rate of 6 ml/min with K-H buffer containing 5.5 mM glucose. After 6 minutes of perfusion, the buffer was switched to one containing $[^3]$H-taurine (25 μM; 0.05 μCi/ml) and, where appropriate, an analog under test. Substrate perfusion was continued for 6 minutes followed by a 6 minute washout period.

Membrane Preparation

The sarcolemma was prepared by the method of Singh et al. (1975). The heparin-treated rats weighing 200-250 g were decapitated and the hearts were quickly removed and perfused through aorta with 20 ml of ice cold 10 mM Tris-HCl, pH 7.4, containing 1 mM EDTA (ethylene-diaminetetraacetic acid). The hearts were then homogenized with 10 volumes of Tris-EDTA buffer. The homogenate was passed through several layers of cheesecloth and centrifuged at 1000 x g for 10 minutes. The sediment was suspended in Tris-HCl (pH 7.4), stirred for 30 minutes, and then centrifuged at 1000 x g for ten minutes. This process was repeated three times and the final sediment was extracted with 0.4 M LiBr in Tris buffer for 45 minutes and centrifuged at 1000 x g for 10 minutes. This sediment was washed three times with 1 mM Tris HCl buffer, pH 7.4, and finally was suspended in this buffer at a final concentration of 2.5 mg protein/ml. The above procedures were carried out at 0-4°C. The repeated washings were done to remove intracellular organelles.

Calcium Binding Study

Calcium binding was performed utilizing a millipore filtration system. Incubation (total volume 2 ml) contained 1.75 ml of
Kreb-Henseleit bicarbonate buffer containing 0.2 μCi/ml $^{45}$Ca, 0.05 ml of taurine solution (40 x final concentration), and 0.2 ml of membrane suspension, such that final protein content was 0.25 mg of protein per assay tube. The Kreb-Henseleit buffer was corrected for volume dilution and the reaction was started by the addition of protein. The reaction tubes were incubated at room temperature to lower dissociation rate, and the reaction was stopped after 4 minutes by rapid filtration under vacuum on cellulose acetate filters (0.5 μm pore size) for 5 seconds. Effluent was counted for radioactivity. Binding calculated from effluent activity was checked by counting the filter paper. In this case, 5 ml of ice cold 1 mM Tris was added to the incubation medium and was filtered rapidly. The filters were washed three times with 5 ml volumes of ice cold Tris and the filter was counted for radioactivity retained.

**Protein Determination**

The protein concentration was determined according to the method of Lowry et al. (1951) with bovine serum albumin serving as the standard.

**Effect of Amrinone on Rate of Taurine Influx**

Hearts from rats weighing 200-250 g or guinea pigs weighing 275-325 g were perfused at a flow rate of 6 ml/min with Kreb-Henseleit bicarbonate buffer. After 6 minutes of perfusion, the buffer was switched to one containing amrinone (10 μg/ml with rat hearts or 15 μg/ml with guinea pig hearts plus $[^3]$H)-taurine (25 μM; 0.05 μCi/ml) and the hearts were perfused for five minutes, followed by a 6 minute
washout. The radioactivity remaining in the heart was determined as described.

**Effect of Amrinone on Contractility and Heart Rate**

Guinea pig hearts were perfused at a flow rate of 6 ml/min with K-H buffer. After six minutes of perfusion, the buffer was switched to one containing 10, 15, or 20 μg/ml amrinone, molecular weight (MW) 187.2, and the hearts were perfused for five minutes followed by a washout of six minutes. The heart's contractile force and rate were measured as described above.

**Influx Rate of Radiolabeled Calcium**

Guinea pig hearts were perfused at a flow rate of 6 ml/min with K-H buffer. After 6 minutes of perfusion, the buffer was switched to one containing $^{45}\text{CaCl}_2$ (0.032 μCi/ml, 2.5 mM) and the hearts were perfused for five minutes followed by a washout of five minutes. The rate of calcium influx was measured as described above.

**Efflux of $^{45}$Calcium**

The male guinea pig hearts were perfused at a flow rate of 6 ml/min with K-H buffer. After 6 minutes of perfusion, the buffer was switched to one containing $^{45}\text{CaCl}_2$ (0.05 μCi/ml, 2.5 mM) and the hearts were perfused for five minutes followed by a washout with K-H buffer for five minutes. During the final washout, 40 drops (1.0 ml) of effluent were collected using a fraction collector (Gilson's Micro Fractionator). Samples of effluents were counted for radioactivity by liquid scintillation counter.
Tissue Preparation for Cyclic AMP Determination

The hearts from male guinea pigs were perfused at a flow rate of 6 ml/min with K-H buffer. After 6 minutes of perfusion, the buffer was switched to one containing either $8 \times 10^{-7}$ M isoproterenol, or 10 and 20 $\mu$g/ml of amrinone MW = 187.2. During perfusion, the contractile force of the heart was measured as described above, and immediately at the end of a five minute perfusion, the hearts were rapidly frozen in liquid nitrogen. The frozen hearts were placed on dry ice for trimming the fat and large vasculature. The hearts, while frozen, were homogenized in 10% trichloroacetic acid, 1.0 g heart per 4.0 ml, at maximal speed with a Polytron for 15 seconds. The homogenized hearts were centrifuged at 40,000 x g for 20 minutes and part of supernatant was prepared for cyclic AMP determination. The supernatant (1 ml) was washed with four volumes of water-saturated ether to extract trichloroacetic acid. This process was repeated five times. The ether extracted supernatant was placed in 40°C water bath for 15 minutes to evaporate the ether. The level of cyclic AMP was determined using a cyclic AMP assay kit.

Cyclic AMP Determination

The principle of the cyclic AMP assay kit (Amersham) is based on the competition between unlabeled cyclic AMP and a fixed quantity of tritium-labeled cyclic AMP for binding to a protein which has a high specificity for cyclic AMP. The amount of labeled cyclic AMP protein complex formed is inversely related to the amount of unlabeled cyclic AMP present in the assay. The concentration of cyclic AMP in the unknown is determined by comparison with a linear standard curve.
Spectral Analysis

The cardiomyopathic (CM) and random bred hamsters were sacrificed by decapitation. The hearts were removed and washed in 0.9% saline solution containing glucose (1.3 g/l). Atria, right and left ventricles were dissected, washed and blot dried before transfer to pre-weighed calcium-free volumetric flasks. The samples were digested for one hour in 65% ultrapure nitric acid (EM Laboratories, Elmford, NY) on a steam bath. Sufficient ionization buffer containing cesium was added to make the final solution 1000 ppm (parts per million) cesium. After cooling, the samples were diluted with distilled deionized water. Precipitated proteins were filtered and the solution analyzed by conventional atomic absorption spectrophotometry with a Varian AA5. An air/acetylene flame was used for iron and a nitrous oxide/acetylene flame for calcium and magnesium. The analytical wave lengths used were 422.7, 285.2, and 248.3 mm, respectively, for calcium, magnesium, and iron.

Taurine Analysis

The cardiomyopathic and random bred hamsters were sacrificed by decapitation and the heart removed, washed in saline, and dissected into areas. The blot dried sections were transferred to pre-weighed microfuge tubes. Each gram of tissue was diluted with 10 milliliters of 3.5% 5-sulfosalicylic acid, sonicated to homogeneity, centrifuged, and a 250 microliter sample of supernatant was analyzed for concentration of taurine using an amino acid analyzer (Model 118C, Beckman).
Receptor Binding Assay

The ventricles from cardiomyopathic and random bred hamsters, after the perfusion of the excised hearts with 10 ml of saline, were homogenized with a polytron homogenizer (Brinkman) at setting #5, for 30 seconds to make a 2.5% homogenate in 0.05 M Na⁺-K⁺ phosphate buffer (pH 7.4). The homogenate was passed through several layers of cheesecloth and centrifuged at 48,000 x g for 20 minutes in a Sorvall RC2-B centrifuge. The supernatant was measured and discarded. The pellets were washed three times with Na⁺-K⁺ phosphate buffer and finally were suspended in Na⁺-K⁺ phosphate buffer in a volume equal to that of the discarded supernatant. The suspension was re-homogenized for five seconds. β-Adrenergic receptor binding was assayed by the method of Bylund and Snyder (1976). Tissue homogenates containing 80 µg protein were incubated for 30 minutes at 25°C in 2 ml of 0.05 M Na⁺-K⁺ phosphate buffer containing 0.25 µM [³H]-dihydroalprenolol (DHA; 58 Ci/m mole) in the presence and absence of 0.1 µM (-)-propranolol. The reaction was terminated by vacuum filtration through GF/B glass fiber filters followed by four 5 ml rinses with buffer kept at 25°C. Bound [³H]-DHA retained on the filter was extracted in 9 ml of a toluene-based scintillation cocktail and radioactivity counted. Specific [³H]-DHA binding was defined as the binding displaceable by 0.1 M (-)-propranolol. The protein determinations for receptor binding studies of the ventricles were performed by the method of Lowry as described above.
Rate of Taurine Influx in Hamsters

The hearts from cardiomyopathic and random bred hamsters were perfused by a Langendorff technique as described above, with slight modifications. The resistance in the lines was lowered (by clamping the lines attached to bubble traps), the flow rate at 4 ml/min, and the hearts were perfused with K-H buffer for five minutes. Then, the buffer was switched to one containing $[^3]$H-taurine (25 μM, 0.05 μCi/ml) and perfused for five minutes. Following the perfusion with substrate, there was a five minute washout. The influx rate was determined in the way described above.

Dietary Taurine Treatment

The cardiomyopathic (CM) hamsters at their pre-lesion age (30-40 days old) were placed on 0.1 M solution of taurine as their drinking water. Such dietary regimen lasted either 30 days or 120 days. The animals were kept one or two to a cage and their water consumption was established prior to the experiment. The control animals were the CM hamsters on regular drinking water.

Sample Preparation for Histology

The cardiomyopathic hamsters that were placed on dietary taurine were sacrificed by decapitation. The hearts were rapidly removed and transferred to ice chilled 0.9% saline. They were perfused with 10 ml of cold saline to remove the blood. The hearts were cut into upper and lower sections by a transverse sectioning. Each time an alternating section was fixed in 10% buffered formalin. The other half was used for ionic and taurine determination. Hematoxalin-eosin stained, six
micron thick sections were prepared from paraffin-embedded tissues. Von Kossa stained sections were prepared for further confirmation of the calcium deposition in the myocardium.

Lesion Evaluation

The microscopic slides were prepared from cardiomyopathic and cardiomyopathic hamsters placed on 0.1 M taurine were evaluated for calcium deposition in a single blind study. Grading was based on a 0-4+ scale, with 4+ representing the highest level. The preparation of slides and scorings was carried out by Dr. Peter Brumbaugh from the Department of Pathology, The University of Arizona.

Materials

Radiolabeled taurine [3H]-taurine (5 mCi/mmol) was obtained from New England Nuclear (NEN). Radiolabeled calcium 45CaCl2 (1 µCi/mmole) and cyclic AMP assay kit were obtained from Amersham (Arlington Heights, IL). Amrinone was a gift from Sterling-Winthrop Research Institute. Ionophores were obtained from Eli Lilly Laboratories. The remainder of the chemicals were from Sigma Company, except for guanidoethyl sulfonate which was synthesized in our laboratory.

Calculations

Statistical analyses and the linear regression for determination of slopes were performed on a Wang Series 700 programmable calculator. All values are expressed as means ± standard error of the mean (SEM) and a difference with probability of < 0.05, as calculated by the Student's paired or unpaired 't' test, were considered significant.
CHAPTER 3

RESULTS

Mechanism of the Adrenergic Stimulation
of Taurine Influx in the Heart

Adrenergic stimulation of the heart produces manifold changes, any one of which could be responsible for the increased influx of taurine. Such changes include increased heart rate, increased cyclic AMP levels, increased calcium influx, and enhanced sodium-calcium exchange. The effect of heart rate and increases in cyclic AMP levels on the rate of taurine influx have been measured. The following experiments attempt to elucidate the involvement of other changes resulting from β-adrenergic receptor stimulation in the heart on rate of taurine influx.

Effect of Glucagon on Taurine Influx

An increase in cell cyclic AMP levels is a necessary component of catecholamine-induced stimulation of taurine influx. Glucagon, a polypeptide hormone from the pancreas, also increases cyclic AMP levels by a non-adrenergic mechanism. I examined the effect of this agent on taurine influx to establish whether adrenergic receptors were necessarily involved in the response. As shown in Table 3, at inotropic concentrations of glucagon, taurine influx is stimulated. Glucagon has a biphasic effect on cardiac contractility. At 0.75 mg/ml, there is an immediate 25-30% increase in contractility which reached its maximum
Table 3. Effect of Glucagon on Taurine Influx Rate in the Heart

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Tension (g)</th>
<th>4 min Tension (g)</th>
<th>Initial Taurine Influx Rate (nmole/min/g wet wt)</th>
<th>4 min Taurine Influx Rate (nmole/min/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.2±0.3</td>
<td>4.8±0.3</td>
<td>2.3±0.2</td>
<td>11.0±0.7</td>
</tr>
<tr>
<td>Glucagon</td>
<td>6.4±0.7</td>
<td>10.1±1.0</td>
<td>3.0±0.1</td>
<td>13.3±0.7</td>
</tr>
</tbody>
</table>

*ap < 0.01 compared to control.

*bp < 0.01 compared to initial.

Hearts were perfused with a Krebs-Henseleit bicarbonate buffer. The perfusate contained 0.75 mg/ml of glucagon, and ³H-taurine (25 μM; 0.05 μCi/ml). The influx rate was determined during positive ionotropic effect of glucagon 4 min from the initiation of perfusion. Data are expressed as means ± SEM for 5 hearts per group.

effect at four minutes. After this, however, a decrease in contractility was observed. The measurements for the rate of taurine influx were carried out at the time of maximum stimulation in contractility.

Effect of Ionophores on Taurine Influx

Ionophores are antibiotic substances capable of inducing the passage of specific ions across biological membranes. Ionophores stimulate the passive movement of ions down their concentration gradient. The effects of three ionophores on the taurine influx rate were examined to unravel the movement of cations and contractility in response to isoproterenol. Prior to testing the effects of ionophores in Langendorff perfused hearts, rats were given IP injection of reserpine to deplete the heart's catecholamines. In reserpine-treated
hearts, the sodium ionophore, monensine, decreased the heart rate by 8-10% and stimulated the contractile force by 10-17%. The calcium ionophore, A23187, beside its vasodilator effect which caused a decrease in aortic perfusion pressure, showed a mild biphasic ionotrophic effect. The contractile force initially decreased by 10-15% followed by an increase. When A23187 was then removed from the perfusate, the contractile force dropped by 27%. There was no significant effect on the heart rate. Valinomycin, a potassium ionophore, has a potent action on contractility. Numerous concentrations were tested in order to find a suitable level at which the heart's contractility decreased without stopping the heart. At $1 \times 10^{-7}$ M, the contractile force was decreased by 90-95% within three minutes and the effect could not be reversed during washout. At $1 \times 10^{-8}$ M valinomycin, there is a 40-50% decrease in contractility within four minutes of perfusion. Again, washout did not reverse the negative inotropy completely. Valinomycin did not have any chronotropic effect. The results shown in Table 4 indicate that no statistically significant changes occurred in the rate of taurine influx in these experiments.

Involvement of Calcium and Inotropy in Taurine Influx

One of the events involved in isoproterenol stimulated hearts is an increase in the rate of calcium influx. Ionophore A23187, which stimulates calcium influx, had no effect on the rate of taurine influx. Verapamil was used to examine the effect of calcium blockade on taurine influx. Electrophysiological studies using voltage clamping have shown that verapamil selectively inhibits the transmembrane flux
Table 4. Effect of Ionophores on Taurine Influx

<table>
<thead>
<tr>
<th>Ionophore</th>
<th>Concentration (M)</th>
<th>Tension (g)</th>
<th>Taurine Influx Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Peak</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>4.91 ± 0.21</td>
<td>4.53 ± 0.33a</td>
</tr>
<tr>
<td>Monensin</td>
<td>$1 \times 10^{-6}$</td>
<td>5.87 ± 0.19</td>
<td>6.77 ± 0.47a*</td>
</tr>
<tr>
<td>A23187</td>
<td>$1 \times 10^{-6}$</td>
<td>6.35 ± 0.31</td>
<td>5.52 ± 0.29b*</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>$1 \times 10^{-8}$</td>
<td>5.46 ± 0.17</td>
<td>2.94 ± 0.22a*</td>
</tr>
</tbody>
</table>

* $p < .001$ compared to initial value.

a Tension development 3-4 min after initiation of perfusion.

b Decrease occurred within 1 min of perfusion, followed by an increase (not included in the table) to 6.58 ± 0.34 g after 3-4 min consistent with biphasic response elicited by A23187 on tension development in the heart. Once A23187 was removed from perfusion medium during final wash, the tension was 5.17 ± 0.5 (not included in the table). Statistical comparison of the tension developed within 3-4 min after initiating perfusion with A23187 (6.58 ± 0.349) with that during final wash (5.17 ± 0.45 g) was significant ($p < 0.001$).

Perfusion medium contained the indicated ionophore and $[^3]$H]taurine (25 μM). Seven hearts were used per group and data reported as means ± SEM.
of calcium (Kohlhardt, Bauer, and Krause, 1972). The blockade of calcium flux with verapamil \(3.4 \times 10^{-7}\) M in isolated perfused rat hearts decreased the contractility by 30-40%, but had no effect on the rate of taurine influx. Co-perfusing the hearts with isoproterenol and verapamil, the calcium movement across the membrane was blocked with verapamil while the \(\beta\)-receptor stimulation in the heart, causing an increase in cyclic AMP levels, was maintained using isoproterenol. Hence, the increased contractility caused by isoproterenol was antagonized. Whereas verapamil alone had no effect on the rate of taurine influx, co-perfusion with verapamil plus isoproterenol increased the rate of taurine influx to the level of that of isoproterenol alone (Table 5). This indicates that increased calcium influx, or any of its consequences, such as positive inotropy, is not needed to observe a stimulation in taurine influx. By a process of elimination, my results indicate that the only factor contributing to the increased rate of taurine influx is stimulation in the levels of cyclic AMP. At the cellular level, the increased levels of cyclic AMP bring about numerous changes. One of these changes is membrane phosphorylation. In Chapter 4, in accord with membrane phosphorylation, I have presented a model describing the increased rate of taurine influx in response to \(\beta\)-adrenergic stimulation.

**Specificity of the Taurine Transporting System in the Heart**

Taurine transport in isolated rat hearts is a saturable process with a \(V_{\text{max}} = 32\) nmoles/g dry wt/min and a \(K_m = 45\) \(\mu\)M (Huxtable and Chubb, 1977). Furthermore, its transport is inhibited by metabolic...
Table 5. Effect of Verapamil, Isoproterenol, and Verapamil Plus Isoproterenol on Taurine Influx

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (x 10^7 M)</th>
<th>Tension (g)</th>
<th>Taurine Influx Rate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Peak</td>
<td>nmole/min/</td>
<td>nmole/min/</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g wet weight</td>
<td>g dry weight</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>5.46 ± 0.63</td>
<td>5.80 ± 0.63^a</td>
<td>2.7 ± 0.1</td>
<td>13.7 ± 0.7</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4</td>
<td>4.13 ± 0.58</td>
<td>5.96 ± 0.79^b**</td>
<td>3.2 ± 0.1*</td>
<td>16.0 ± 0.2*</td>
</tr>
<tr>
<td>Verapamil</td>
<td>3.4</td>
<td>5.14 ± 0.50</td>
<td>2.31 ± 0.21^a**</td>
<td>2.8 ± 0.2</td>
<td>13.2 ± 0.8</td>
</tr>
<tr>
<td>Isoproterenol plus Verapamil</td>
<td>4</td>
<td>4.48 ± 0.59</td>
<td>4.02 ± 0.50^a</td>
<td>3.2 ± 0.2*</td>
<td>16.7 ± 1.0*</td>
</tr>
</tbody>
</table>

^p < .05 relative to control.

^^p < .001 relative to initial values.

^Measurement after 4 min from initiation of perfusion,

bMeasurement after 0.5 min perfusion,

Perfusion medium contained indicated compound and [3H]taurine (25 μM). Eight to ten hearts were used per group, and data are reported as means ± SEM.
inhibitors (Kromphardt, 1963; Gaut and Nauss, 1976). Such findings indicate that taurine influx occurs by a carrier mediated transport process (Lehninger, 1975). This type of process involves a number of steps. First, taurine binds to an active carrier site on the membrane. Then translocation of the taurine carrier complex occurs, followed by taurine release from the carrier. This carrier has recently been isolated from rat heart sarcolemma by Kulakowski, Maturo, and Schaffer (1978). Carrier-mediated transport sites usually show a structural specificity for their substrate. To examine the structural specificity for taurine transport, I studied the effect of various taurine analogs in isolated perfused rat hearts, for their competition with the taurine carrier. Among the analogs tested (Fig. 3), hypotaurine, β-alanine, β-guanidoethyl sulfonate, and β-guanidopropionate showed statistically significant inhibition of taurine transport (Table 6). The effective inhibitors had a close resemblance to taurine.

Transport of β-Guanidoethyl Sulfonate

Among the analogs that inhibit taurine transport, β-guanidoethyl sulfonate was tested further. The choice of selection was based on the absence of this compound in mammalian heart (Guidotti and Costagli, 1970), being a rather potent transport inhibitor, and was claimed (Guidotti and Costagli, 1970) not to have pharmacological actions of its own. β-Guanidoethyl sulfonate shows a saturable transport by the isolated heart with a $K_m$ of 153 μM and a $V_{max}$ of 64.6 nmole/min/g dry weight (Fig. 4).
Taurine Analogs

\[
\begin{align*}
\text{Hypotaurine} & : H_2N\cdot CH_2\cdot CH_2\cdot SO_2H \\
\text{\(\beta\)-Alanine} & : H_2N\cdot CH_2\cdot CH_2\cdot CO_2H \\
\text{2-Aminoethyl phosphonic acid} & : H_2N\cdot CH_2\cdot CH_2\cdot PO_3H_2 \\
\text{Guanidoethyl-sulfonate} & : H_2N\cdot C\cdot NH\cdot CH_2\cdot CH_2\cdot SO_3H \\
\text{Guanidopropionate} & : H_2N\cdot C\cdot NH\cdot CH_2\cdot CH_2\cdot CO_2H \\
\text{Nicotinic Acid} & : \text{CH}_3\cdot \text{C}\cdot \text{NH}\cdot \text{CH}_2\cdot \text{CO}_2H \\
\text{Acetimidoyl glycine} & : H_3C\cdot \text{CH}--\text{CH}\cdot \text{CH}_3 \\
\text{2-Aminocylohexane sulfonate} & : \text{H}_2\text{N}\cdot \text{CH}_2\cdot \text{CH}_2\cdot \text{SO}_3H \\
\text{3-Aminobutene-2-sulfonate} & : \text{H}_2\text{N}\cdot \text{CH}--\text{CH}\cdot \text{CH}_3
\end{align*}
\]

Fig. 3. Taurine Analogs
Table 6. Effect of Co-Perfused Analogs on Taurine Influx in the Heart

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μM)</th>
<th>Influx Rate (nmole/min/g dry weight)</th>
<th>% Change</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (n)</td>
<td>Treated (m)</td>
<td></td>
</tr>
<tr>
<td>Hypotaurine</td>
<td>150</td>
<td>10.6 ± 0.6 (4)</td>
<td>1.8 ± 0.5 (4)</td>
<td>-82.3</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>500</td>
<td>21.4 ± 1.2 (4)</td>
<td>3.6 ± 0.8 (4)</td>
<td>-59.5</td>
</tr>
<tr>
<td>β-Guanidoethyl sulfonate</td>
<td>150</td>
<td>18.4 ± 0.5 (6)</td>
<td>10.9 ± 0.3 (8)</td>
<td>-40.6</td>
</tr>
<tr>
<td>β-Guanidopropionate</td>
<td>150</td>
<td>13.2 ± 0.4 (9)</td>
<td>10.4 ± 0.1 (11)</td>
<td>-21.3</td>
</tr>
<tr>
<td>3-Aminobutane-2-sulfonate</td>
<td>150</td>
<td>13.8 ± 0.2 (5)</td>
<td>12.3 ± 0.6 (5)</td>
<td>+10.7</td>
</tr>
<tr>
<td>2-Aminocyclohexane sulfuronate</td>
<td>150</td>
<td>10.6 ± 0.6 (5)</td>
<td>10.3 ± 0.6 (5)</td>
<td>+3.0</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>150</td>
<td>10.8 ± 0.4 (3)</td>
<td>12.0 ± 0.8 (3)</td>
<td>+11.4</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>10.0 ± 0.2 (8)</td>
<td>12.4 ± 0.3 (7)</td>
<td>+24.9</td>
</tr>
<tr>
<td>Acetimidoyl glycine</td>
<td>150</td>
<td>18.0 ± 0.7 (5)</td>
<td>19.8 ± 0.8 (5)</td>
<td>+8.9</td>
</tr>
<tr>
<td>2-Aminoethyl phosphonate</td>
<td>150</td>
<td>15.2 ± 0.5 (4)</td>
<td>15.6 ± 0.5 (5)</td>
<td>+2.6</td>
</tr>
</tbody>
</table>

Perfusion medium contained the indicated analog and $[^3]$H]taurine (25 μM). Data are reported as means ± SEM. Number of animals per group given in parentheses.
Fig. 4. Guanidoethyl sulfonate Lineweaver-Burk Plot — Hearts were perfused with β-guanidoethyl sulfonate over a concentration range of 25 to 100 μM with a Krebs-Henseleit buffer containing [³H]guanidoethyl sulfonate specific activity 4.20 mCi/mmole. Slope is derived from a linear regression analysis of the data. Each point represents Mean ± SEM from 4-6 hearts.

$V_{\text{max}} = 64.6 \text{nmole/min/g}$

$K_m = 153 \mu M$
Effect of Isoproterenol on β-Guanidoethyl Sulfonate Influx

β-Guanidoethyl sulfonate inhibits taurine transport by competing with taurine for the same carrier protein. Since isoproterenol stimulates the rate of taurine influx, it was of interest to test the effect of isoproterenol on the influx rate of β-guanidoethyl sulfonate. Isoproterenol at $4 \times 10^{-7}$ M, a concentration at which the maximal effect on the rate of taurine influx is achieved (Chubb and Huxtable, 1978a), stimulated the influx of β-guanidoethyl sulfonate (Table 7).

Positive Inotropic Effect of Taurine and β-Guanidoethyl Sulfonate

There are numerous reports as to the positive inotropic effect of taurine; however, such studies have employed high concentrations of taurine. Since calcium has no effect on taurine transport as shown above, the effect of taurine on calcium flux was examined. Perfusing of the hearts with a normal Krebs-Henseleit buffer containing a more reasonable concentration of taurine or β-guanidoethyl sulfonate (0.1 mM), there was a decrease in contractility within one minute from initiating perfusion followed by an increase in contractility. However, these changes were not pronounced. Perfusion of the hearts with a Krebs-Henseleit buffer containing 0.5 mM calcium (1/5 of normal calcium) and 0.1 mM taurine or β-guanidoethyl sulfonate, a similar but more pronounced biphasic response was elicited (Fig. 5). Such results were interpreted as to the effect of these compounds on cardiac membrane(s), resulting in the modification of calcium influx. The
Table 7. Effect of Isoproterenol on β-Guanidoethyl Sulfonate Influx

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>β-Guanidinoethyl Sulfonate Influx</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>β-Guanidinoethyl Sulfonate Influx</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n mole/min/g wet wt</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>1.98 ± 0.05</td>
<td>10.60 ± 0.63</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>5</td>
<td>2.40 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.93 ± 1.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> <i>p < 0.05</i>.

Hearts were perfused with Krebs-Henseleit buffer containing 4 x 10<sup>-7</sup> M isoproterenol and 25 μM [3H] guanidoethyl sulfonate (0.05 μCi/ml). Data are expressed as mean ± SEM.
Fig. 5. Inotropic Effect of Taurine and Guanidoethyl sulphonate (GES) in Rat Hearts — Hearts were perfused with Krebs-Henseleit buffer containing 0.5 mM calcium for 4 min, then switched to a low calcium buffer containing 0.1 mM taurine or β-guanidoethyl sulphonate, and perfused for 3 min. Following perfusion, hearts were washed with low calcium buffer for 4 min and again perfused with low calcium buffer containing 0.1 mM taurine or β-guanidoethyl sulphonate. Data are expressed as means ± SEM for 4 hearts per group. In statistical analysis 'a' was taken in reference to initial baseline contractility, 'b' taken in reference to the baseline contractility at final washout.
increase of contractility by taurine was higher than that produced by 
β-guanidoethyl sulfonate.

Effect of Taurine on Sarcolemmal Calcium Binding

There are a number of reports indicating taurine to have no
effect on calcium binding, or release from cardiac sarcoplasmic 
reticulum (Chubb and Huxtable, 1978b; Entman et al., 1977; Remtulla et 
al., 1978). Based on my results presented above, the inotropy resulting 
from taurine and β-guanidoethyl sulfonate may result from the effect of 
these substrates in modulating calcium influx at the sarcolemma. To 
test the membrane effect of taurine, a sarcolemma calcium binding in 
the presence of taurine was performed. Taurine at concentrations 
ranging from 1-10 mM decreases calcium binding to the sarcolemma in a 
dose-dependent relation (Fig. 6). The difference in the inhibition of 
calcium binding to the sarcolemma at concentrations of 5 and 10 mM 
taurine is not statistically significant (Table 8). This implies that 
the effect of taurine in modifying calcium binding to the cardiac 
membrane(s) is saturable. My results are in disagreement with those of 
Chovan et al. (1979). They have reported that taurine enhances the 
binding of calcium to the low affinity sites on the sarcolemma. However, 
taurine decreasing the binding of calcium to the sarcolemma is con­ 
sistent with the positive inotropy produced by taurine.

Effect of Amrinone on Cyclic AMP Levels

The positive inotropic effect of amrinone in dogs has been shown 
to be independent of increased cyclic AMP levels (Farah and Alousi,
Fig. 6. Effect of Taurine on Calcium Binding to Sarcolemma -- Calcium binding was determined by a millipore filtration technique. Incubation medium (final volume 2 ml) contained Krebs-Henseleit buffer, indicated concentration of taurine, and 0.2 μCi/ml 45Ca. The reaction was started by the addition of a sarcolemmal suspension, such that final protein concentration was 0.25 mg/assay tube. The reaction tubes were incubated at room temperature for 4 min. The reaction was stopped by rapid filtration under vacuum on cellulose acetate filter (0.05 μM pore size) for 5 sec and effluent was counted for radioactivity. Data points are the means of 4 samples in each group, and values are given ± SEM.
Table 8. Effect of Taurine on Calcium Binding to Sarcolemma

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>nmole (^{45}\text{Ca/mg protein bound} )</th>
<th>% decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>204.6 ± 17.7</td>
</tr>
<tr>
<td>Taurine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>141.8 ± 16.5(^a)</td>
<td>30.7</td>
</tr>
<tr>
<td>2.5</td>
<td>81.60 ± 10.7(^b)</td>
<td>60.2</td>
</tr>
<tr>
<td>5.0</td>
<td>51.30 ± 7.3(^b)</td>
<td>74.9</td>
</tr>
<tr>
<td>10.0</td>
<td>42.30 ± 10.4(^b)</td>
<td>79.3</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.025.  
\(^b\) p < 0.01.

Calcium binding was determined by a millipore filtration technique. Incubation medium (final volume 2 ml) contained Krebs-Henseleit buffer, indicated concentration of taurine, and 0.2 μCi/ml \(^{45}\text{Ca}\). The reaction was started by the addition of a sarcolemmal suspension, such that final protein concentration was 0.25 mg/assay tube. The reaction tubes were incubated at room temperature for four minutes. The reaction was stopped by rapid filtration under vacuum, on cellulose acetate filters (0.05 μM pore size) for 5 seconds and effluent was counted for radioactivity. Data points are the means of four samples in each group and values are given as ± SEM.
A determination of the effect of this compound on cyclic AMP levels in guinea pig was performed. At concentrations of 10 and 20 μg/ml in perfused guinea pig hearts, amrinone produced no effect on the levels of cyclic AMP even though these doses produced maximum inotropic effect (Table 9). Isoproterenol induces its inotropic effect through increased cyclic AMP levels which are responsible for stimulating calcium influx. Compared to isoproterenol which stimulates cyclic AMP levels 1.5-fold and contractility by 78%, the non-cyclic AMP dependent inotropy by amrinone at 100-fold higher concentration compared to the isoproterenol only produces 36% stimulation in contractility.

Effect of Amrinone on Contractility and Heart Rate

In order to establish a dose-response relation for amrinone and to measure the effect of this drug on the heart rate, concentrations of amrinone from 10-20 μg/ml MW = 187.2 were used. The effect of this drug in stimulating contractility appears to have reached its maximum at 10 μg/ml. Further increases beyond 10 μg/ml do not have a pronounced effect on contractility (Table 10). Comparing the effect of amrinone on contractility with that of other agents such as glucagon and isoproterenol, amrinone at twice the concentration for the maximal effect (20 μg/ml) does not alter contractility, but it does not produce a toxic response either. Isoproterenol and glucagon beyond the doses at which their maximal stimulation of contractility is achieved, induce a toxic effect. Their toxic effect is manifested in fibrillation, decreased contractility, and very often the contracture of cardiac muscle or irreversible contracture.
Table 9. Effect of Amrinone on Cyclic AMP Levels

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Tension (g)</th>
<th>Cyclic AMP (nmole/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>5 min</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>5.08 ± 0.2</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>8 x 10^{-7}</td>
<td>5.07 ± 0.4</td>
</tr>
<tr>
<td>Amrinone</td>
<td>5.3 x 10^{-5}</td>
<td>5.01 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>10.7 x 10^{-5}</td>
<td>4.75 ± 0.2</td>
</tr>
</tbody>
</table>

*Contractile force recorded prior to exposure to amrinone.

*bContractile force recorded after five minutes of perfusion with amrinone.

*c_p < 0.025.

Isolated guinea pig hearts were perfused with amrinone for five minutes. At the end of this time, they were rapidly frozen in liquid nitrogen. The frozen hearts were trimmed, homogenized in 10% trichloroacetic acid (TCA), centrifuged, and the TCA from supernatant was extracted with water saturated ether. The cyclic AMP levels were determined using cyclic AMP assay kit from Amersham. Data shown as means ± SEM for five hearts per group except in case of isoproterenol which there were four hearts.
<table>
<thead>
<tr>
<th>Amrinone Concentration (µg/ml)</th>
<th>Tension (g)</th>
<th>Heart Rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 min&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>5.15 ± 0.2</td>
<td>5.09 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>5.12 ± 0.2</td>
<td>6.64 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>4.99 ± 0.5</td>
<td>6.49 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>4.89 ± 0.4</td>
<td>6.52 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Contractile force and heart rate prior to exposure to amrinone.

<sup>b</sup> Contractile force and heart rate after 5 minutes of perfusion with amrinone.

<sup>c</sup> p < 0.01.

Isolated guinea pig hearts were perfused by Langendorff technique with Krebs-Henseleit buffer. Hearts were perfused for six minutes. The buffer was switched to one containing indicated concentration of amrinone and the hearts were perfused for five minutes followed by a washout of six minutes. Data shown as means ± SEM for 7-8 hearts per group.
Effect of Amrinone on Rate of Taurine Influx

Previously, it was stated that in rat hearts the increased rate of taurine influx is independent of ionic flux and inotropy, but depends on increased levels of cyclic AMP. Since amrinone was claimed not to have a significant electrophysiological effect in heart (Farah and Alousi, 1978), has an inotropic effect, and does not alter the levels of cyclic AMP, it was of interest to test the effect of amrinone on the rate of taurine influx. In isolated perfused rat hearts, amrinone at 10 μg/ml had no effect on the rate of taurine influx (Table 11). However, in isolated perfused guinea pig hearts, amrinone stimulates the rate of taurine influx (Table 12). Comparing the rate of taurine influx in these species, the rate of uptake in control guinea pigs is 40% higher than that in rats. Guinea pigs are herbivores and because of a lack of taurine in the plant world, they must synthesize their own taurine. By contrast, rats maintain their cardiac taurine levels by an active transport. Therefore, the different results obtained in rats versus guinea pigs could be due to inherent differences between these two species and might be due to a different mechanism(s) involved in taurine transport.

Calcium Efflux from Amrinone Treated Hearts

Amrinone produces an inotropic effect without alterations in the levels of cyclic AMP. Since increased levels of cyclic AMP lead to an increase in the rate of calcium influx into the heart, it was interesting to see whether amrinone could modify calcium kinetics
Table 11. Effect of Amrinone on Rate of Taurine Influx in Rat Hearts

<table>
<thead>
<tr>
<th>Influx Rate</th>
<th>Heart Weight Wet</th>
<th>Heart Weight Dry</th>
<th>nmole/min/g wet wt</th>
<th>nmole/min/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.70 ± .03</td>
<td>0.10 ± .00</td>
<td>3.30 ± 0.1</td>
<td>22.5 ± 0.4</td>
</tr>
<tr>
<td>Amrinone</td>
<td>0.68 ± .01</td>
<td>0.11 ± .00</td>
<td>3.72 ± 0.2</td>
<td>21.7 ± 1.3</td>
</tr>
</tbody>
</table>

Isolated rat hearts were perfused with Krebs-Henseleit buffer for six minutes, the buffer was switched to one containing 10 µg/ml amrinone plus 25 µM taurine (0.05 µCi/ml), and hearts were perfused for 6.0 minutes followed by a washout at six minutes. The rate of influx was determined from radioactivity left in the heart after the washout period. Data shown as mean ± SEM for eight hearts per group.

Table 12. Effect of Amrinone on Rate of Taurine Influx in Guinea Pig Hearts

<table>
<thead>
<tr>
<th>Influx Rate</th>
<th>Heart Weight Wet</th>
<th>Heart Weight Dry</th>
<th>nmole/min/g wet wt</th>
<th>nmole/min/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00 ± 0.1</td>
<td>0.19 ± .01</td>
<td>6.02 ± 0.6</td>
<td>30.3 ± 2.9</td>
</tr>
<tr>
<td>Amrinone</td>
<td>1.14 ± 0.1</td>
<td>0.22 ± .06</td>
<td>8.40 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.2 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.01.

Isolated guinea pig hearts were perfused with Krebs-Henseleit buffer for six minutes, the buffer was switched to one containing 10 µg/ml amrinone plus 25 µM taurine (0.05 µCi/ml), and hearts were perfused for 6.0 minutes followed by a washout of six minutes. The rate of taurine influx was determined from radioactivity left in the heart after the washout period. Data shown as mean ± SEM for 11 hearts per group.
independent of cyclic AMP. The isolated guinea pig hearts were perfused with Krebs-Henseleit buffer containing $^{45}\text{CaCl}_2$ (0.05 μCi/ml, 2.5 mM) for five minutes, then washed for five minutes with regular Krebs-Henseleit buffer. During the five minute washout, fractions were collected for analysis of radiolabeled calcium. The slopes of washout either from extracellular or intracellular regions are not different from the amrinone treated versus control hearts (Fig. 7). The apparent half-life of washout also was not altered (Table 13). However, the interstitial pool size (A) shows a statistically significant increase in amrinone treated hearts, while the intracellular pool size (B) is not significantly altered (Table 13). Repeating the experiment in a similar fashion as above and measuring the residual radioactive calcium left in the heart, I found that the amrinone treated hearts show higher levels of calcium. This residual calcium may be due to the calcium left in pool A (Table 14).

**Localization of Calcium Overload**

In cardiomyopathic (CM) hamsters, the calcium overload is responsible for cardiomyopathy (Bajusz, 1969). Furthermore, it is not clear whether alterations in the levels of iron and magnesium are the cause or the result of cardiomyopathy. I made a regional cation analysis in 30-40 day old hamsters (pre-lesioning age) in order to localize the site for calcium overload. Among the regions studied, the left ventricle in the CM animals showed a higher calcium concentration, compared to the random bred (RB) animals. The difference in the calcium
Fig. 7. Calcium Efflux from Amrinone-Treated Guinea Pig Hearts -- Isolated guinea pig hearts were perfused at a flow rate of 6 ml/min with Krebs-Henseleit buffer. After 6 min of perfusion, the buffer was switched to one containing $^{45}\text{CaCl}_2$ (0.05 μCi/ml, 2.5 mM) plus 15 μg/ml amrinone. The hearts were perfused for five min. During the final washout with Krebs-Henseleit buffer effluent was collected and measured for radioactivity. Data show as means of five control and seven experimental hearts.
Table 13. Calcium Efflux from Amrinone Treated Guinea Pig Hearts

<table>
<thead>
<tr>
<th></th>
<th>Apparent Half-Life of Washout (min)</th>
<th>Pool Size (nmole/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.28 ± 0.0</td>
</tr>
<tr>
<td>Amrinone</td>
<td>7</td>
<td>0.27 ± 0.0</td>
</tr>
</tbody>
</table>

\[a_p < 0.025.\]

Isolated guinea pig hearts were perfused at flow rate of 6 ml/min with Krebs-Henseleit buffer. After six minutes of perfusion, the buffer was switched to one containing \( ^{45}\text{CaCl}_2 \) (0.05 μCi/ml, 2.5 mM) plus 15 μg/ml amrinone. The hearts were perfused for five minutes followed by a washout with Krebs-Henseleit buffer for five minutes. During the final washout, effluent were collected. Samples of effluents were determined for radioactivity. Data shown as means ± SEM.

Table 14. Effect of Amrinone on Residual Calcium in Guinea Pig Hearts

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>nmole/g Wet Weight</th>
<th>nmole/g Dry Weight</th>
<th>nmole/min/g Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>65.9 ± 2.5</td>
<td>351.8 ± 12.1</td>
<td>70.4 ± 2.4</td>
</tr>
<tr>
<td>Amrinone</td>
<td>8</td>
<td>89.3 ± 9.2</td>
<td>445.3 ± 42.5</td>
<td>89.1 ± 8.5</td>
</tr>
</tbody>
</table>

\[a_p < 0.05.\]

Isolated guinea pig hearts were perfused at flow rate of 6 ml/min with Krebs-Henseleit buffer. After six minutes of perfusion, the buffer was switched to one containing \( ^{45}\text{CaCl}_2 \) (0.05 μCi/ml, 2.5 mM) plus μg/ml amrinone. The hearts were perfused for five minutes followed by a washout with Krebs-Henseleit buffer for five minutes. At the end of washout, the hearts were homogenized and evaluated for levels of radioactivity. Data shown as means ± SEM.
level was not very pronounced, but it was statistically significant (Table 15). There was no difference in the levels of iron or magnesium.

**Regional Concentration of Taurine**

Elevation in the levels of taurine has been reported in animals and humans. In dogs with right-sided congestive heart failure, Peterson et al., (1973) have reported an increase in methionine and taurine concentrations in the right ventricle. Huxtable and Bressler (1974) have reported a 100% elevation in the levels of taurine in humans dying from congestive heart failure. I studied various regions in the heart from the CM and the RB animals to see whether there are any changes in the concentration of taurine. In 30-40 day old hamsters, the left ventricles from the CM versus the RB did not show any significant difference in the concentration of taurine (Table 16).

**Effect of Advancing Cardiomyopathy on Cation Concentrations**

In CM hamsters, lesioning becomes more pronounced around 60-70 days. I analyzed again various regions in the heart in order to establish the changes in cation concentrations. The calcium overload was only in the left ventricle and compared to the RB animals, there is a 14-fold increase in calcium concentrations. The level of the other cations, magnesium and iron, was not affected (Table 17). Comparing the concentrations of iron and magnesium in 30-40 day old hamsters versus 60-70 day old ones, magnesium shows somewhat of a decrease and iron an increase. Since there is no difference in concentrations of
Table 15. Regional Cation Concentrations in Hearts from 30-40 Day Old Hamsters

<table>
<thead>
<tr>
<th>Location</th>
<th>Wet Tissue Weight (mg)</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right &amp; left atria</td>
<td>8.7 ± 0.4</td>
<td>45 ± 5</td>
<td>223 ± 23</td>
<td>not detectable</td>
</tr>
<tr>
<td>right ventricle</td>
<td>31.5 ± 1.8</td>
<td>68 ± 6</td>
<td>253 ± 1</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>left ventricle</td>
<td>124.5 ± 4.0</td>
<td>40 ± 3</td>
<td>233 ± 21</td>
<td>41 ± 3</td>
</tr>
<tr>
<td>CM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>right &amp; left atria</td>
<td>9.4 ± 0.5</td>
<td>59 ± 11</td>
<td>239 ± 12</td>
<td>not detectable</td>
</tr>
<tr>
<td>right ventricle</td>
<td>40.0 ± 3.5</td>
<td>68 ± 5</td>
<td>247 ± 1</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>left ventricle</td>
<td>133.0 ± 5.6</td>
<td>68 ± 8</td>
<td>246 ± 3</td>
<td>51 ± 2</td>
</tr>
</tbody>
</table>

\[a_p < 0.025.\]

The cardiomyopathic (CM) and random bred (RB) animals were sacrificed by decapitation. Atria, right and left ventricles were dissected, washed, and transferred to pre-weight calcium free volumetric flasks. The samples were digested in nitric acid and sufficient ionization buffer was added to make the final solution 1000 PPM (parts per million) cesium. The samples were diluted with distilled water to equal volumes. Precipitated proteins were filtered and the solution analyzed by atomic absorption spectrophotometry. The analytical wave lengths used were 422.7, 285.2, and 248.3 nm respectively for calcium, magnesium, and iron. Data shown as means ± SEM for four hearts per group.
Table 16. Regional Concentration of Taurine in 30-40 Day Old Hamsters

<table>
<thead>
<tr>
<th>Location</th>
<th>RB</th>
<th>CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium</td>
<td>26.4 ± 3.3</td>
<td>36.8 ± 9.3</td>
</tr>
<tr>
<td>Left atrium</td>
<td>26.7 ± 1.7</td>
<td>33.5 ± 1.0^a</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>36.3 ± 5.9</td>
<td>35.0 ± 2.0</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>26.9 ± 3.6</td>
<td>29.4 ± 1.8</td>
</tr>
<tr>
<td>Total Taurine'</td>
<td>28.5 ± 2.3</td>
<td>30.8 ± 2.8</td>
</tr>
</tbody>
</table>

^a_p < 0.025.

The cardiomyopathic (CM) and random bred (RB) animals were sacrificed by decapitation. Atria, right and left ventricles were dissected, and washed in saline. Each gram of tissue was diluted with 10 milliliters of 3.5% 5-sulfosalicylic acid, sonicated to homogeneity, centrifuged, and a 250 µl sample of supernatant was analyzed for concentration of taurine using an amino acid analyzer. Data shown as mean ± SEM for four hearts per group.
Table 17. Regional Cation Concentrations in Hearts from 60-70 Day Old Hamsters

<table>
<thead>
<tr>
<th>Location</th>
<th>Wet Tissue Weight (mg)</th>
<th>µg/g Tissue</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>left &amp; right atria</td>
<td>13.4 ± 1.0</td>
<td>67 ± 6</td>
<td>205 ± 4</td>
<td>54 ± 2</td>
</tr>
<tr>
<td></td>
<td>right ventricle</td>
<td>54.2 ± 1.0</td>
<td>52 ± 2</td>
<td>236 ± 6</td>
<td>56 ± 1</td>
</tr>
<tr>
<td></td>
<td>left ventricle</td>
<td>208.0 ± 6.5</td>
<td>36 ± 0</td>
<td>221 ± 3</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>CM</td>
<td>left &amp; right atria</td>
<td>14.0 ± 1.5</td>
<td>81 ± 8</td>
<td>197 ± 1</td>
<td>61 ± 7</td>
</tr>
<tr>
<td></td>
<td>right ventricle</td>
<td>43.8 ± 1.0</td>
<td>63 ± 4</td>
<td>222 ± 2</td>
<td>56 ± 2</td>
</tr>
<tr>
<td></td>
<td>left ventricle</td>
<td>190.0 ± 5.8</td>
<td>510 ± 93</td>
<td>217 ± 5</td>
<td>47 ± 0</td>
</tr>
</tbody>
</table>

\[ a_p < 0.001. \]

The cardiomyopathic (CM) and random bred (RB) animals were sacrificed by decapitation. Atria, right and left ventricles were dissected, washed, and transferred to pre-weight calcium free volumetric flasks. The samples were digested in nitric acid and sufficient ionization buffer was added to make the final solution 1000 PPM (parts per million) cesium. The samples were diluted with distilled water to equal volumes. Precipitated proteins were filtered and the solution analyzed by atomic absorption spectrophotometry. The analytical wavelengths used were 422.7, 285.2, and 248.3 nm respectively for calcium, magnesium, and iron. Data shown as means ± SEM for four hearts per group.
these ions among age matched groups, the changes described above were attributed to a difference in age rather than cardiomyopathy.

**β-Receptor Binding in the Heart**

β-Adrenergic stimulation in the heart leads to an increase in calcium influx. Hartman and Booth (1960) have suggested that sympathetic overactivity is responsible for calcium overload and myocardial damage. Although numerous studies have been conducted on energy utilization and its formation in cardiomyopathic hamsters, the cause of calcium overload is not clear. A β-receptor binding study in the hearts from the CM versus the RB hamsters could provide evidence as to the involvement of the adrenergic system in calcium overload. Furthermore, β-stimulation in the heart leads to an increase in the rate of taurine transport. The β-receptor binding study also partially elucidates the lack of a significant difference in taurine concentrations in the left ventricles (Table 16). Using 90 day old hamsters, a binding study was carried out by the method of Bylund and Snyder (1976). The binding of $[^3H]$-dihydroalprenolol to the heart was displaced by (-)-propranolol in order to establish specific binding. The CM hamsters utilized in this study were suffering from cardiac hypertrophy evidenced by a higher heart to body ratio (Table 18). The specific binding in the hearts from the CM is higher than that in the RB hamsters. However, when such binding results are corrected for the cardiac hypertrophy, the difference in binding is not statistically significant.
Table 18. β-Adrenergic Receptor Binding in Hearts from 90 Day Old Hamsters

<table>
<thead>
<tr>
<th></th>
<th>Heart:Body Ratio x 10^{-3}</th>
<th>Wet Heart Wt (g)</th>
<th>fmole $^3$H-DHA bound/mg protein</th>
<th>fmole $^3$H-DHA bound/g tissue</th>
<th>fmole $^3$H-DHA bound/heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>3.3 ± 0.1</td>
<td>0.395 ± 0.00</td>
<td>6.0 ± 0.3</td>
<td>334.8 ± 32.4</td>
<td>131.4 ± 10.7</td>
</tr>
<tr>
<td>CM</td>
<td>4.0 ± 0.1</td>
<td>0.533 ± 0.02</td>
<td>8.5 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>371.2 ± 26.4</td>
<td>197.4 ± 15.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.05.

The excised hearts were perfused with 10 ml of saline, then homogenized to make a 2.5% homogenate in 0.05 M Na$^+-$K$^+$ phosphate buffer. Homogenate was passed through several layers of cheesecloth and centrifuged at 48,000 x g for 20 minutes. The pellets were suspended in 0.05 M Na$^+-$K$^+$ phosphate buffer to make a 2.5% homogenate. The suspension was re-homogenized and tissue homogenates containing 80 μg protein were incubated for 30 minutes at 25°C in 2 ml of 0.05 M Na$^+-$K$^+$ phosphate buffer containing 0.25 M $[^3]$H dihydroalprenolol (58 Ci/mmol) in the presence and absence of 0.1 μM (-)-propranolol. The reaction was terminated by vacuum filtration through GF/B glass fiber filters followed by four 5 ml rinses with buffer kept at 25°C. Bound $[^3]$H-DHA retained on the filter was counted for radioactivity. Data shown as mean ± SEM for five hearts per group.
Effect of Taurine on Cation Concentrations

The overload of calcium in the CM hamsters is not dependent on the overactivity of the β-adrenergic system. Singh et al. (1975) have shown decreased enzymatic activity in the sarcolemma. Thus, sarcolemma could be a defective site through which calcium passively leaks into the myocardial cells. There are numerous suggestions as to the membrane stabilizing effect of taurine; therefore, the CM hamsters were supplemented with taurine in their drinking water to see whether there is a protection against calcium overload. The CM hamsters, 30-40 days old, were placed on 0.1 M taurine for 30 days and showed a 60% decrease in concentrations of calcium (Table 19) in their heart compared to the CM hamsters placed on water. There was no significant difference in the concentrations of other ions. To establish the role of taurine in controlling the calcium overload more specifically, hearts from the CM hamsters on taurine and those on regular water were cut to small pieces and one portion was analyzed for taurine and the other for calcium. In addition, the serum from the CM animals on taurine versus the serum from the CM animals on water were tested for their concentrations of taurine. The CM hamsters on taurine showed a statistically significant increase in taurine concentrations in the heart and a significant 70% decrease in calcium concentrations (Table 20). The serum concentrations of taurine in the CM animals were higher than those in the controls, but the difference is not statistically significant.
Table 19. Effect of Taurine on Cation Concentrations

<table>
<thead>
<tr>
<th>Wet Heart Weight (g)</th>
<th>µg/g Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td>Control</td>
<td>0.220 ± 0.00</td>
</tr>
<tr>
<td>CM</td>
<td>0.230 ± 0.00</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.025.

The cardiomyopathic (CM) hamsters 30-40 days old were placed on 0.1 M taurine as their drinking water for one month. The control animals were the CM animals on regular water. After one month, animals were sacrificed and the cation concentrations were evaluated as indicated in Table 16. Data shown as means ± SEM for six hearts per group.

Table 20. Effect of Taurine on Calcium and Taurine Concentration in the Heart

<table>
<thead>
<tr>
<th>N</th>
<th>Serum Taurine (µmole/ml)</th>
<th>Cardiac Taurine (µmole/g dry wt)</th>
<th>Cardiac Calcium (µg/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 6</td>
<td>0.64 ± .05</td>
<td>133.8 ± 5.7</td>
<td>1467 ± 283</td>
</tr>
<tr>
<td>CM 9</td>
<td>0.80 ± .07</td>
<td>154.5 ± 5.8(^a)</td>
<td>600 ± 128(^b)</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.025.

\(^b\) p < 0.010.

The cardiomyopathic (CM) hamsters 30-40 days old were placed on 0.1 M taurine as their drinking water for one month. The control animals were the CM animals on regular water. After one month, animals were sacrificed and the hearts were removed. The hearts were perfused with 10 ml of 0.9% saline and were cut to small pieces. One portion was used for determination of calcium as indicated in Table 16; the other portion was used for determination of taurine as indicated in Table 17. The serum concentration of taurine after removal of red blood cells was evaluated in a similar procedure described for tissue taurine. Data shown as mean ± SEM.
Effect of Taurine on Controlling Cardiac Lesions

The calcium overload is responsible for myocardial damage. Taurine reduces the calcium overload by 60-70%. Then, what effect does taurine have on lesion severity? The CM hamsters at a pre-lesioning age of 30-40 days old were placed on 0.1 M taurine for one month. At the end of this time, the animals were sacrificed and evaluated for calcium and the severity of the calcium dependent necrosis (lesion). All the tissue evaluations and scoring were conducted by Dr. Peter Brumbaugh of the Department of Pathology, The University of Arizona, in a single blind study. Taurine decreases calcium concentrations and such a decrease protects against myocardial necrosis (Table 21, Figs. 8, 9, 10, 11).

Effect of Taurine on Cation Concentrations in Advanced Stages of Cardiomyopathy

Taurine protects against myocardial damage at early stages of cardiomyopathy. Could taurine offer a similar protection in the advanced stages of cardiac disorder? The CM hamsters at pre-lesioning age were placed on 0.1 M taurine for four months. At the end of this time, hearts were evaluated for concentrations of various cations and taurine. The calcium overload in the heart of the hamsters without taurine was severe. The CM hamsters on taurine showed lower concentrations of calcium in the heart and less myocardial damage; however, the protective effects of taurine overall are not as pronounced as in the earlier stages of cardiomyopathy. Comparing the changes in other cations, magnesium is increased and iron is decreased which is statistically significant (Table 22). Since the levels of magnesium and iron
Table 21. Effect of Taurine on Controlling Cardiac Lesions

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Lesion Score</th>
<th>Calcium Concentration (µg/g tissue)</th>
<th>Per Cent Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>3.0⁺ ± 0.4</td>
<td>814 ± 113</td>
<td>0</td>
</tr>
<tr>
<td>CM</td>
<td>18</td>
<td>1.8⁺ ± 0.3ᵃ</td>
<td>353 ± 74ᵇ</td>
<td>41</td>
</tr>
</tbody>
</table>

ᵃ⁺p < 0.025,
ᵇ⁺p < 0.010.

The cardiomyopathic hamsters (CM) 30-40 days old were placed on 0.1 M taurine as their drinking water for one month. The control animals were the CM animals on regular water. After one month, animals were sacrificed and the hearts were removed. The hearts were perfused with 10 ml of 0.9% saline and were cut in a transverse section to upper and lower parts. Each time an alternating section was used for histological tissue preparation, while the other half was used for calcium determination. The calcium determination was performed as indicated in Table 16. The alternating sections of the heart were fixed in 10% buffered formalin. Hematoxalin-eosin stained six micron sections were prepared from parafin-embedded tissues. Grading was based on a 0-4⁺ scale with 4⁺ representing the highest level. The scores were assigned by Dr. Peter Braumbaugh in a single blind study. Data shown as mean ± SEM.
Fig. 8. The Heart from 60-70 Day Old Cardiomyopathic Hamster without Taurine -- In a single blind study a grade of 3+ was assigned for severity of necrosis.
Fig. 9. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with Grade of 0 Assigned to the Severity of Necrosis in a Single Blind Study
Fig. 10. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with Grade of 1+ Assigned to the Severity of Necrosis in a Single Blind Study
Fig. 11. The Heart from 60-70 Day Old Cardiomyopathic Hamster Placed on Taurine for 30 Days, with a Grade of 2+ Assigned to the Severity of Necrosis in a Single Blind Study
Table 22. Effect of Taurine on Cation Concentrations in Advanced Stages of Cardiomyopathy

<table>
<thead>
<tr>
<th></th>
<th>µg/g dry wt Ca</th>
<th>µg/g dry wt Mg</th>
<th>µg/g dry wt Fe</th>
<th>µmole/g dry wt Taurine</th>
<th>Lesion Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>172 ± 18</td>
<td>858 ± 11</td>
<td>206 ± 2</td>
<td>104.9 ± 10</td>
<td>0.0</td>
</tr>
<tr>
<td>CM without Taurine</td>
<td>3775 ± 221\textsuperscript{c}</td>
<td>1008 ± 8\textsuperscript{a}</td>
<td>171 ± 4\textsuperscript{b}</td>
<td>107.2 ± 6</td>
<td>3.25\textsuperscript{+} ± 0.5</td>
</tr>
<tr>
<td>CM Plus Taurine</td>
<td>2472 ± 543\textsuperscript{c}</td>
<td>1006 ± 22\textsuperscript{a}</td>
<td>191 ± 4\textsuperscript{b}</td>
<td>142.2 ± 11</td>
<td>2.62\textsuperscript{+} ± 0.4</td>
</tr>
</tbody>
</table>

\[\textsuperscript{a}p < 0.001.\]
\[\textsuperscript{b}p < 0.025.\]
\[\textsuperscript{c}p < 0.01.\]

The cardiomyopathic hamsters (CM) 30-40 days old were placed on taurine as their drinking water for four months. At the end of the four months, the animals were sacrificed, and the hearts were removed. The hearts were perfused with 10 ml of 0.9% saline and were cut in a transverse section to upper and lower parts. Each time an alternating section was used for histological tissue preparation, as described in Table 21. The other half was cut to small pieces. One portion was used for calcium determination as described in Table 15 and the other portion was used for taurine determination as described in Table 16. Data shown as mean ± SEM for four hearts in RB and CM without taurine group and six hearts in CM plus taurine group.
were unaltered in the earlier stages (60-70 days old), such changes are the consequence of advancing cardiac disorder. Comparing the cardiac concentrations of taurine in the CM animals in the advanced stages with those in Table 20, there is a significant decrease. The concentration of taurine in the CM hamsters without taurine supplementation decreased from 134 µmole/g dry wt to 107 µmole/g dry wt. Such decreases are probably due to the enhanced efflux of taurine.

**Rate of Taurine Influx**

Comparison of the concentrations of taurine in Table 21 and those in Table 22 indicates that decreased levels of taurine may result either from lower influx or enhanced efflux rates. To examine the influx rate, the hearts from the CM and RB animals were perfused with buffer containing radiolabeled taurine. The rate of taurine influx is higher in the CM hamsters (Table 23). Thus, the lower concentration of taurine observed in the CM hamsters at the advanced stages of cardiomyopathy must result from enhanced efflux.
Table 23. Rate of Taurine Influx

<table>
<thead>
<tr>
<th></th>
<th>Wet Heart Weight (g)</th>
<th>Dry Heart Weight (g)</th>
<th>Influx Rate nmole/min/ g wet wt</th>
<th>Influx Rate nmole/min/ g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>0.39 ± 0.0</td>
<td>0.086 ± 0.00</td>
<td>3.08 ± 0.2</td>
<td>14.0 ± 0.8</td>
</tr>
<tr>
<td>CM</td>
<td>0.41 ± 0.0</td>
<td>0.084 ± 0.00</td>
<td>4.60 ± 0.3</td>
<td>22.6 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>p < 0.005.

The isolated hearts from 70-80 day old hamsters were perfused by Langendorff technique. The hearts were perfused at a flow rate of 4 ml/min with Krebs-Henseleit buffer for five minutes. Then buffer was switched to one containing 25 µM taurine (0.05 µCi/ml) and perfused for five minutes. Following the perfusion with substrate, there was a five minute washout. The rate of influx was determined from the radioactivity remaining in the heart. Data shown as mean ± SEM for five hearts in each group.
CHAPTER 4

DISCUSSION

It will take some time before the enigma about the presence of high concentration of taurine in excitable tissues and its function(s) in such tissues can be completely answered. This work has attempted broad elucidation of the function of taurine in the heart. In this regard separate studies have been made examining the pharmacology of taurine in the heart, its relationship to other cardiotonic agents, and the role it plays in cardiomyopathy.

Taurine in Heart

Adrenergic stimulation of the heart produces manifold changes, any one of which could be responsible for the increased influx of taurine. Such changes include increased heart rate, increased levels of cyclic 3',5'-adenosine monophosphate (cyclic AMP), and increased calcium influx in response to β-stimulation of the heart. Previously, Huxtable and Chubb (1977) studied the effect of the heart rate on taurine flux. In isolated perfused rat hearts, with ablated sinoatrial node, the pacing of the hearts at rates up to 450 beats/min had no effect on the rate of taurine influx. Thus, chronotropy is not a factor contributing to increased taurine influx. More recently, Chubb and Huxtable (1978a) have shown that the isoproterenol stimulation of taurine influx can be blocked by propranolol, a β-receptor blocker. They have also achieved stimulation in the rate of taurine influx.
using either dibutyryl cyclic AMP (a diffusible analog of cyclic AMP),
or theophylline, a phosphodiesterase inhibitor (enzyme responsible for
degradation of cyclic AMP). Considering such findings, the above
authors have suggested that increased concentrations of cyclic AMP
within the cardiac cells mediate increased taurine influx. This is
verified by the observation that glucagon, a polypeptide hormone from
the pancreas, which leads to an increase in the levels of cyclic AMP
by activating adenylate cyclase independent of adrenergic receptor
stimulation in isolated perfused rat hearts, also stimulates the
taurine influx rate.

There are a number of reports linking increased levels of cyclic
AMP with increased contractility. The increased contractility is
either associated with an increase in the rate of calcium influx during
the plateau phase of cardiac action potential (Williamson, 1966;
Robison, Butcher, and Sutherland, 1967; Gardner and Allen, 1976), or by
augmentation of the quantity of calcium released from the sarcoplasmic
reticulum after membrane excitation (Skelton, Levey, and Epstein, 1970).
Thus, it is not clear whether the stimulation in the rate of taurine
influx in response to an increase in the levels of cyclic AMP is an
independent phenomenon or is a function of enhanced inotropy, calcium
influx, or possibly of sodium-calcium exchange.

The present data show that the inotropic status of the heart
does not influence taurine influx. If the positive inotropy produced by
isoproterenol is blocked by concomitant perfusion with verapamil, the
stimulated influx of taurine is still observed (Table 5). The
positive inotropy produced by monensin or A23187 and the negative
inotropy produced by valinomycin likewise have no effect on influx rate (Table 4).

The studies with verapamil also eliminate calcium as a mediator of taurine influx. Electrophysiological studies using voltage clamping have shown that verapamil selectively inhibits the transmembrane flux of calcium without reducing the rapid transient sodium current (Kohlhardt et al., 1972). Thus, verapamil reverses the inotropy of isoproterenol by antagonizing calcium flux. Such lowering of calcium by verapamil, or increasing the passive flux of calcium into the cell by calcium ionophore A23187, does not affect taurine transport.

In hearts from reserpine-treated animals, monensin increases sodium current into the cell. An increase in the level of intracellular sodium will facilitate the sodium-calcium exchange (Langer, 1974b) leading to an inotropic effect. However, monensin has no effect on taurine influx. Valinomycin increases potassium flux, causing a fall in membrane potential and increased excitability. Again, taurine transport is unaffected. The cyclic AMP stimulated uptake of amino acids into a variety of other tissues including bone (Phang, Downing, and Weiss, 1970), kidney cortex (Weiss, Morgan, and Phang, 1972), myometrium (Griffin and Szego, 1968), liver (Tews, Woodcock, and Harper, 1970), and intestine (Kinzie, Grimme, and Alpers, 1976) has been reported. In some cases, the cyclic AMP associated amino acid uptake merely reflected altered intermediary metabolism of amino acids. For example, in liver slices incubated with cyclic AMP, the uptake of some amino acids decreased while others increased (Tews et al., 1970). In other cases, the changes were related to enhanced
electrolyte transport, especially in kidney and intestine and reflected altered electrochemical gradients across the cell. Amino acid transport is dependent upon extracellular sodium and the higher the external sodium concentration, the greater the capacity of transport systems to transport amino acids (Wisemann, 1968). Therefore, alterations in sodium fluxes may be responsible for the generalized stimulation of amino acid influx. Based on the results shown, enhanced electrolyte transport induced by ionophores does not appear to have a role on the increased rate of taurine influx. Thus, it is reasonable to conclude that the only consequence of adrenergic receptor activation that is involved in the regulation of taurine influx is the increase in cyclic AMP levels. In contrast to the transport of other amino acids described above, the cyclic AMP dependent influx of taurine is unique to β-amino acids.

To explain the β-adrenergic stimulation of cation transport in amphibian bladder (DeLorenzo et al., 1973) and avian erythrocytes (Greengard, 1975), DeLorenzo et al. and Greengard have offered the following explanation. Isoproterenol activates membrane bound adenylate cyclase, leading to the formation of cyclic AMP. Cyclic AMP, in turn, activates a membrane bound protein kinase causing the phosphorylation of a specific membrane protein. As a result of membrane phosphorylation, allosteric changes occur in the membrane which facilitate increased transport. In light of recent findings by Kulakowski et al. (1978) who have reported the isolation of a membrane protein from rat sarcolemma, identified as a taurine receptor, the following modified explanation could be given for enhanced taurine
transport. The taurine carrier protein (taurine receptor) is responsible for the active transport of taurine under normal conditions. Under isoproterenol stimulation, the activated membrane protein kinase phosphorylates the taurine carrier protein. Since the taurine carrier is an allosteric protein, its transport activity is modulated through the non-covalent association of taurine at the sites on the protein other than the catalytic site. Furthermore, the change in the receptor protein's environment, resulting from membrane phosphorylation, increases the dissociation of non-covalently bound taurine. The increased association of taurine with the carrier protein, concomitant with facilitated disassociation leads to an increase in taurine influx in response to β-stimulation.

Taurine transport is an active process, and it is stimulated in response to an increased cyclic AMP level. Like other actively transported substrates, the taurine carrier protein must have a structural requirement in order to recognize its substrate. Such structural specificities are shown when analogs of taurine are tested for their competition for the taurine carrier protein. As shown in Table 6, hypotaurine, β-alanine, β-guanidopropionate, and β-guanidoethyl sulfonate are inhibitors of taurine transport. The inhibitors, hypotaurine and β-alanine both occur naturally in the rat heart and both are straight chain β-amino acids, as is taurine. Hypotaurine is the sulfinic acid analog and β-alanine is the carboxylic acid analog of taurine. Guanidoethyl sulfonate and guanidopropionate, the amidino derivatives of taurine and β-alanine respectively, are also transport inhibitors of taurine with the former being more potent. 2-Aminoethyl
phosphonic acid (Fig. 3) which differs from taurine in its acidic moiety, being dibasic compared to taurine which is monobasic, has no inhibitory effect. 2-Aminocyclohexane, which does not have a planar conformation, does not affect taurine transport. Acetimidoyl glycine in which the separation between the acidic and basic moiety is less than two carbon atom, also has no effect on taurine transport. 3-Aminobutane-2-sulfonate in which the α-carbon (carbon next to the acidic moiety) is substituted with a methyl group, also has no effect on the transport of taurine. The stimulation in taurine transport seen at a higher concentration of nicotinic acid, is probably due to the ability of this compound to alter the membrane conformation, thus affecting cellular transport in general, and not an event specific to taurine. There are other reports substantiating the preciseness of the taurine transporting system. Hruska et al. (1978) have studied the effect of 2-aminoethyl phosphonic acid (a taurine analog in which the acidic moiety is dibasic) and 2-aminoethyl hydrogen sulfate (analog with larger acidic moiety) in rat brain synaptosomes for their effect on taurine transport, neither compound shows any significant effect. Furthermore, other analogs such as 2-methyl-cyclohexane sulfonic acid (devoid of basic end and a non-planar compound) and 1,2-diemthyltaurine (taurine analog with methyl substitution in α and β carbons) in isolated rat hearts have no effect on taurine transport (Huxtable and Chubb, 1977; Chubb and Huxtable, 1978c).

Structural comparison (Fig. 3) in addition to the reports by other investigators, indicates that analogs of taurine with a significant inhibitory effect at the taurine transport site fulfill the
following criteria: There is a minimum requirement of two carbon atoms between the acidic and basic ends of the molecule. The acidic end must be unsubstituted at the α-carbon and must be monobasic. By contrast, the amino end may be substituted. Finally, the ability to assume a planar conformation appears to be necessary.

Hypotaurine, although a potent inhibitor, at the cellular level is readily oxidized to taurine. Therefore, it cannot be of any benefit in studying the physiological effects of lowering intracellular concentrations of taurine. β-Alanine has potential usefulness as a transport inhibitor. However, it has physiological and pharmacological actions of its own. Recently, Huxtable (1979) has shown in isolated perfused rat hearts that there is a non-saturable component to its uptake by the heart. Guanidoethyl sulfonate, on the other hand, does not occur in mammalian heart (Guidotti and Costagli, 1970) and its transport is saturable. Compared to taurine which has a $K_m = 45 \mu M$ and $V_{max} = 32$ nmole/min/g, the guanidoethyl sulfonate has a somewhat lower affinity of $153 \mu M$ and a $V_{max} = 64.6$ nmole/min/g. Furthermore, its transport is stimulated in response to β-adrenergic stimulation similar to that of taurine. This compound may be a useful tool in studying the role of increased taurine concentration in animal models of congestive heart failure that show elevated taurine levels.

In Chapter 1, the difficulty in altering the tissue taurine levels, especially those in the heart was mentioned. Recently, Huxtable et al. (in press) have shown that rats and mice, given free access to drinking water containing 1% guanidoethyl sulfonate, show marked decreases in the concentration of taurine in all tissues.
examined, regardless of the taurine content of their chow. In rats, within 9 days in the brain, depletions range from 52% in the frontal cortex to 30% in the cerebellum and inferior colliculus. The spinal cord shows the smallest decrease at 14%. Concentrations of free amino acids apart from taurine are unaltered in the brain or the heart. Thus, the simplicity by which guanidoethyl sulfonate can lower taurine levels in various tissues will open a new era in studying the physiological role of taurine in the brain as a neurotransmitter or neuromodulator and other tissues such as the heart. Finally, the variable effect of guanidoethyl sulfonate in lowering the taurine level in rat brain might be associated with the affinity at the various areas for this compound or the role of taurine in these areas. In light of recent evidence, β-guanidoethyl sulfonate appears to be the most promising in studying the effects of lowering tissue taurine concentrations.

Inasmuch as the function of taurine in the heart has yet to be clearly defined, the function of cyclic AMP regulation of influx is uncertain. It may be related to the effects of taurine or calcium movements in the heart (Dolara et al., 1973; Chubb and Huxtable, 1978b). In this regard, the decreased binding of calcium to sarcolemma in the presence of taurine is of interest (Fig. 6). If this effect resulted in increased release of calcium from the sarcolemma under physiological conditions, the free calcium pool within the cell would increase, making more calcium available for binding to the contractile elements and thereby producing enhanced contractility. Taurine has also been found to decrease the binding of calcium to rat brain microsomes.
(Izumi et al., 1977). The binding result reported is in contradiction with those of Chovan et al. (1979) in which 10 mM taurine induced a slight increase in calcium binding to the sarcolemma. The discrepancy in these findings may be related to methodological differences, two of which are the methods of isolation of sarcolemma and the incubation buffer. Chovan et al. (1979) did not include EDTA (ethylenediaminetetraacetic acid) to remove endogenous calcium and they have used a different incubation medium. Their incubation medium contained a lower concentration of sodium and had no phosphate and bicarbonate which are included in the Krebs-Henseleit buffer.

Consistent with the effect of taurine in decreasing calcium binding to the sarcolemma, is the biphasic contractile response elicited by taurine or β-guanidoethyl sulfonate in perfused rat hearts. Taurine or β-guanidoethyl sulfonate at concentrations up to 0.25 mM in isolated perfused rat hearts, produced no observable changes in contractility or heart rate. However, when the hearts were perfused with a Krebs-Henseleit buffer containing 0.5 mM calcium (1/5 of normal concentration) plus 0.1 mM taurine or β-guanidoethyl sulfonate, there is a potentiation of contractility (Fig. 4). Consistent with a mechanism discussed below, it appears at low concentration of calcium, the competition from taurine or β-guanidoethyl sulfonate for calcium exchange site(s) increases.

In order to explain how the positive inotropy can be generated at the sarcolemma, I will discuss below the relation of taurine to the $\text{Na}^+ - \text{Ca}^{2+}$ exchange model proposed by Langer (1971). In Fig. 12, cycle one depicts the path of the carrier involved with the sodium
Fig. 12. Sodium Calcium Exchange Mechanism -- A model for inter-relation among sodium, potassium, and calcium (Langer, 1971, p. 1065).
pump and regulated by the Na\(^+\)-K\(^+\) activated ATPase. Cycle number two represents the path for the proposed carrier system for sodium and calcium ions (Na\(^+\)-Ca\(^{2+}\)). The box within the sarcolemma represents the sites for which Na\(^+\) and Ca\(^{2+}\) are in competition. These sites are in rapid reversible equilibrium with Na\(^+\) and Ca\(^{2+}\) in the extracellular space. In this model, the inward movement of calcium is coupled with the movement of sodium from intracellular regions to the box in the sarcolemma. The energy for sodium movement is derived from the inward movement of calcium down its concentration gradient. The calcium at the sarcolemma is responsible for supplying calcium for the coupling contraction with excitation. These calcium sites are replenished from superficial calcium stores localized in the basement membrane. "The basement membrane represents the binding and exchange region for calcium near the sarcolemma which is in rapid equilibrium with interstitium and from which calcium is released (through the sarcolemma) to activate the myofilaments" (Langer, 1971, p. 1068).

Taurine is a stable zwitterion with a high dipole moment, it may interact with the zwitterionic phospholipid structure of membranes and cause a conformational change by virtue of stabilizing charge separation (Huxtable, 1976). The alterations in cation affinity are a consequence of membrane conformational change. Taurine causes a conformation change at the basement membrane which will shift the calcium binding and exchange equilibrium toward the interstitium. This is consistent with the result that taurine decreases the binding of calcium to the sarcolemma prepared from rat hearts (Fig. 6). In isolated perfused hearts, taurine exerts its effect as follows:
1. Through its effect at the sarcolemma (the site responsible for immediate release of calcium to the myofilaments), taurine transiently shifts the equilibrium for calcium toward the extracellular space resulting in a decrease in the calcium content at the sarcolemma. This is consistent with a decrease in the contractility seen within one minute of initiating perfusion of isolated rat hearts with Krebs-Henseleit buffer containing 0.5 mM calcium plus 0.1 mM taurine or guanidoethyl sulfonate.

2. At the basement membrane, taurine, by shifting the calcium binding equilibrium toward intersstitium, results in an increase in the calcium levels at the interstitium. This increase facilitates augmentation of releasable calcium pools at the sarcolemma.

With increased calcium content at the easily releasable site in the sarcolemma, more calcium is released to the myofilaments per unit time at each excitation. The increased availability of calcium to the myofilaments will result in a positive inotropic effect. This is consistent with the positive inotropy seen following a decrease in contractility when rat hearts are perfused with 0.1 mM taurine or guanidoethyl sulfonate. To explain the fate of $\text{Na}^+$, increased calcium release from sarcolemma to the interior of the cells generates more energy for transport of sodium. The increased energy will transport more sodium to the $\text{Na}^+\text{-Ca}^{2+}$ exchange site at the sarcolemma. The consequence of increased sodium at the sarcolemma will be shifting the sodium equilibrium toward the extracellular space and eventually a
decrease in sodium current. This is consistent with observations made by Diacono and Dietrich (1976).

I have proposed a number of models which the following experiments could help in verifying:

1. To establish that membrane phosphorylation is responsible for enhanced taurine transport in response to isoproterenol, perfuse the hearts with $^{32}$P (orthophosphate) to equilibrate intracellular phosphate pools with labeled phosphate. Stimulate the hearts with isoproterenol, isolate the membrane bound taurine carrier, verify its phosphorylation, and examine the taurine binding affinity of this phosphorylated protein versus the control.

2. To establish taurine interaction (binding) to basement membrane, isolate sarcolemma and test the effect of Lanthanum on taurine binding. Lanthanum displaces calcium binding from the basement membrane. If taurine exerts its effect at these calcium binding sites, Lanthanum should inhibit the taurine binding to these sites.

**Amrinone**

Amrinone [5-amino-3,4'-bipyridin-6 (IH)-one] is a non-glycoside, non-catecholamine, positively inotropic agent with an unknown mechanism of action. In the Langendorff perfused guinea pig heart, amrinone at concentrations 10-20 µg/ml, increased the contractile force from 29-33% with no significant effect on the heart rate. The onset of maximal response was within two minutes of initiating perfusion and lasted for
about 2-3 minutes during post perfusion washout. The lack of dose-response in the guinea pig heart in response to amrinone is probably due to having reached the limit of the drug's effectiveness at 10 μg/ml. A similar result has been reported in dog hearts (Farah and Alousi, 1978). Amrinone neither alleviated nor aggravated the spontaneous arrhythmias in the guinea pig heart. The absence of the anti-arrhythmic effect in the guinea pig is consistent with the report by Farah and Alousi (1978) that amrinone does not significantly affect electrophysiological events in cat papillary muscle or the Na⁺-K⁺ ATPase in dogs. Using isoproterenol as a marker for the induction of positive inotropy and increased cyclic AMP levels in response to β-adrenergic stimulation, amrinone at 10 and 20 μg/ml concentrations increases the contractile force by 32-36% with no effect on the levels of cyclic AMP. However, isoproterenol stimulates the contractile force by 78% and the levels of cyclic AMP increased 1.5 fold. Thus, it is clear that the positive inotropy resulting from amrinone treatment in the guinea pig is independent of increased cyclic AMP levels. This is consistent with the results reported in dogs. Finally, in dogs (Alousi and Farah, 1978; Farah and Alousi, 1978), it has been reported that amrinone works independently of β-adrenergic receptors and it does not have any significant changes in catecholamine metabolism. I also have observed no change in the cyclic AMP level or a change in heart rate. Based on their findings, the above authors have suggested that amrinone works directly on myocardial contractile elements to bring about its positive inotropic effect. However, they have not shown
either intracellular localization of amrinone nor have they tested their proposal at the molecular level.

Increases in the intensity of the active state or the rate of maximal tension development could be brought about by a number of factors: namely, reduction of extracellular $K^+$ (Reiter, Seibel, and Stickel, 1971), increased intracellular sodium, or decreased extracellular sodium (Langer, 1974a). Most such alterations are associated either with ionic loss or a change in the heart rate. Based on the lack of electrophysiologic effects of amrinone on cat papillary muscle (Farah and Alousi, 1978) and non-significant changes in the heart rate, involvement of such events in inotropy can be eliminated. One of the major factors influencing the intensity of active state is the release of more calcium to the myofilaments per unit time (Langer, 1974b). Such release could be brought about either from sarcolemma or sarcotubular assembly. The result of such stimulated release is an increase in the rate of tension development ($dp/dt$) which has been shown in dog hearts by Farah and Alousi (1978). In a preliminary study of calcium kinetics in amrinone treated hearts versus the controls (Fig. 7), the slopes for either rapid (A) or slow (B) washout of the radioactive calcium from the heart are not altered among the two groups. However, extrapolation of these slopes to their intercepts and using the values obtained from such intercepts with a correction for the difference in the heart weight to determine calcium concentration, revealed a significant increase in the calcium pool size in amrinone treated hearts (Table 13). Such an increase in calcium could arise from increase in calcium concentration at the intracellular
level, interstitial (rapidly washable) spaces, or both. The intracellular calcium concentration is not altered (Table 13), therefore, the increase in calcium pool size must be due to an increase in calcium concentration at extracellular spaces. Based on my preliminary results, I suggest that the positive inotropic effect of amrinone in the guinea pig heart is mediated through calcium regulation.

The increase in the calcium levels at the interstitium brings about augmentation of the releasable calcium pools which are located in the sarcolemma. Upon excitation, the amount of calcium made available per unit time to the myofilaments increases resulting in a positive inotropy. This is consistent with the observation made by Langer (1974a) in which increasing extracellular calcium to 4-5 mM causes a rise in tension development. Langer (1974a) has attributed the increase in tension development to the augmentation of calcium at the sarcolemmal releasing sites. The increased interstitial calcium levels can also explain the observations made during perfusion and post perfusion washout with amrinone. Compared to the isoproterenol perfused guinea pig hearts in which the onset of maximum inotropy is within seconds of initiating perfusion, and the effect is washed out within seconds during final washout, amrinone shows a different time course. The positive inotropy with amrinone starts within one minute of initiating perfusion, reaching its maximum within 2-3 minutes and the positive inotropy lasts for about 2-3 minutes during post perfusion wash. The delay in the onset of the inotropic effect could be attributed to time needed to augment the interstitial calcium levels. The persistence of positive inotropy during the final washout could be
explained as follows. The increased calcium concentration in the interstitium (Table 13) may indicate that more interstitial calcium is available to the intracellular calcium pool during washout. In accord with this is the increased residual calcium left in heart after 6 min washout period (Table 14).

Previously, it was stated that an increase in the levels of cyclic AMP is responsible for increased taurine influx. I tested amrinone on the rate of taurine influx in rats and guinea pigs. In rats, amrinone showed no effect on the rate of taurine influx. I have not measured the levels of cyclic AMP in rat hearts in response to amrinone, but based on the ineffectiveness of this compound to elicit any change in the levels of cyclic AMP in dog and guinea pig, it is reasonable to assume amrinone will not alter cyclic AMP levels in the rat hearts. Based on such assumptions, the earlier proposal as to the role of cyclic AMP in mediating increased taurine influx is further substantiated. In guinea pig, however, there is stimulation in the rate of taurine influx. Such response in guinea pig might be due to species differences. Guinea pig, being a herbivore, does not receive any dietary taurine, and the concentration of taurine which is very low in guinea pig is probably achieved by synthesis rather than transport as is the case in rats. This is consistent with a very recent observation by Huxtable (1979) that guanidoethyl sulfonate cannot lower the taurine level in guinea pigs. There are other differences such as the responsiveness to calcium and the response to cardiac glycoside. Rat hearts are insensitive to cardiac glycosides (Allen and Schwartz, 1969) and increased extracellular calcium (Guilbault et al., 1962).
Furthermore, calcium and taurine concentrations are lower in guinea pig hearts compared to rats (Dietrich and Diacono, 1971), and there are the possible ultrastructural differences among the hearts from these two species.

In conclusion, based on my results as to the effect of amrinone on calcium kinetics in guinea pig hearts, and lack of inotropic response by amrinone in rat hearts, I suggest amrinone induces its inotropy by mechanism(s) other than a direct action of amrinone on the cardiac myofilaments as suggested by Farah and Alousi (1978). One possible mechanism is that amrinone, being a polar compound, could bind to calcium binding sites (the charged carboxyl groups from sialic acid residues) at the basement membrane, and prevent calcium exchange at such sites. Inhibition of calcium exchange leads to an increase in the level of calcium at the extracellular spaces resulting in an inotropic effect as described above. More work both at the cellular level and intact heart is required before solid conclusions can be drawn.

**Cardiomyopathy and Taurine**

In cardiomyopathic (CM) golden Syrian hamsters, the level of calcium in the left ventricles of the affected animals in the pre-lesion period (30-40 days) is higher than that in random bred (RB) animals. The difference, although not great, is statistically significant. The levels of taurine in age matched left ventricles of the CM versus the RB animals are not significantly different. The levels of calcium in the left ventricles of older animals (60-70 days old) show a significant difference. Calcium increases 14-fold in the
left ventricles of the CM animals, compared to the RB animals. The levels of iron and magnesium are not different at either 30-40 days or 60-70 days. Thus, calcium overload which is mainly in the left ventricle appears to be responsible for the cardiac necrosis. Such data are consistent with the proposal by Fleckenstein et al. (1974, 1975) as to the role of calcium overload in myocardial necrosis. The calcium overload could be either due to enhanced calcium influx resulting from β-adrenergic receptor supersensitivity in the heart (Hartman and Booth, 1960), a genetically transmitted defect in sarcolemma (Dhalla, 1976), or defective cardiac cell organelles incapable of removing calcium. β-Adrenergic receptor binding in the hearts from the CM and the RB animals (Table 18) eliminated the adrenergic involvement in calcium overload. The absence of adrenergic involvement also explains the unaltered levels of taurine among RB versus CM in the 30-40 or 140-150 day old hamsters. Taurine levels increase in a response to an increase in the cyclic AMP levels resulting from β-adrenergic receptor stimulation. Furthermore, the lack of taurine alteration suggests that taurine is probably elevated in those forms of cardiomyopathic disorders in which the β-adrenergic receptor over-stimulation is involved. Based on the results by Singh et al. (1975), sarcolemma appears to be affected in the cardiomyopathic hamsters. They have found a decrease in the activities of Ca$^{2+}$-ATPase, Mg$^{2+}$-ATPase, and Na$^+$,K$^+$-ATPase in the sarcolemma prepared from cardiomyopathic hamsters.

Based on Huxtable's suggestion as to the membrane stabilizing effect of taurine and the relationship between taurine and calcium, the
animals were placed on 0.1 M solution of taurine, as their drinking water. The 30-40 day old cardiomyopathic animals placed on taurine for 30 days in contrast to those on regular water showed marked changes. The heart taurine concentrations were higher, the calcium concentrations in the heart were reduced by 60%, and there was a 40% reduction in lesion severity (necrosis). There were no changes in the levels of iron or magnesium and serum taurine concentration in the animals on taurine was higher, but the difference was not statistically significant. The lack of statistically significant change in serum taurine levels in the CM animals on taurine solution versus those on water alone could be either due to enhanced uptake of taurine by various tissues including heart, or due to increased excretion of taurine. At earlier stages of cardiomyopathy (60-70 days) it appears increased uptake is involved, because the CM hamsters on taurine (Table 20) show a higher cardiac taurine level. Elevation of cardiac taurine in the CM hamsters on taurine, plus a higher rate of taurine influx in the CM versus the RB animals (Table 23) without activation from β-adrenergic receptor in the heart, could result from one or more of the following factors: impaired efflux of taurine from the heart, increased adenylate cyclase activity (enzyme responsible for formation of cyclic AMP from adenosine triphosphate), or decreased phosphodiesterase activity (enzyme responsible for degradation of cyclic AMP). Either of these enzymatic alterations will lead to an increase in cyclic AMP levels which, in turn, leads to an increased influx of taurine.

If the taurine levels are compared in the CM animals 60-70 days old placed on taurine for one month versus those of 150-160 days
old placed on taurine for four months, the older animals show lower levels of taurine. Comparing the older animals (Table 22), there were no significant differences between the levels of taurine among RB versus the cardiomyopathic ones that were not on taurine. With the knowledge of higher taurine uptake in the CM versus the RB animals, it appears that, lower levels of taurine in the heart as described above are due to increased efflux of taurine from the heart. Lossnitzer and Bajusz (1973) have shown, at advanced stages of cardiomyopathy, the CM hamsters develop a kidney disorder. The reabsorption of ions and amino acids by kidney is decreased resulting in a higher excretion. Thus, increased excretion of taurine by kidney lowers the serum concentration of taurine, which leads to enhanced efflux of taurine from various tissues, including heart, to the serum. The efflux of taurine from the heart at advanced stages of cardiomyopathy must be greater than the influx of taurine, because the overall result is lowered cardiac taurine concentration. Consistent with possible alterations in the efflux of taurine in cardiomyopathic animals, there were two animals in a group of 10 CM animals on taurine (for 30 days) that showed very low taurine concentrations in the serum and the heart. The calcium concentrations in the hearts of these animals were also very high. The consequence of lowered serum taurine concentration due to increased excretion of taurine, is lesser protection offered by taurine in controlling the calcium overload and calcium dependent necrosis. Taurine stabilizes the defective sarcolemma in the CM hamsters and prevents calcium overload, with a decreased concentration of taurine in the serum, such protection is lost.
In advanced stages of cardiomyopathy, there is a statistically significant increase in the levels of magnesium and a decrease in iron, probably the consequence of destroyed or leaky mitochondria. However, at earlier stages of cardiomyopathy, there is no difference in the levels of these cations among the CM versus the RB. Therefore, the changes in iron and magnesium are the result, not the cause, of cardiomyopathy in Syrian golden hamsters. There is also an interesting finding as to the level of iron. The CM hamsters on taurine show a lesser decrease in iron level compared to the CM hamsters on water (Table 23). This could be attributed to the membrane effect of taurine. Taurine stabilizes mitochondrial membrane preventing its lysis. Since mitochondria is the site for a number of iron dependent enzymes, the stabilization of their membrane by taurine will slow the leakage of iron to the cytoplasm, the site from which iron is extruded to the extracellular and eventually excreted. This stabilizing effect of taurine may also be involved in protecting mitochondrial energy formation.

The cardiomyopathy in the Syrian golden hamsters is due to a membrane defect which leads to a calcium overload. The overload of calcium is responsible for the myocardial lesions and fibrosis. The calcium overload leads to activation of a number of calcium dependent ATPases, leading to an energy depletion and impairment of energy production. Taurine protects against the progress of myocardial disorder by stabilizing the membrane and lowering the calcium influx. Taurine does not appear to induce its protective role by increasing the affinity of calcium for various intracellular structures. This is
because there are no correlations between intracellular taurine concentration and that of calcium. Finally, taurine, being an endogenous substance, could be useful in combating cardiomyopathic disorder(s) that are associated with a defective membrane and/or calcium regulation. Taurine may be a useful agent in the treatment of cardiomyopathy associated with Friedreich's ataxia.
CHAPTER 5

SUMMARY

In conclusion this work has established the following points.

1. Stimulated taurine transport in rat hearts in response to \(\beta\)-adrenergic receptor activation is independent of enhanced movement of cations, including calcium, or inotropy, but it is a direct function of increased cyclic AMP levels. The sites responsible for taurine transport in the heart have a precise structural requirement for substrate binding. Analogs capable of competing with taurine for such transport sites must have an acidic end devoid of any alteration or substitution at the \(\alpha\)-carbon, a chain length the same as taurine, and a planar conformation. In rat hearts, taurine has a modulating effect on calcium binding. Such modulation is induced at the sarcolemma, and one result is potentiation of inotropy at low calcium concentration.

2. Amrinone, a non-catecholamine, non-glycoside cardiotonic agent, has a positive inotropic effect on guinea pig heart. Such inotropy is independent of alteration in cyclic AMP levels. The inotropy resulting from amrinone treatment may be due to an alteration in calcium regulation. Amrinone does not affect the rate of taurine influx in rat hearts. However, in guinea pigs there is a stimulation of taurine transport.
3. The cardiomyopathy associated with Syrian golden hamsters, is independent of ions such as magnesium and iron, but it is calcium dependent. The site of major calcium overload is localized in the left ventricle, and such overload is not the result of β-adrenergic receptor involvement. In contrast to some forms of congestive heart failure which show elevated taurine concentration in the heart, taurine levels in advanced stages of myopathy in the cardiomyopathic hamsters are unaltered. Dietary taurine supplementation increases cardiac taurine content, which in turn protects against calcium overload and calcium dependent cardiac necrosis. With advancing myopathy, the protective role of taurine against necrosis is somewhat diminished. This lower protection is due to a decrease in cardiac taurine concentration. Since uptake is higher in the cardiomyopathic animals, the lower cardiac taurine may be due to enhanced efflux of taurine from the heart.
LIST OF REFERENCES


Cloetta, A. "Uber das vorkommen von inosin hermsaure, taurin und

Collins, C. G. S. "The rates of synthesis, uptake and disappearance
of $[^{14}C]$-taurine in eight areas of the rat central nervous

Collu, R., G. Charpenet, and M. Clermont. "Antagonism by taurine of
Sci. 5:139-142 (1978).

related to $\gamma$-aminobutyric acid," Pharmacol. Rev. 17:347-391
(1965).

Cusworth, D. C., and C. E. Dent. "Renal clearance of amino acids in
normal adults and in patients with aminoaciduria," Biochem. J.

Davison, A. N. "Amino acid decarboxylases in rat brain and liver,"


vesicles from mammalian cerebral cortex: a reappraisal," J.

DeGuzman, N. T., O. Munoz, and R. F. Palmer. "A clinical evaluation of
amrinone—a new inotropic agent," Circl. 58:Supplz:IX-183-II-188
(1978).

DeLorenzo, R. J., K. G. Walton, P. F. Curran, and P. Greengard,
"Regulation of phosphorylation of a specific protein in toad­
bladder membrane by antidiuretic hormone and cyclic AMP, and
its possible relationship to membrane permeability changes," Proc.

Derouaux, M., E. Puil, and R. Naguet. "Antiepileptic effect of
taurine in photosensitive epilepsy," Electroen-cephalogr.

Dhalla, N. S. "Involvement of membrane systems in heart failure due to
Cardiol. 8:662-666 (1976).

Dhalla, N. S., P. V. Sulakhe, M. Fedeleova, and J. C. Yates.
"Molecular abnormalities in cardiomyopathy," Advances in


Huxtable, R. Professor of Pharmacology, University of Arizona, personal communication (1979).


Rasmussen, H., D. B. Goodman, and A. Tenenhouse. "The role of cyclic

Rassin, D. K., and J. A. Sturman. "Cysteine sulfinic acid decarboxylase
in rat brain: Effect of Vitamin B6 deficiency on soluble and


Read, W. O., and R. D. Welty. "Effect of taurine on epinephrine and
digoxin induced irregularities of the dog heart," J. Pharmacol.

potential of guinea pig papillary muscle," in Taurine, Eds. R.
Huxtable and A. Barbeau, Raven Press, New York, pp. 173-177
(1976).

Reiter, M., K. Seibel, and F. J. Stickel. "Sodium dependence of the
inotropic effect of a reduction in cellular potassium
(1971).

Remtulla, M. A., S. Katz, and D. A. Applegarth. "Effect of taurine on
ATP-dependent calcium transport in guinea pig cardiac muscle,"

Roberts, E., I. Low, M. Chanin, and B. Jelinek. "Free or easily
extractible amino acids of the heart muscle of various

as an adrenergic receptor," Ann. NY Acad. Sci. 139:703-723
(1967).

Rouser, G., K. Kelly, B. Jelinek, and D. Heller. "Free amino acids in
the blood of man and animals. II. Normal individuals and
patients with chronic granulocytic leukemia and polycytemia," in Amino Acid Pools, Ed. J. T. Holden, Elsevier, Amsterdam,

Ryan, W. L., and M. J. Carver. "Free amino acids of human foetal and

Sanchez-Casis, G., M. Coté, and A. Barbeau. "Pathology of the heart in
Friedreich's ataxia: Review of the literature and report of one


