INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book. These are also available as one exposure on a standard 35mm slide or as a 17” x 23” black and white photographic print for an additional charge.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.
Cardiac electrophysiology and cardiotoxicity of halothane

Zhang, Ke, Ph.D.
The Ohio State University, 1989

Copyright ©1989 by Zhang, Ke. All rights reserved.
CARDIAC ELECTROPHYSIOLOGY AND CARDIOTOXICITY OF HALOTHANE

DISSertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of the Ohio State University

By

Ke Zhang, B.S., M.S.

* * * * *

The Ohio State University

1989

Dissertation Committee:
R.L. Hamlin
S.M. Strauch
W.W. Muir
R. Stradley

Approved by

Adviser

Department of Veterinary Physiology and Pharmacology
ACKNOWLEDGMENTS

I wish to thank my advisers, Drs. S. M. Strauch and R. L. Hamlin for their unfailing encouragement and guidance throughout these studies. Completion of these studies would not have been possible without their help. Special thanks goes to Dr. S. M. Strauch for his enthusiasm and friendship. His input into my career goes far beyond these studies. I am also grateful to the other members of my graduate committee, Drs. W. W. Muir and R. P. Stradley for their valuable advice, suggestion and comments.

I would like to express appreciation to Jan Sally for technical assistance.

I would like to extend grateful appreciation to Drs. W. W. Muir and R. Fertel for the use of their laboratories and Dr. S.A. McCune for the use of her rats.

I am extremely appreciative of the persons within the Department of Veterinary Physiology and Pharmacology who have made possible my participation in the program.

To my mother, De-Yu Sun, and father, Ji-Xuan Zhang,
who stimulated in me the interest, desire, and impetus to pursue these higher educational goals, I offer sincere appreciation for their support and encouragement.

To my wife, Chen X. Zhang, for the many sacrifices she has made to help me attain this goal and for her constant encouragement and understanding throughout my pursuit for this degree.
VITA

January 6, 1958 ............. Born - Shenyang, China

1977-1982 ................... B.S. in Medicine, 
                                   Jin-Zhou College of Medicine, 
                                   Jin-Zhou, China

1982-1985 ................... M.S. in Pharmacology, 
                                   China Medical University, 
                                   Shenyang, China

1985-present ............... Graduate Research Associate 
                                   Department of Veterinary, 
                                   Physiology and Pharmacology, 
                                   The Ohio State University, 
                                   Columbus, Ohio

PUBLICATIONS

Zhang, K: The Effects of Cimetidine on Action Potentials of 
Canine Cardiac Purkinje Fibers and Experimental 
Arrhythmias. Abstract. Pharmacological Communications of 

Zhang, K. and Strauch, S.M: The Role of Cyclic AMP in 


Zhang, K. and Strauch, S.M: Myocardial Contractile and 
Electrical Performance in Spontaneous Cardiomyopathy-
Congestive Heart Failure Rats. Abstract. The Proceedings 
for the First Conference of the Chinese Association of 
Agricultural Student and Scholars. June 24-25, 1989.

FIELDS OF STUDY

Cardiac Electrophysiology 
Cardiovascular Pharmacology
# TABLE OF CONTENTS

ACKNOWLEDGMENTS ........................................ ii
VITA .................................................. iv
LIST OF TABLES .................................. vii
LIST OF FIGURES .................................. x
ABBREVIATIONS ........................................ xv

## CHAPTER PAGE

<table>
<thead>
<tr>
<th>I. THE DIFFERENTIAL EFFECTS OF HALOTHANE ON ACTION POTENTIAL DURATION IN PURKINJE FIBERS AND VENTRICULAR MUSCLE</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Literature Review</td>
<td>4</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>8</td>
</tr>
<tr>
<td>Results</td>
<td>17</td>
</tr>
<tr>
<td>Discussion</td>
<td>57</td>
</tr>
<tr>
<td>List of References</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. THE ROLE OF CYCLIC AMP IN HALOTHANE SENSITIZATION</th>
<th>77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>77</td>
</tr>
<tr>
<td>Literature Review</td>
<td>79</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>86</td>
</tr>
<tr>
<td>Results</td>
<td>101</td>
</tr>
<tr>
<td>Discussion</td>
<td>131</td>
</tr>
<tr>
<td>List of References</td>
<td>143</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III. THE EFFECTS OF CALCIUM AND HALOTHANE ON ELECTROPHYSIOLOGICAL PROPERTIES OF HYPERTROPHIED VENTRICULAR PAPILLARY MUSCLE OF CONGESTIVE HEART FAILURE RATS</th>
<th>151</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>151</td>
</tr>
<tr>
<td>Literature Review</td>
<td>153</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>157</td>
</tr>
<tr>
<td>Results</td>
<td>163</td>
</tr>
<tr>
<td>Discussion</td>
<td>188</td>
</tr>
<tr>
<td>List of References</td>
<td>197</td>
</tr>
</tbody>
</table>
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and overshoot (OS) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>24</td>
</tr>
<tr>
<td>2. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>24</td>
</tr>
<tr>
<td>3. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and overshoot (OS) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>29</td>
</tr>
<tr>
<td>4. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>29</td>
</tr>
<tr>
<td>5. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and overshoot (OS) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>36</td>
</tr>
<tr>
<td>6. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>36</td>
</tr>
<tr>
<td>7. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and overshoot (OS) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle</td>
<td>45</td>
</tr>
</tbody>
</table>
8. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle...

9. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and overshoot (OS) for each treatment administered to canine cardiac Purkinje fibers and ventricular muscle...

10. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers...

11. Determination of absolute binding percentage...

12. Means and standard errors of the means of action potential amplitude (APA), overshoot (OS), resting membrane potential (RMP), and maximum diastolic potential (MDP) for each treatment administered to canine cardiac Purkinje fibers...

13. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) for each treatment administered to canine cardiac Purkinje fibers...

14. Means and standard errors of the means of action potential amplitude (APA), overshoot (OS), resting membrane potential (RMP), and maximum diastolic potential (MDP) for each treatment administered to canine cardiac Purkinje fibers...

15. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) for each treatment administered to canine cardiac Purkinje fibers...

16. Means and standard errors of the means of cyclic AMP (cAMP) level for each treatment administered to canine Purkinje fibers...

17. Means and standard errors of the means of escape time (ET), spontaneous rate (SR), and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers...
18. Means and standard errors of the means of escape time (ET), spontaneous rate (SR), and maximum rate of rise of phase 0 (Vmax) for each treatment administered to canine cardiac Purkinje fibers ... 126

19. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and maximum rate of rise of phase 0 (Vmax) for each treatment administered to ventricular papillary muscle ......................... 169

20. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) and heart weight (HW) for each treatment administered to ventricular papillary muscle .............................................. 170

21. Means and standard errors of the means of resting membrane potential (RMP), action potential amplitude (APA), and maximum rate of rise of phase 0 (Vmax) for each treatment administered to ventricular papillary muscle ......................... 179

22. Means and standard errors of the means of action potential duration at 50% and 90% repolarization (APD50 and APD90) for each treatment administered to ventricular papillary muscle ..................... 180

23. Effects of Ca++ on action potential duration at 50% repolarization (APD50) at different conditions ......................................................... 187

24. Effects of Ca++ on action potential duration at 90% repolarization (APD90) at different conditions ......................................................... 187

25. Computer program for analysis of cyclic AMP concentration ......................................................... 206
LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Schematic diagram of electronic equipment</td>
<td>12</td>
</tr>
<tr>
<td>2. Action potentials of Purkinje fiber and ventricular muscle</td>
<td>14</td>
</tr>
<tr>
<td>3. Action potentials recorded from Purkinje fibers and ventricular muscle before and after halothane 2% and 4%</td>
<td>26</td>
</tr>
<tr>
<td>4. Effects of halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle</td>
<td>28</td>
</tr>
<tr>
<td>5. Effects of halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle</td>
<td>28</td>
</tr>
<tr>
<td>6. Action potentials recorded from Purkinje fibers and ventricular muscle before and after verapamil 3 X 10^{-6} M</td>
<td>31</td>
</tr>
<tr>
<td>7. Action potentials recorded from Purkinje fibers and ventricular muscle before and after verapamil 3 X 10^{-6} M and combination of verapamil and halothane 2% and 4%</td>
<td>33</td>
</tr>
<tr>
<td>8. Effects of verapamil 3 X 10^{-6} M on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle</td>
<td>35</td>
</tr>
<tr>
<td>9. Effects of verapamil 3 X 10^{-6} M and halothane 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle</td>
<td>35</td>
</tr>
<tr>
<td>10. Action potentials recorded from Purkinje fibers and ventricular muscle before and after tetrodotoxin (TTX) 5 X 10^{-7} M</td>
<td>38</td>
</tr>
<tr>
<td>11. Action potentials recorded from Purkinje fibers and ventricular muscle before and after TTX 5 X 10^{-7} M and halothane 2% and 4%</td>
<td>40</td>
</tr>
</tbody>
</table>
12. Effects of TTX $5 \times 10^{-7}$ M and halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle .......................... 42

13. Effects of TTX $5 \times 10^{-7}$ M and halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle ................................ 42

14. Percentage changes of action potential durations at 50% repolarization (APD50) after TTX $5 \times 10^{-7}$ M and halothane 2% and 4% .................................. 44

15. Percentage changes of action potential durations at 90% repolarization (APD90) after TTX $5 \times 10^{-7}$ M and halothane 2% and 4% .................................. 44

16. Action potentials recorded from Purkinje fibers and ventricular muscle before and after tetraethylammonium (TEA) $1 \times 10^{-2}$ M .................. 47

17. Action potentials recorded from Purkinje fibers and ventricular muscle before and after TEA $1 \times 10^{-2}$ M and combination of TEA $1 \times 10^{-2}$ M and halothane 2% and 4% .............................. 49

18. Effects of TEA $1 \times 10^{-2}$ M and halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle ............................. 51

19. Effects of TEA $1 \times 10^{-2}$ M and halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle ............................. 51

20. Effects of halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle at stimulating interval = 500 mSec ................................. 54

21. Effects of halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle at stimulating interval = 500 mSec ................................. 54

22. Combination of effects of halothane 2% and 4% on action potential duration at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle at stimulating intervals = 500 mSec and 1000 mSec ................................. 56
23. Combination of effects of halothane 2% and 4% on action potential duration at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle at stimulating intervals = 500 mSec and 1000 mSec .................................................. 56

24. Schematic diagram of electronic equipment .............. 89

25. Action potentials of paced and unpaced Purkinje fibers ............................................... 93

26. Action potentials recorded from Purkinje fibers before and after halothane 2% and 4% ............. 111

27. Action potentials recorded from Purkinje fibers before and after aminophylline 5 X 10^-6 to 5 X 10^-4 M ............................................. 113

28. Action potentials recorded from Purkinje fibers before and after isoproterenol 1 X 10^-8 to 1 X 10^-6 M ............................................. 115

29. Action potentials recorded from Purkinje fibers before and after halothane 2% and aminophylline 5 X 10^-6 to 5 X 10^-4 M ............................................. 117

30. Action potentials recorded from Purkinje fibers before and after halothane 2% and combination of halothane 2% and isoproterenol 1 X 10^-8 to 1 X 10^-6 M ............................................. 119

31. Percentage changes of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) from control after halothane 1%, 2% and 4%............................ 121

32. Percentage changes of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) from control after combined treatments ........................................ 121

33. Percentage changes of cyclic AMP (cAMP) from control after combined treatments ........... 124

34. Action potentials recorded from Purkinje fibers before and after halothane 2% and 4% .......... 128

35. Percentage changes of escape time (ET) and spontaneous rate (SR) from control after halothane 1%, 2% and 4% ........................................ 130

36. Percentage changes of escape time (ET) and spontaneous rate (SR) from control after combined treatments ........................................ 130
37. Action potential of ventricular papillary muscle .. 160
38. Action potentials recorded from ventricular papillary muscle ....................... 172
39. Action potentials recorded from ventricular papillary muscle ....................... 174
40. Effects of increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solutions at 50% repolarization (APD50) of ventricular papillary muscle ....................... 176
41. Effects of increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solutions at 90% repolarization (APD90) of ventricular papillary muscle ....................... 176
42. Percentage change of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solutions .............. 178
43. Percentage change of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solutions .............. 178
44. Effects of halothane 2% and 4%, increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ after halothane 4% on action potential duration at 50% repolarization (APD50) of ventricular muscle ....................... 182
45. Effects of halothane 2% and 4%, increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ after halothane 4% on action potential duration at 90% repolarization (APD90) of ventricular muscle ....................... 182
46. Percentage changes of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle after halothane 2% and 4% ................. 184
47. Percentage changes of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle after halothane 2% and 4% ................. 184
48. Percentage changes of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle with and without pretreatment by halothane 4% after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ ...................... 186
49. Percentage changes of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle with and without pretreatment by halothane 4% after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ .......................... 186

50. Flow chart of computer program for analysis of cyclic AMP concentration ............................ 205
ABBREVIATIONS

RMP = Resting membrane potential
MDP = Maximal diastolic potential
APA = Action potential amplitude
OS = Overshoot
APD = Action potential duration
APD_{50} = Action potential duration at 50% repolarization
APD_{90} = Action potential duration at 90% repolarization
Vmax = Maximal rate of rise of fast depolarization phase
ERP = Effective refractory period
ET = Escape time
SR = Spontaneous rate
I_{si} = Slow inward current
I_{x} = Outward potassium current
I_{x1} = Early component of outward potassium current
I_{x2} = late component of outward potassium current
I_{k1} = Background outward current
I_{k2} = Outward potassium current in diastolic depolarization
I_{f} = Hyperpolarization-induced inward current in diastolic depolarization
cAMP = Cyclic AMP
cGMP = Cyclic GMP

xv
G-protein = Guanine-nucleotide binding regulatory protein

Gs = Stimulatory guanine-nucleotide binding protein

Gi = Inhibitory guanine-nucleotide binding protein

TTX = Tetrodotoxin

TEA = Tetraethylammonium

VER = Verapamil

HAL = Halothane

ISO = Isoproterenol

AMI = Aminophylline

SHR = Spontaneous hypertensive rat
CHAPTER I

THE DIFFERENTIAL EFFECTS OF HALOTHANE ON ACTION POTENTIAL DURATION IN PURKINJE FIBERS AND VENTRICULAR MUSCLE

INTRODUCTION

It has been long recognized that halothane sensitizes the heart to the arrhythmogenic effects of catecholamines (cardiac sensitization).\(^1\)\(^\text{-}\)\(^{24}\) The mechanism(s) of cardiac sensitization, however, is(are) still under investigation, but ultimately results from changes in impulse generation and/or impulse conduction. Investigators \(^9\)\(^,\)\(^{22}\) have shown that halothane decreases the action potential duration and refractory period in canine cardiac Purkinje fibers whereas it has little effect on the action potential of the ventricular muscle. This can result in a disparity in the refractoriness between Purkinje fibers and ventricular muscle, which favors formation of premature stimuli or reentry (arrhythmogenic effects). A decrease in cardiac contractile force and intracellular Ca++ levels in cardiac muscle by halothane has been shown in various species \(^17\).
and the depressant effect of verapamil (Ca++ blocker) and halothane on myocardial contractility is additive, suggesting that halothane might depress the heart by inhibiting the slow inward Ca++ current (I_{si}). This current is the major current responsible for the plateau phase of action potential. In addition to I_{si}, there are at least two other ionic currents responsible for the action potential duration in Purkinje fibers, i.e. a delayed outward potassium current (I_{k}) and a tetrodotoxin-sensitive inward sodium current (Na+ window current). In ventricular muscle, however, the Na+ window current plays little or no role in the action potential repolarization. Therefore, the Na window current may be partly responsible for the longer action potential duration in Purkinje fibers compared to ventricular muscle. Whether or not the differential effects of halothane on action potential duration and refractoriness in canine Purkinje fibers and ventricular muscle are due to the differential effects of halothane on these ionic currents has not been tested. The purpose of this study is to determine the differential effects of halothane on the action potential duration in both Purkinje fibers and ventricular muscle. The effects of halothane in combination with tetrodotoxin (TTX), Na+ channel blocker, and tetraethylammonium (TEA), K+ channel blocker, or verapamil (VER), Ca++ channel blocker were investigated in both Purkinje fibers and ventricular muscle. The effects of halothane on changes in action
potential duration by altering stimulus frequency in both Purkinje fibers and ventricular muscle were also tested.
LITERATURE REVIEW

Halothane sensitizes the heart to the arrhythmogenic effects of catecholamines (cardiac sensitization).\textsuperscript{1-24} Hauswirth in 1969 reported that halothane decreased the action potential duration in Purkinje fibers.\textsuperscript{5} Interestingly, halothane and catecholamines are mutually antagonistic in most of their cardiovascular effects,\textsuperscript{32} but the decrease in action potential duration is an action shared by both halothane and catecholamines. It is believed that the effects of halothane and catecholamines on action potential duration could be an important factor in the induction of arrhythmias.

The ionic currents responsible for the formation of duration of Purkinje fiber action potential include at least three currents, i.e. slow inward Ca++ current, $I_{Si}$, delayed outward potassium current, $I_{X}$ and tetrodotoxin-sensitive inward sodium current, Na+ window current. $I_{Si}$ is the major current responsible for action potential duration in both Purkinje fibers and ventricular muscle. The Na+ window current is absent in ventricular muscle.

Reynolds reported in 1983 that halothane has little effect on the action potential of papillary muscle but it
markedly decreases the effective refractory period of the Purkinje fiber action potential.\(^5\),\(^9\) This results in a disparity in the refractory period between Purkinje fiber and myocardial muscle. Therefore, a premature stimulus from an ectopic focus will encounter the early excitable tissue which facilitates the formation of the reentry arrhythmias. It has been reported that halothane depresses cardiac contractility in a dose-dependent manner.\(^{18,33}\) Lynch et al. found that halothane depresses the slow action potential and decreases the slow inward Ca++ current, \(I_{\text{Si}}\) in guinea pig papillary muscle and thus decreases action potential duration.\(^{34-36}\) \(I_{\text{Si}}\) is responsible for both the slow action potential and the plateau phase of the fast action potential under physiological conditions. The cardiac depressant effect of halothane can be overcome by addition of calcium and potentiated by the calcium blocker, verapamil. Hirota et al. used whole cell voltage clamp technique to study the effects of halothane on the \(I_{\text{Si}}\) and \(I_X\) in single cells from bullfrog atrium.\(^{37}\) They found that halothane significantly increases the action potential duration and decreases the action potential plateau. They also demonstrated that halothane inhibits \(I_X\) and \(I_{\text{Si}}\). The inhibition by halothane on these currents is probably responsible for the increase in action potential duration and decrease in the plateau. A slight increase in action potential duration in rabbit atrial cells by halothane was also reported.\(^5\)
The action potential duration is longer in Purkinje fibers than in ventricular muscle.\textsuperscript{38-39} The maximum action potential duration is found in the few millimeters proximal to the terminal Purkinje fiber. This forms a gating mechanism which determines functional refractory period of the conducting system and plays a critical role in protecting the heart from prematurity under physiological conditions.\textsuperscript{40} Although the mechanism(s) for the greater action potential duration in Purkinje fibers compared to ventricular muscle is still under investigation, it was reported that a steady-state tetrodotoxin-sensitive inward sodium current persists in Purkinje fibers which may be partly responsible for these differences.\textsuperscript{30-31} Coraboeuf \textit{et al.} showed that TTX shortens action potential duration much more markedly in Purkinje fibers than in ventricular muscle at a TTX concentration much lower than the TTX-induced decrease in maximum rate of rise of action potential (V$_{\text{max}}$).\textsuperscript{30}

Action potential depolarization, caused by a large, fast transient of inward sodium current, is maintained as the plateau because of a balance between inward and outward currents.\textsuperscript{41} The inward current is carried by Ca++ ($I_{\text{Si}}$) and the outward current predominantly by K+ ($I_{\text{x}}$). Depending on the relative effects on $I_{\text{Si}}$ and $I_{\text{x}}$, either a longer or a shorter duration of the action potential will prevail.\textsuperscript{41}
The preceding cycle length is a very important determinant of cardiac action potential duration. Alteration of stimulating frequency or heart rate changes the action potential duration in both Purkinje fibers and ventricular muscle. Although the increase in stimulating frequency or heart rate can lead to a decrease in the action potential duration from both types of tissues, the response in ventricular muscle is usually more complex than Purkinje fibers. In Purkinje fibers, a sudden decrease in cycle length shortens the action potential by reducing the plateau duration with relatively little effect on the time course of the late repolarization whereas the response of ventricular muscle includes an initial elevation, prolongation of the plateau phase and a marked steepening of the final repolarization limb and then a constant decrease in the duration.
MATERIALS and METHODS

Adult mongrel dogs (15-35 kg) were anesthetized with sodium pentobarbital, 30 mg/kg. The hearts were removed rapidly via a lateral thoracotomy and placed in carbongenated (95% O₂, 5% CO₂, Liquid Carbonic, Chicago, IL) Tyrode's solution at room temperature to let them pump free of blood. The Tyrode's solution contained the following composition in millimoles per liter: NaCl, 137.0; NaHCO₃, 12.0; CaCl₂ 1.8; MgCl₂, 0.5; NaH₂PO₄, 1.8; KCl, 4.0; and glucose, 5.5.

Left ventricular Purkinje fibers were uncovered by cutting the left ventricle from base to apex along the interpapillary free wall. Purkinje fiber and a myocardial pad in the anatomically distal end of the fiber were carefully cut and placed in a beaker of Tyrode's solution bubbled with carbogen (95% O₂ and 5% CO₂).

Right ventricular Purkinje fibers were uncovered after cutting the right ventricle from base to apex along the anterior interventricular sulcus and prepared as above.

About three to four Purkinje fibers were obtained from each heart. One Purkinje fiber was mounted in a tissue bath to record action potential characteristics. The Purkinje fiber was mounted on a molded acrylic tissue holder with
the anatomically proximal end of the fibers overlying bipolar platinum extracellular electrodes. The holder was fixed to the bottom of the tissue bath by nylon screws. The bath was perfused with carbongenated (95% O₂ and 5% CO₂) Tyrode’s solution. The perfused solution was temperature (37°C) and pH (7.4) controlled. The preparations were stimulated at a constant rate (60 times/minutes) through the stimulating electrodes by rectangular pulses of 2-msec duration at twice the threshold voltage.

The Purkinje fiber with a myocardial pad for recording action potential characteristics was impaled with microelectrodes at both the Purkinje fiber and ventricular muscle recording sites. Microelectrodes with resistances ranging from 10 to 30 MΩ were prepared on a horizontal magnetic electrode puller (Narishige Scientific Instrument Laboratories, Tokyo, Japan). Microelectrodes were filled with 2.5 M KCl and then coupled by an Ag-AgCl cell to an amplifier (S-7071A, WP Instruments, Inc., New Haven CT) with high input impedance and input capacity compensation. The output of the amplifier was connected to an oscilloscope (Model 5111A with one 5A18N four channel and 5B12N dual time base, Tektronix, Inc., Beaverton, OR) with storage capabilities. The first derivatives of the action potentials were obtained electronically using a differentiator. The measurement is linear from 0 to 1000
volts/second. The action potentials (Figure 1.) and their derivatives were displayed on the first and second channels of the oscilloscope, respectively. Records of Purkinje fiber membrane potentials and their derivatives were calibrated by passing 100 mV and 600 volts/second signals, respectively, between the microelectrodes and the Ag-AgCl ground reference cell. The membrane potentials and their derivatives were also recorded on a VCR (General Electric) and Polaroid film for analysis.

The basic protocol was to record normal membrane characteristics after an equilibration period of 30 minutes. During a test period, a drug was introduced and the same membrane characteristics were determined for comparison to controls. The membrane characteristics which were determined during the control and test periods were as follows:

Action Potential Characteristics (Figure 2):

1. Resting Membrane Potential, RMP (mV): the potential just before rapid depolarization phase.

2. Action Potential Amplitude, APA (mV): the height of rapid depolarization phase from RMP to peak potential.

3. Overshoot, OS (mV): the peak positive potential from zero potential to peak potential.
Figure 1.
Schematic Diagram of Electronic Equipment
Figure 1
Figure 2.

Action potentials of Purkinje fiber and ventricular muscle.

a: Action potential of Purkinje fiber
b: Action potential of ventricular muscle
Figure 2
4. Action Potential Duration, APD (msec): time from the beginning of rapid depolarization to 50% (APD$_{50}$), and 90% (APD$_{90}$) of the action potential repolarization.

5. Maximum rate of rise of rapid depolarization phase, Vmax or dV/dt (V/sec): the derivative of rapid depolarization phase.

After the control period, the following tests were made:

1. Halothane (Halocarbon Laboratories, Inc)  
   2% and 4%

2. Verapamil (American Reagent Laboratories, Inc.)  
   $3 \times 10^{-6}$ M, followed by halothane (2% and 4%)

3. Tetrodotoxin (Sigma Chemical Company) $5 \times 10^{-7}$ M,  
   followed by halothane (2% and 4%)

4. Tetraethylammonium (Eastman Kodak Company) $1 \times 10^{-2}$ M,  
   followed by halothane (2% and 4%)

5. Halothane (Halocarbon Laboratories, Inc)  
   2% and 4% at Stimulus interval of 500 mSec  
   In all experiments, halothane was carried by O$_2$
via a calibrated vaporizer (FLUOTEC 3, CYPRANE Keighley, England) and introduced to the perfusate reservoir through a glass dispersion tube. Monitoring of $P_{O_2}$, $P_{CO_2}$ and pH were performed so as to maintain them at predetermined levels. Two 1.0 ml bath samples were drawn and injected into sealed vials at the conclusion of each anesthetic exposure for gas chromatographic analysis (Gas Chromatograph, Model 3700, Varian Instrument Group, Sunnyvale, CA). Standard curves were constructed daily from known amounts of vapor. The chromatographic peaks of halothane from the drawn sample were compared with peaks from standard curve. A scavenging system for limiting exposure of personnel to anesthetics was employed.

TTX stock solution was made up in acetic acid at a concentration of $5 \times 10^{-4}$ M and 1 ml of the stock solution was added to 1 L volume of perfusion reservoir. TEA stock solution was made up in double distill water at a concentration of 2 M.

The statistical significance of differences in mean values for electrophysiological data was assessed using analysis of variance and Duncan's multiple range test. Differences of $P < 0.05$ were considered significant.
RESULTS

Effects of Halothane on Action Potentials of Purkinje Fibers and Ventricular Muscle

Means and standard errors of the means of Purkinje fiber and ventricular muscle electrophysiology after each treatment are listed in Tables 1 and 2. The effects of 2% and 4% halothane on the Purkinje fibers and ventricular muscle are compared in Figure 3. Panel a shows normal action potentials of the Purkinje fiber and ventricular muscle. Panel b shows the effects of 2% halothane on the action potentials of the Purkinje fiber and ventricular muscle. Two percent halothane shortens the action potential duration of the Purkinje fiber, but has no effect on ventricular muscle. The effect of 4% halothane on the action potentials of the Purkinje fiber and ventricular muscle is illustrated in Panel c. Four percent halothane further decreases the action potential duration of the Purkinje fiber significantly (P < 0.05) and slightly increases the action potential duration at 90% repolarization in ventricular muscle. Quantitative summaries of the effects of halothane on action potential durations of the Purkinje fiber and ventricular muscle are presented graphically in Figures 4 and 5.
Effects of Halothane on the Action Potential of Purkinje fiber and Ventricular Muscle Pretreated by Verapamil

Means and standard errors of the means of Purkinje fiber and ventricular muscle electrophysiology after each treatment are listed in Tables 3 and 4. Table 4 also shows the percentage decreases of the action potential duration at 50% repolarization by halothane (2% and 4%) in the presence and absence of verapamil. The effects of $3 \times 10^{-6}$ M verapamil on the action potential of the Purkinje fiber and ventricular muscle are shown in Figure 6. Panel a shows the normal action potential of both Purkinje fiber and ventricular muscle. Panel b shows the effects of verapamil on the action potentials. Verapamil depresses the action potential plateau phase and decreases the action potential duration at 50% repolarization and increases the duration at 90% repolarization of the Purkinje fiber while it decreases action potential duration at both 50% and 90% repolarization in ventricular muscle. The effects of 2% and 4% halothane on the Purkinje fiber and ventricular muscle pretreated with verapamil are compared in Figure 7. Panel a shows normal action potentials of the Purkinje fiber and ventricular muscle. Panel b shows the effects of verapamil on the action potentials of the Purkinje fiber and ventricular muscle. Panel c shows the combined effects of verapamil and 2% halothane. Two percent
halothane decreases the action potential duration of both Purkinje fiber and ventricular muscle. Panel d shows the combined effects of verapamil and 4% halothane on the action potential duration. Four percent halothane further decreases the action potential duration of both Purkinje fiber and ventricular muscle. Quantitative summaries of the effects of verapamil and/or halothane on action potential duration of the Purkinje fiber and ventricular muscle are presented graphically in Figures 8 and 9.

Effects of Halothane on the Action Potential of Purkinje fiber and Ventricular Muscle Pretreated by Tetrodotoxin (TTX)

Means and standard errors of the means of Purkinje fiber and ventricular muscle electrophysiology after each treatment are listed in Tables 5 and 6. The effects of $5 \times 10^{-7}$ M TTX on the action potential on the Purkinje fiber and ventricular muscle are shown in Figure 10. Panel a shows the normal action potential of both Purkinje fiber and ventricular muscle. Panel b shows the effects of TTX on the action potentials. TTX depresses the action potential plateau phase and decreases the action potential duration of Purkinje fiber significantly while it has no effects on action potential duration of ventricular muscle.
The effects of 2% and 4% halothane on the Purkinje fiber and ventricular muscle pretreated with TTX are compared in Figure 11. Panel a shows normal action potentials of the Purkinje fiber and ventricular muscle. Panel b shows the effects of TTX on the action potentials of the Purkinje fiber and ventricular muscle. Panel c shows the combined effects of TTX and 2% halothane. Two percent halothane decreases the action potential duration of Purkinje fiber while it slightly increases the action potential duration of ventricular muscle. Panel d shows the combined effects of TTX and 4% halothane on the action potential duration. Four percent halothane further decreases the action potential duration of the Purkinje fiber significantly while it slightly increases the duration of ventricular muscle. Quantitative summaries of the effects of TTX and/or halothane on action potential duration of the Purkinje fiber and ventricular muscle are presented graphically in Figures 12 and 13. Figures 14 and 15 show the percentage change of action potential duration at 50% and 90% repolarization after halothane (2% and 4%), TTX 5 × 10⁻⁷ M and the combination of halothane and TTX. Halothane at 2% and 4% decreases action potential duration at 50% repolarization about 10% and 20% (#2 in Figure 14), respectively whereas halothane at 2% and 4% in the presence of TTX decreases the duration about 29% and 39% (#3 in Figure 14), respectively. However, TTX itself decreases the duration about 17% (#1 in Figure 14). If the effect due
to TTX (#1) is subtracted from the effect of halothane in the presence of TTX (#3), the net effect of halothane at 2% and 4% is about 12% and 22% which are very close to the effects of halothane in the absence of TTX, 10% and 20%. Likewise, for action potential duration at 90% repolarization the net effect of halothane at 2% and 4% is about 5.0% and 10.0% which are very close to the effects of halothane in the absence of TTX, 6.0% and 11.0% (Figure 15).

Effects of Halothane on the Action Potential of Purkinje fiber and Ventricular Muscle Pretreated by Tetraethylammonium (TEA)

Means and standard errors of the means of Purkinje fiber and ventricular muscle electrophysiology after each treatment are listed in Tables 7 and 8. The effects of 1 X 10^{-2} mM TEA on the action potential on the Purkinje fiber and ventricular muscle are shown in Figure 16. Panel a shows the normal action potential of both Purkinje fiber and ventricular muscle. Panel b shows the effects of TEA on the action potentials. TEA elevates the action potential plateau phase and increases the duration of both Purkinje fiber and ventricular muscle, but these effects are more prominent in the Purkinje fiber. The effects of 2% and 4% halothane on the Purkinje fiber and ventricular
muscle pretreated with $1 \times 10^{-2}$ mM TEA are compared in Figure 17. Panel a shows normal action potentials of the Purkinje fiber and ventricular muscle. Panel b shows the effects of TEA on the action potentials of the Purkinje fiber and ventricular muscle. Panel c shows the combined effects of TEA and 2% halothane. Two percent halothane decreases the action potential duration of Purkinje fiber significantly while it increases the action potential duration of ventricular muscle significantly. Panel d shows the combined effects of TEA and 4% halothane on the action potential duration. Four percent halothane further decreases the action potential duration of the Purkinje fiber significantly while it increases the duration of ventricular muscle significantly. Quantitative summaries of the effects of TEA and/or halothane on action potential duration of the Purkinje fiber and ventricular muscle are presented graphically in Figures 18 and 19.

Effects of Halothane on the Action Potential of Purkinje fiber and Ventricular Muscle at a Stimulating Interval of 500 mSec

Means and standard errors of the means of Purkinje fiber and ventricular muscle electrophysiology at a stimulating interval of 500 mSec are listed in Tables 9 and 10. Quantitative summaries of the effects of 2% and 4%
halothane on action potential duration at 50% and 90% repolarization of the Purkinje fiber and ventricular muscle at a stimulating interval 500 mSec are shown graphically in Figures 20 and 21, respectively. Two percent and four percent halothane decrease the action potential duration of Purkinje fiber while it slightly increases the action potential duration of ventricular muscle. The effects of 2% and 4% halothane on the action potential durations at 50% and 90% repolarization of the Purkinje fiber and ventricular muscle at both stimulating intervals 500 mSec and 1000 mSec are compared in Figures 22 and 23, respectively. The figures show parallel decreases in the action potential durations of the Purkinje fiber at both 50% and 90% repolarization by halothane in a dose dependent manner at both stimulating intervals. However, the durations of ventricular muscle do not change significantly.
Table 1.
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Overshoot (OS) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP(-mV)</th>
<th>APA(mV)</th>
<th>OS(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90.25±2.29</td>
<td>120.08±1.84</td>
<td>29.83±1.18</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>89.50±2.76</td>
<td>117.92±2.13</td>
<td>28.42±0.97</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>86.92±3.53</td>
<td>115.42±2.63</td>
<td>28.46±1.16</td>
</tr>
<tr>
<td></td>
<td>Ventricular Muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93.67±0.98</td>
<td>116.45±1.89</td>
<td>22.78±1.32</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>95.62±0.75</td>
<td>119.28±2.02</td>
<td>23.67±1.32</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>94.70±1.46</td>
<td>117.72±2.16</td>
<td>23.02±1.31</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) and Maximum rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>Vmax(v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>205.0±8.86</td>
<td>315.5±13.27</td>
<td>526.7±53.71</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>183.7±10.98</td>
<td>296.3±14.14</td>
<td>477.1±45.55</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>162.0±10.49*</td>
<td>280.3±14.47</td>
<td>402.5±44.41</td>
</tr>
<tr>
<td></td>
<td>Ventricular Muscles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>139.1±9.48</td>
<td>188.7±8.49</td>
<td>241.2±33.59</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>142.0±11.10</td>
<td>191.4±9.24</td>
<td>243.7±40.94</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>146.4±10.55</td>
<td>200.1±9.14</td>
<td>243.0±44.98</td>
</tr>
</tbody>
</table>

* = P < 0.05 compared with control, N = 6
Figure 3.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after halothane 2% and 4%

a: Control
b: Halothane 2%
c: Halothane 4%
Figure 4.

Effects of halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fibers and ventricular muscle. * compared with control

Figure 5.

Effects of halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fibers and ventricular muscle
Figure 4

Figure 5
Table 3.
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Overshoot OS for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP(-mV)</th>
<th>APA(mV)</th>
<th>OS(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purkinje Fibers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>90.33±2.59</td>
<td>127.75±1.79</td>
<td>37.42±1.12</td>
</tr>
<tr>
<td>VER 3X10-6 M</td>
<td>89.67±2.24</td>
<td>128.42±2.46</td>
<td>38.75±0.69</td>
</tr>
<tr>
<td>VER + 2% HAL</td>
<td>89.20±2.04</td>
<td>127.15±2.44</td>
<td>38.00±0.76</td>
</tr>
<tr>
<td>VER + 4% HAL</td>
<td>88.38±2.42</td>
<td>126.30±2.55</td>
<td>37.92±1.08</td>
</tr>
</tbody>
</table>

| **Ventricular Muscles** |            |            |           |
| Control               | 88.27±1.27 | 112.62±1.72| 24.35±2.21|
| VER 3X10-6 M          | 87.32±1.28 | 110.72±2.09| 23.40±1.78|
| VER + 2% HAL          | 88.05±1.30 | 110.73±1.24| 22.68±1.26|
| VER + 4% HAL          | 88.55±0.96 | 111.30±0.79| 22.77±1.14|

N = 6
VER = Verapamil
HAL = Halothane

Table 4.
Means and Standard Errors of the Means Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) and Maximum rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>Vmax(v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purkinje Fibers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>187.2±29.06</td>
<td>292.3±27.55</td>
<td>528.0±66.57</td>
</tr>
<tr>
<td>VER 3X10-6 M</td>
<td>140.0±22.04</td>
<td>313.2±32.16</td>
<td>532.0±66.45</td>
</tr>
<tr>
<td>VER + 2% HAL</td>
<td>a116.5±16.25*</td>
<td>295.7±30.69</td>
<td>523.3±65.39</td>
</tr>
<tr>
<td>VER + 4% HAL</td>
<td>b90.50±10.37**</td>
<td>277.7±28.33</td>
<td>471.2±69.43</td>
</tr>
</tbody>
</table>

| **Ventricular Muscles** |            |            |           |
| Control               | 168.20±15.31 | 219.67±9.21 | 221.0±39.06|
| VER 3X10-6 M          | 160.80±15.47 | 213.67±9.61 | 212.5±25.80|
| VER + 2% HAL          | 152.30±15.10 | 206.33±9.94 | 205.5±40.57|
| VER + 4% HAL          | 148.70±13.59 | 201.80±11.10| 208.3±39.96|

* = P < 0.05, ** = P < 0.01 compared with control, N = 6
a = % decrease in APD50 from control: 38.0 at VER + 2% HAL
b = % decrease in APD50 from control: 52.0 at VER + 4% HAL
% decrease in APD50 from control: 10.0 at 2% HAL (Table 2)
% decrease in APD50 from control: 21.0 at 4% HAL (Table 2)
VER = Verapamil
HAL = Halothane
Figure 6.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after verapamil 3 X 10^{-6} M

a. Control
b. Verapamil 3 X 10^{-6} M
Figure 7.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after verapamil $3 \times 10^{-6}$ M and combination of verapamil $3 \times 10^{-6}$ M and halothane 2% and 4%.

a. Control
b. Verapamil $3 \times 10^{-6}$ M
c. Verapamil $3 \times 10^{-6}$ M + halothane 2%
d. Verapamil $3 \times 10^{-6}$ M + halothane 4%
Figure 7
Figure 8.

Effects of verapamil 3 X 10⁻⁶ M and halothane 2% and 4% on action potential durations at 50% repolarization (APD₅₀) of Purkinje fiber and ventricular muscle. * and ** compared with control

Figure 9.

Effects of verapamil 3 X 10⁻⁶ M and halothane 2% and 4% on action potential durations at 90% repolarization (APD₉₀) of Purkinje fiber and ventricular muscle.
Table 5.
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Overshoot (OS) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP(-mV)</th>
<th>APA(mV)</th>
<th>OS(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>91.15±2.88</td>
<td>125.4±4.12</td>
<td>34.27±2.07</td>
</tr>
<tr>
<td>TTX 5X10^-7 M</td>
<td>87.42±3.74</td>
<td>121.5±4.90</td>
<td>34.15±1.87</td>
</tr>
<tr>
<td>TTX + 2% Hal</td>
<td>86.75±3.73</td>
<td>123.1±3.47</td>
<td>34.22±1.89</td>
</tr>
<tr>
<td>TTX + 4% Hal</td>
<td>86.27±4.27</td>
<td>121.6±5.66</td>
<td>33.17±3.29</td>
</tr>
<tr>
<td>Ventricular Muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93.27±2.33</td>
<td>113.1±2.01</td>
<td>19.90±1.96</td>
</tr>
<tr>
<td>TTX 5X10^-7 M</td>
<td>93.25±2.70</td>
<td>113.5±2.11</td>
<td>19.35±2.09</td>
</tr>
<tr>
<td>TTX + 2% Hal</td>
<td>92.85±2.64</td>
<td>111.3±2.33</td>
<td>18.48±2.09</td>
</tr>
<tr>
<td>TTX + 4% Hal</td>
<td>92.93±3.24</td>
<td>110.0±3.75</td>
<td>18.20±2.53</td>
</tr>
</tbody>
</table>

N = 6
TTX = Tetrodotoxin, HAL = Halothane

Table 6.
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) and Maximum Rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>Vmax(v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>240.0±24.82</td>
<td>337.8±20.16</td>
<td>570.3±47.10</td>
</tr>
<tr>
<td>TTX 5X10^-7 M</td>
<td>199.2±15.92</td>
<td>291.7±11.60</td>
<td>528.7±42.12</td>
</tr>
<tr>
<td>TTX + 2% Hal</td>
<td>169.9±10.12*</td>
<td>273.5±8.03**</td>
<td>495.8±55.14</td>
</tr>
<tr>
<td>TTX + 4% Hal</td>
<td>144.5±13.71**</td>
<td>256.5±6.490**</td>
<td>500.3±54.57</td>
</tr>
</tbody>
</table>

T = Tetrodotoxin, Hal = Halothane
Figure 10.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after tetrodotoxin (TTX) 5 X 10^{-7} M.

a. Control

b. TTX 5 X 10^{-7} M
Figure 10
Figure 11.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after TTX 5 x 10^{-7} M and combination of TTX 5 x 10^{-7} M and halothane 2% and 4%.

a. Control
b. TTX 5 x 10^{-7} M
c. TTX 5 x 10^{-7} M + halothane 2%
d. TTX 5 x 10^{-7} M + halothane 4%
Figure 12.

Effects of TTX 5 X 10^-7 M and halothane 2% and 4% on the action potential durations at 50% repolarization (APD50) of Purkinje fiber and ventricular muscle. * and ** compared with control

Figure 13.

Effects of TTX 5 X 10^-7 M and halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fiber and ventricular muscle. ** compared with control
Figure 12

Figure 13
Figure 14.

Percentage changes of action potential durations at 50% repolarization (APD50) after TTX 5 x 10^{-7} M and halothane 2% and 4% from control.

Figure 15.

Percentage changes of action potential durations at 90% repolarization (APD90) after TTX 5 x 10^{-7} M and halothane 2% and 4% from control.
Figure 14

% CHANGES OF APD50 OF PURLINE FIBERS

(1) TTX 5x10^-7 M
(2) HAL 2% OR 4%
(3) TTX + HAL 2% OR 4%
(4) (3) - (1)

Figure 51

% CHANGES OF APD90 OF PURLINE FIBERS

(1) TTX 5x10^-7 M
(2) HAL 2% OR 4%
(3) TTX + HAL 2% OR 4%
(4) (3) - (1)
### Table 7.
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Overshoot (OS) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP (mV)</th>
<th>APA (mV)</th>
<th>OS (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purkinje Fibers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>94.03±1.85</td>
<td>127.55±3.40</td>
<td>33.62±2.34</td>
</tr>
<tr>
<td>TEA 1X10⁻² M</td>
<td>95.67±1.99</td>
<td>130.37±2.81</td>
<td>32.45±2.26</td>
</tr>
<tr>
<td>TEA + 2% HAL</td>
<td>94.77±2.87</td>
<td>126.68±6.59</td>
<td>31.92±4.34</td>
</tr>
<tr>
<td>TEA + 4% HAL</td>
<td>93.17±2.20</td>
<td>119.95±4.91</td>
<td>28.45±4.14</td>
</tr>
<tr>
<td><strong>Ventricular Muscles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93.08±1.31</td>
<td>111.58±2.60</td>
<td>18.50±1.65</td>
</tr>
<tr>
<td>TEA 1X10⁻² M</td>
<td>92.92±2.45</td>
<td>112.73±4.11</td>
<td>21.48±2.34</td>
</tr>
<tr>
<td>TEA + 2% HAL</td>
<td>89.25±0.99</td>
<td>107.58±2.62</td>
<td>18.33±2.57</td>
</tr>
<tr>
<td>TEA + 4% HAL</td>
<td>88.43±2.80</td>
<td>107.00±2.96</td>
<td>18.57±2.36</td>
</tr>
</tbody>
</table>

N = 6
TEA = Tetraethylammonium
HAL = Halothane

### Table 8.
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD₅₀ and APD₉₀) and Maximum rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD₅₀ (mSec)</th>
<th>APD₉₀ (mSec)</th>
<th>Vmax (v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purkinje Fibers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>301.4±24.20</td>
<td>391.8±28.29</td>
<td>683.0±64.37</td>
</tr>
<tr>
<td>TEA 1X10⁻² M</td>
<td>404.8±15.59**</td>
<td>517.4±21.22**</td>
<td>622.5±52.78</td>
</tr>
<tr>
<td>TEA + 2% HAL</td>
<td>389.2±16.65*</td>
<td>536.6±29.47**</td>
<td>626.8±91.96</td>
</tr>
<tr>
<td>TEA + 4% HAL</td>
<td>328.0±34.61</td>
<td>530.6±55.53**</td>
<td>616.3±94.04</td>
</tr>
<tr>
<td><strong>Ventricular Muscles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>178.67±8.96</td>
<td>224.42±9.85</td>
<td>242.5±20.98</td>
</tr>
<tr>
<td>TEA 1X10⁻² M</td>
<td>218.00±11.92</td>
<td>281.70±13.10*</td>
<td>232.5±26.78</td>
</tr>
<tr>
<td>TEA + 2% HAL</td>
<td>237.30±13.76*</td>
<td>313.00±20.41**</td>
<td>249.0±27.35</td>
</tr>
<tr>
<td>TEA + 4% HAL</td>
<td>274.30±19.59**</td>
<td>367.80±27.88**</td>
<td>239.7±42.12</td>
</tr>
</tbody>
</table>

* = P < 0.05, ** = P < 0.01 compared with control, N = 6
TEA = Tetraethylammonium
HAL = Halothane
Figure 16.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after tetraethylammonium $1 \times 10^{-2}$ M (TEA).

a. Control
b. TEA $1 \times 10^{-2}$ M
Figure 17.

Action potentials recorded from Purkinje fiber and ventricular muscle before and after TEA $1 \times 10^{-2}$ M and combination of TEA $1 \times 10^{-2}$ M and halothane 2% and 4%.

a. Control
b. TEA $1 \times 10^{-2}$ M
c. TEA $1 \times 10^{-2}$ M + halothane 2%
d. TEA $1 \times 10^{-2}$ M + halothane 4%
Figure 18.

Effects of TEA $1 \times 10^{-2}$ M and halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fiber and ventricular muscle. * and ** compared with control.

Figure 19.

Effects of TEA $1 \times 10^{-2}$ M and halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fiber and ventricular muscle. * and ** compared with control.
Figure 18

Figure 19
Table 9.
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Overshoot (OS) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP(-mV)</th>
<th>APA(mV)</th>
<th>OS(mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87.7±1.94</td>
<td>119.32±4.65</td>
<td>31.58±4.49</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>86.18±2.06</td>
<td>118.45±5.83</td>
<td>32.27±4.64</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>84.80±2.64</td>
<td>116.40±5.96</td>
<td>31.60±4.32</td>
</tr>
<tr>
<td>Ventricular Muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>85.52±2.12</td>
<td>110.93±2.75</td>
<td>20.20±2.58</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>85.68±2.44</td>
<td>110.90±2.90</td>
<td>19.82±2.38</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>85.32±2.25</td>
<td>113.40±2.76</td>
<td>20.98±3.93</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* Stimulus Interval = 500 mSec

Table 10.
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) and Maximum Rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Canine Cardiac Purkinje Fibers and Ventricular Muscles*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>Vmax(v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purkinje Fibers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>172.2±15.50</td>
<td>264.4±12.98</td>
<td>455.8±67.14</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>157.2±13.27</td>
<td>252.0±11.32</td>
<td>429.8±85.02</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>131.2±16.73</td>
<td>235.3±11.16</td>
<td>407.5±88.04</td>
</tr>
<tr>
<td>Ventricular Muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>131.6±11.51</td>
<td>177.0±13.88</td>
<td>188.3±42.24</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>127.0±11.39</td>
<td>173.6±13.43</td>
<td>234.3±43.84</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>134.4±13.14</td>
<td>182.4±15.31</td>
<td>233.8±48.37</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* Stimulus Interval = 500 mSec
Figure 20.

Effects of halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fiber and ventricular muscle at stimulating interval = 500 mSec.

Figure 21.

Effects of halothane 2% and 4% on action potential durations at 90% repolarization (APD90) of Purkinje fiber and ventricular muscle at stimulating interval = 500 mSec.
Figure 20

Figure 21
Figure 22.

Comparison of effects of halothane 2% and 4% on action potential durations at 50% repolarization (APD50) of Purkinje fiber and ventricular muscle at stimulating intervals = 500 mSec and 1000 mSec.

Figure 23.

Comparison of effects of halothane 2% and 4% on action potential duration at 90% repolarization (APD90) of Purkinje fiber and ventricular muscle at stimulating intervals = 500 mSec and 1000 mSec.
Figure 22

Figure 23
DISCUSSION

The purpose of this present study is to determine the differential effects of halothane on the action potential duration in the Purkinje fiber and ventricular muscle. The results show that halothane does produce different effects on the action potential durations of the Purkinje fiber and ventricular muscle and the differential effects are also found when the action potentials are treated concurrently with a slow inward calcium channel blocker, verapamil, a sodium channel blocker, TTX, and a potassium channel blocker, TEA.

The normal action potential duration in the Purkinje fiber is longer than the one in ventricular muscle. Results of the present study indicate that halothane depresses the action potential plateau phase and shortens the duration in the Purkinje fiber in a dose dependent manner whereas halothane at 2% has little or no effect on the duration and at 4% increases the duration at 90% repolarization. A similar result was reported in 1983. They found that at 2%, halothane decreased the action potential duration significantly in the Purkinje fiber and had no effect on the ventricular muscle. There are at least three ionic currents responsible for the Purkinje fiber action potential repolarization, i.e. slow
inward Ca++ current, $I_{si}$, delayed outward potassium current, $I_x$, and TTX-sensitive inward sodium current (Na+ window current) whereas there are only two currents, $I_{si}$ and $I_x$ in ventricular muscle responsible for the formation of the action potential duration. The Na+ window current may be responsible for the longer duration in the Purkinje fiber. The two inward currents, $I_{si}$ and Na+ window current, maintaining the action potential duration and plateau, are overcome by the outward current, $I_x$. Increasing the inward currents increases the duration and increasing the outward current decreases the duration and hence completes the repolarization. Therefore, depending on the relative effects on the inward or outward currents, either a longer or a shorter duration will result. Consequently, the decrease in action potential duration by halothane may be due to either a decrease in the inward currents or an increase in the outward current or both. Lynch et al. reported that halothane depresses the slow action potential and decreases the slow inward Ca++ current, $I_{si}$ in guinea pig papillary muscle and thus decreases the action potential duration. Therefore, the depression of plateau phase in the Purkinje fiber by halothane may result from its inhibitory effect on the $I_{si}$ and the decrease in the action potential duration may be mainly due to the inhibition of $I_{si}$ and/or increase in $I_x$. Hauswirth et al. found that halothane decreases the action potential duration in sheep Purkinje fiber and ventricular
muscle and slightly increases the duration in rabbit atrial
cells. Our results in which the action potential durations
in canine ventricular muscle are prolonged are different
from Hauswirth's. The difference may result from the
different species.

Figures 4 and 5 show that in ventricular muscle
halothane (2% and 4%) slightly increases the action
potential duration at 50% and 90% repolarization. Hirota et
al. used whole cell voltage clamp technique to investigate
the effects of halothane on the $I_{s1}$ and $I_x$ in single cells
from bullfrog atrium. They found that halothane
significantly increases action potential duration and
depresses action potential plateau. They also demonstrated
that halothane inhibits $I_x$ and $I_{s1}$. The inhibition by
halothane on these currents may be responsible for the
increase in action potential duration and depression of the
plateau. The inhibition of $I_x$ by halothane may explain our
results in which the action potential duration was
prolonged by halothane in the canine ventricular muscle.
However, the inhibitory effect requires very high
concentration of halothane (4%).

Hauswirth, Noble and Tsien studied the $I_x$ by
using voltage clamp technique and they found that the $I_x$
could be separated into two components, an early rapid
component-\textit{I}_x^1$, and a slow late component-\textit{I}_x^2$. The \textit{I}_x^1 is the major component which is responsible for terminating the plateau by overcoming the depolarizing effect of the inward currents. The later component, \textit{I}_x^2, combined with the \textit{I}_x^1 then terminates the action potential. Halothane has more effect on the duration at 90\% repolarization than the duration at 50\% repolarization in ventricular muscle. This may be because halothane mainly effects the \textit{I}_x^2.

Figure 6 demonstrates that verapamil at $3 \times 10^{-6}$ M depresses the plateau phase and decreases the duration of action potential at 50\% repolarization and increases the duration at 90\% repolarization in the Purkinje fiber. Verapamil is a calcium antagonist which blocks the \textit{I}_{si} and then depresses the plateau and decreases the duration at 50\% repolarization. The \textit{I}_x is Ca++ dependent; increasing the intracellular Ca++ concentration $[\text{Ca}^{++}]_{in}$ can increase the \textit{I}_x, a process called Ca++ trigger \textit{I}_x.\textsuperscript{50} Verapamil blocks the Ca++ channel and decreases the $[\text{Ca}^{++}]_{in}$ and thus decreases the Ca++ trigger force for the \textit{I}_x and therefore \textit{I}_x decreases. The decrease in \textit{I}_x may explain the prolongation of action potential duration at 90\% repolarization. Figures 7, 8 and 9 show that in the ventricular muscle halothane at 2\% and 4\% decreases the durations of the action potentials at both 50\% and 90\% repolarization. It is very interesting that in the absence
of verapamil halothane prolongs the action potential duration at 90% repolarization at 4% whereas in the presence of verapamil halothane decreases the duration constantly at both 2% and 4%. As discussed above, verapamil and halothane can inhibit the $I_{sl}$.\textsuperscript{34,51-52} The inhibitory effects are additive and thus halothane in the presence of verapamil decreases the action potential duration. Although halothane may inhibit $I_X$ and hence increase the duration, the effect may be overcome by the additive inhibitory effects of verapamil and halothane on the $I_{sl}$. As a result, the durations are decreased. Figures 7, 8 and 9 show both action potential durations at 50% and 90% repolarization in the Purkinje fiber are decreased by halothane. This may be mainly due to its inhibitory effects on the $I_{sl}$ and/or increase in the $I_X$. The additive depressant effects of halothane and verapamil on cardiac contractility have been reported.\textsuperscript{29} Our results show that in the Purkinje fiber, the percentage decrease of the duration at 50% repolarization by halothane in the presence of verapamil (38.0 and 52.0 for 2% and 4%, respectively in Table 4) is more than the one by halothane in the absence of verapamil (10.0 and 21.0 for 2% and 4%, respectively in Table 2). Therefore, the additive effects of verapamil and halothane on $I_{sl}$ may explain that halothane, in the presence of verapamil, causes a further decrease in the action potential duration. In addition, halothane may
increase the $I_x$. This is supported by the fact that halothane decreases the verapamil prolonged duration at 90% repolarization.

It is reported $^{31}$ that $3.3-6.6 \times 10^{-7}$ M TTX shortens the action potential and decreases the amplitude of the plateau without any change in the maximum rate of rise of the phase 0 (Vmax). It is believed $^{30-31}$ that at this concentration TTX can block a steady-state TTX sensitive inward sodium current, the Na+ window current in Purkinje fiber, and thus decrease the duration and plateau. The Na+ window current is absent in ventricular muscle and hence TTX at this concentration has no effect on the ventricular muscle. As expected, our results show that $5 \times 10^{-7}$ M TTX depresses the plateau and decreases the action potential duration at 50% and 90% repolarization (Table 6 and Figure 10) in Purkinje fiber and has no effect on ventricular muscle, indicating that the Na+ window current exits in the Purkinje fiber, but not in the ventricular muscle. In the presence of TTX, however, halothane at 2% and 4% further decreases the action potential duration at both 50% and 90% repolarization significantly, but it does not change the duration in ventricular muscle significantly (Figures 11, 12 and 13). Because there is no Na+ window current in ventricular muscle, it is not surprising that the effect of halothane in the presence of TTX is observed similarly to the one of halothane alone. In the Purkinje fiber, however,
the effects of halothane and TTX on the action potential duration are additive (Figures 14 and 15). Figures 14 and 15 show the percentage change of action potential duration at 50% and 90% repolarization after 2% and 4% halothane, TTX at $5 \times 10^{-7}$ M and the combination of halothane and TTX. Halothane at 2% and 4% decreases action potential duration at 50% repolarization about 10% and 20% (#2 in Figure 14), respectively whereas halothane at 2% and 4% in the presence of TTX decreases the duration about 29% and 39% (#3 in Figure 14), respectively (additive effect). However, TTX itself decreases the duration about 17% (#1 in Figure 14). If we subtract the effect of TTX (#1) from the effect of halothane in the presence of TTX (#3), the net effect of halothane at 2% and 4% is about 12% and 22% which are very close to the effects of halothane in the absence of TTX, 10% and 20%. Likewise, for action potential duration at 90% repolarization the net effects of halothane at 2% and 4% are about 5.0% and 10.0% which are very close to the effects of halothane in the absence of TTX, 6.0% and 11.0% (Figure 15). Therefore, these results indicate that halothane has little or no effect on the duration for which the Na+ window currents are responsible.

TEA is a K+ channel blocker which blocks $I_X$ and prolongs the action potential duration.31, 51 Our results indicate that TEA prolongs the action potential duration
more in Purkinje fiber than in the ventricular muscle (Table 8, Figures 16 and 17). The differential effects may be due to the different densities or different kinetics of K+ channels in the Purkinje fiber and ventricular muscle. In the Purkinje fiber, halothane decreases the TEA prolonged action potential duration at 50% repolarization significantly but not 90% repolarization. As we discussed above, in the Purkinje fiber, halothane decreases the duration due to its inhibitory effect on I_{si} or potentiating effect on I_{x} or both. The explanation may be that halothane decreases the TEA prolonged duration at 50% repolarization because halothane inhibits the I_{si}. TEA, however, totally blocks the effect of halothane on the action potential duration at 90% repolarization. In other words, TEA, K+ channel blocker, blocks the potentiating effect of halothane on I_{x}.

In ventricular muscle, halothane at 2% and 4% increases the TEA increased action potential duration at 50% and 90% repolarization significantly (Figures 18 and 19). Hirota et al. 37 demonstrated that halothane inhibits I_{x} and I_{si} and thus prolongs action potential and depresses the plateau. TEA blocks the I_{x} and increases the duration. If our hypothesis in which halothane inhibits the I_{x} and hence prolongs the action potential is correct, it is plausible that halothane increases the TEA prolonged duration because both halothane and TEA inhibit the I_{x}. Our
results that halothane increases the TEA prolonged duration support our hypothesis. However, voltage clamp or patch clamp studies in which the $I_x$ is directly measured need to be done to confirm the hypothesis.

It was demonstrated that a sudden increase in stimulation frequency results in an initial rapid change in duration followed by a slow change in duration which requires about 1 min before a new steady state was achieved.\textsuperscript{45-48} However, the initial rapid change and slow change occur in opposite direction in ventricular muscle \textsuperscript{45} \textsuperscript{48} and in same direction in the Purkinje fiber.\textsuperscript{45-48} A sudden increase in stimulation frequency shortens the action potential by reducing the plateau phase in Purkinje fiber \textsuperscript{45-48} whereas in ventricular muscle \textsuperscript{45-48} the increased frequency induces an initial prolongation of the action potential in a few beats and then a constant decrease in the duration.

In our study, the treatment was introduced 30 minutes after the stimulus interval was reduced from 1000 mSec to 500 mSec to ensure that steady state was achieved. Tables 9, 10 and Figures 20 and 21 summarize the effects of halothane on the action potential at a stimulus interval of 500 mSec. Just as at stimulus interval of 1000 mSec, halothane decreases the action potential duration at 50\% and 90\% repolarization in the Purkinje fiber when the
stimulus interval is 500 mSec, and halothane (4%) slightly increases the duration in ventricular muscle. Figures 22 and 23 compare the effects of halothane on the duration at the two stimulus intervals (500 and 1000 mSec). We found that in ventricular muscle halothane does not change the duration significantly although it slightly increases the duration whereas in Purkinje fiber, parallel decreases in the action potential duration at the two intervals are found. These results indicate that the effects of halothane on the action potential duration are not frequency dependent.

Although the mechanism responsible for the cycle length-dependence of cardiac action potential duration is not clear, an acceptable hypothesis 54-55 emphasizes that stimulation at a rapid frequency decreases the action potential via an increase in K+ permeability or Ix which is mediated by Ca++ accumulation inside the cells. The increased outward current can be reduced by administration of calcium blockers, verapamil and D600,56 suggesting that these alterations depend on Ca++ entry via the slow inward current channel. These Ca++ blockers also inhibit Ix.57 Other evidence supporting the hypothesis is that the shortened action potential at high frequency is associated with an increase of peak tension. The accumulated Ca++ intracellularly at high frequency is responsible for the increase in the peak tension and for the increase in the
Similar to a stimulus interval of 1000 mSec, the inhibition of $I_{si}$ and/or increase in $I_x$ may explain the decrease in the action potential in Purkinje fiber whereas in ventricular muscle the slight increase in the duration may be due to the inhibition of $I_x$ by halothane.

Halothane treated Purkinje fiber show decreased overshoot, resting membrane potential, amplitude of action potential (Table 1) and the maximum rate of rise of phase 0 (Table 2) although no significant change is found. Resting membrane potential is primarily maintained by the electrochemical gradient of potassium across the sarcolema. Because of the concentration gradient, outward movement of the potassium creates a net negative charge inside the cell and an outward current, named background outward current, $I_{k1}$. The decreased potassium permeability and thus the $I_{k1}$ may explain the depressant effect of halothane on resting membrane potential. The depolarization of phase 0 is related to the influx of Na+ though its channel by virtue of a sudden increase in Na+ conductance. The maximum rate of rise of phase 0 depends on the percentage of available Na+ channels and the sodium electrochemical potential and thus the Na+ current. The decreased maximum rate of rise of phase 0 may result from its inhibition of the Na+ current due to either decrease in the number of channels available or in the opening time of
the channel (kinetics). Bean 58 and Rupperberg 59 reported separately that halothane inhibits the Na+ current in crayfish giant axon and human skeletal muscle. The decrease in maximum rate of rise of phase 0 leads to a decrease in the action potential amplitude and overshoot (Table 1).

Clinical Significance

It is well known that the action potential duration in Purkinje fiber is longer than that of ventricular muscle if enough time is allowed to achieve a steady state at any given frequency of stimulation.43 The difference between Purkinje fiber and ventricular muscle is increased at low frequencies of stimulation or slow heart rate and the temporal dispersion of recovery of excitability is presumably enhanced.43 However, the enhanced temporal dispersion of recovery of excitability is of importance in the genesis of re-entrant arrhythmias.60 Han et al.60 demonstrated that the agents which increased the degree of non-uniformity of recovery of excitability reduced the ventricular fibrillation thresholds whereas the antiarrhythmic agents,43 such as propranolol, decrease the duration of action potential in Purkinje fiber, but do not change those in ventricular muscle. This can decrease the discrepancy between action potentials and thus make it
harder to elicit graded responses and decremental conduction and thus treat re-entrant arrhythmias.\textsuperscript{61} Our results indicate that halothane decreases the action potential duration recorded from the Purkinje fiber, but it does little to that of ventricular muscle. This can reduce the discrepancy between the action potentials. Therefore, like propranolol, halothane may play a role in prevention and treatment of re-entrant arrhythmias (antiarrhythmic effect). Luk \textit{et al.} found in 1988 that halothane inhibits delayed afterdepolarizations in human atrial muscle, oscillatory potentials induced by excessive intracellular calcium and called triggered activities.\textsuperscript{62} It has been found that halothane also prevents the arrhythmias induced by ouabain and occlusion and reperfusion.\textsuperscript{63–64} It is believed now that halothane has both arrhythmic and antiarrhythmic properties.\textsuperscript{1,21}

Slowing of impulse propagation plays a critical role in the pathogenesis of ventricular fibrillation, particularly in the setting of acute myocardial ischemia.\textsuperscript{65} It has been demonstrated \textsuperscript{66} that conduction slowing and block occur shortly after the coronary occlusion and precede the onset of ventricular fibrillation. The rate of the action potential upstroke is an important determinant of conduction velocity, \textsuperscript{67} although the decrease in the rate of action potential upstroke is not the only factor responsible for the propagation delay. The decreased
maximum rate of rise of phase 0 (Table 2) by halothane may decreases the conduction. It is reported 3 that halothane reduces the conductivity of the AV node and His-Purkinje system in the dog. Halothane decreases the action potential duration (Table 2, Figures 3, 4 and 5) and refractory period.5 However, shortening refractoriness and slowing conduction may facilitate re-entrant arrhythmias (arrhythmogenic effect).
LIST OF REFERENCES


33. Roizen, M.P. and W. C. Stevens: Multiform Ventricular Tachycardia due to Interaction of Aminophylline and


46. Gettes, L.S., N. Morehouse, and B. Surawicz: Effect of


CHAPTER II

THE ROLE OF CYCLIC AMP IN HALOTHANE SENSITIZATION

INTRODUCTION

Halothane, and certain other halogenated hydrocarbons, sensitize the heart to the arrhythmogenic effects of catecholamines.\textsuperscript{1-24} Catecholamines by themselves are capable of producing cardiac arrhythmias. However, in the presence of halothane the dose of epinephrine which will produce cardiac arrhythmias may be decreased significantly. This has been described as "cardiac sensitization".\textsuperscript{9-10} Halothane dilates bronchial smooth muscle and is an acceptable choice for anesthetizing patients with asthma.\textsuperscript{25} Intravenous aminophylline, a phosphodiesterase inhibitor and epinephrine have been advocated for treatment of an asthmatic attack during anesthesia. However, cardiac arrhythmias frequently occur during halothane anesthesia, especially with the concurrent administration of catecholamines.\textsuperscript{3-4,6-7,13-14} It has been reported that the combination of epinephrine and aminophylline causes greater
Cyclic AMP (cAMP), as second messenger, mediates the actions of adrenergic transmitters to regulate certain cardiac function and cAMP may have a role in the induction of cardiac arrhythmias. Aminophylline increases cAMP concentration by inhibiting the enzyme phosphodiesterase, which degrades cAMP. However, the role of cAMP in cardiac sensitization has not been defined. The aim of this study is to determine whether cardiac Purkinje fiber cAMP level is correlated with changes found in electrophysiological properties of Purkinje fibers exposed to halothane alone and in combination with isoproterenol and/or aminophylline. In this study, therefore, electrophysiological parameters and cAMP concentrations were measured in canine cardiac Purkinje fibers exposed to halothane alone and halothane followed by isoproterenol and/or aminophylline.
Literature Review

Halothane has been long considered as potential cause for cardiac arrhythmias because of its effects on normal electrical properties of the heart and its capacity to sensitize the heart to the arrhythmogenic actions of catecholamines.\textsuperscript{1-24} Catecholamines are capable of producing cardiac arrhythmias in unanesthetized patients. In the presence of halothane, the dose of epinephrine which will produce cardiac arrhythmias may be decreased significantly. This has been described as "cardiac sensitization".\textsuperscript{9-10} It has been reported that cardiac arrhythmias frequently occur during halothane anesthesia, especially with the concurrent administration of catecholamines.\textsuperscript{3-4,6-7,13-14} Intravenous aminophylline and epinephrine have been used for treatment of asthmatic attacks during anesthesia.\textsuperscript{25-26} Halothane is a potent bronchodilator and thus it is an acceptable choice, probably the best choice for anesthetizing patients with asthma. The concurrent administration of halothane and aminophylline can result in severe ventricular arrhythmias in experimental animals\textsuperscript{27-28} and human patients.\textsuperscript{25-26} It has been reported that the combination of epinephrine and aminophylline causes greater incidence of arrhythmias during halothane anesthesia.\textsuperscript{25-30}

Hauswirth \textit{et al}. in 1967 studied the effects of
halothane on action potentials of single rabbit sinoatrial (SA) nodal fibers. \(^3^4\) They found that halothane alone decreases the slope of phase 4 depolarization and maximum diastolic potential, and increases threshold potential in sinus node. These effects are not modified by atropine or propranolol. Others have shown that halothane depresses the maximum rate of rise of the Ca\(^{++}\)-dependent, slow action potential in a dose-dependent manner, whereas the maximum rate of rise of the fast action potentials is not affected by halothane. \(^3^5\)–\(^3^7\) However, the duration and amplitude of the action potential in both fast and slow action potentials are depressed by halothane. These effects are associated with a decrease in the development of tension by the contracting muscle. Some of the depressant effects could be overcome by addition of calcium and verapamil, a calcium-channel blocker, potentiates the depressant effects, suggesting that halothane might inhibit the slow Na\(^{+}\)-Ca\(^{++}\) channels which mediate the slow action potentials.

It is reported that halothane decreases action potential duration, effective refractory period and slope of phase 4 diastolic depolarization in Purkinje fibers \(^5\) whereas there is little effect on the action potential of the ventricular muscle. \(^9\) This can result in a disparity in the refractory period between Purkinje fiber and regular myocardial muscle. \(^5\), \(^9\) Therefore, a premature stimulus from
an ectopic focus will encounter the early excitable tissue and this also facilitates the formation of the reentry arrhythmias. Interestingly, in most of their cardiovascular effects, halothane and adrenaline are mutually antagonistic, but the decrease in the duration of action potential and effective refractory period of the Purkinje fibers is an effect which is shared by both halothane and adrenaline. Consequently, the effective refractory period of Purkinje fibers is dramatically decreased, which favors the formation of the premature or ectopic beats. Therefore, the decrease in the action potential duration and refractory period is a very important factor in the induction of arrhythmias.

The effects of a combination of halothane and adrenaline were examined by simultaneously recording action potentials from spontaneously active, isolated preparations, containing both SA and AV nodes. Exposure to adrenaline results in a marked increase in the rate of firing of the SA node, and the AV node following by the SA node's pace, whereas halothane alone decreases the phase 4 diastolic depolarization of SA node and thus slows the spontaneous rate. At 3.0% halothane concentration, however, AV nodal fibers still continue to fire spontaneously after complete cessation of activity in SA nodes. Exposure to both adrenaline and halothane can result in a pacemaker shift to an AV junction site. This is
because the combination of halothane and adrenaline provides differential effects on fibers of the SA node and AV node. Therefore, it is proposed that in the presence of the anesthetic agent, halothane, the SA node fails to respond fully to the positive chronotropic effect of adrenaline and loses its role of primary pacemaker, and the more halothane resistant AV node assumes the role of the pacemaker. This has been described as "pacemaker migration", which is also very important in the cardiac sensitization. In in-vivo studies, however, halothane has been shown to increase 39-40, decrease 41-42 or not alter heart rate.43

It is generally accepted that cyclic nucleotides, as second messengers, mediate the actions of adrenergic and cholinergic transmitters to regulate cardiac function. A study indicates that cAMP may be the chemical mediator of cardiac arrhythmias induced by the catecholamine31. The arrhythmias can be abolished by the beta adrenergic antagonist propranolol.

As mentioned above, cardiac arrhythmias frequently occur during halothane anesthesia, especially with the concurrent administration of epinephrine,3-4,6-7,13-14 and the combination of epinephrine and aminophylline causes greater incidence of arrhythmia during halothane
anesthesia.\textsuperscript{25,30} Epinephrine, through beta receptor interaction, activates adenyl cyclase to increase cAMP levels and thus initiate certain cardiac actions.\textsuperscript{31-33} Aminophylline increases cAMP concentration by inhibiting the enzyme phosphodiesterase which breaks down cAMP. Therefore, cAMP may play a major role in the cardiac sensitization. Gangat and Bernstein reported that halothane has no effect on the affinity of canine myocardial beta-adrenergic receptor for $^3$H-DHA or 1-isoproterenol, nor does it change the number of available receptors at binding equilibrium. Halothane depresses the inotropic state of the heart, possibly by decreasing the rate of formation of cAMP through inhibition of adenyl cyclase in cardiac muscle.\textsuperscript{44-46} However, it was found in 1982 that bromochlorodifluoromethane (1211), structurally related to halothane, increases cAMP concentration in Purkinje fiber while it decreases action potential duration, effective refractory period and automaticity.\textsuperscript{47} It was also reported that halothane decreased cAMP levels in cardiac muscle.\textsuperscript{44-46}

A pair of homologous guanine-nucleotide-binding regulatory proteins (G-proteins), stimulatory guanine-nucleotide-binding protein (Gs) and inhibitory guanine-nucleotide-binding protein (Gi), act as transducer between the receptors and the enzymes, adenyl cyclase or guanyl cyclase and regulate their activation and thus the concentration of the cyclic nucleotide.\textsuperscript{48-50} Evidences
indicate that halothane interferes with signal transduction of the receptor at the level of G-protein. It has been recently reported that halothane increases the basal activity of adenyl cyclase and depresses the muscarinic inhibition of forskolin- and isoproterenol-stimulated activity of adenyl cyclase in rat cardiac membranes. Since both muscarinic and beta receptors affect the activities of G proteins, halothane may affect the G proteins. Halothane inhibits muscarinic inhibition of stimulated adenyl cyclase activity. It is possible that halothane interferes with either the muscarinic receptor or Gi protein. Other results show that halothane does not affect the binding of muscarinic agonist. Therefore, halothane may selectively affect Gi protein and the increased cAMP concentration induced by halothane might be due to, in part, interfering with Gi protein.

On the other hand, however, a recent study shows that halothane decreases cardiac cAMP level and increased cGMP level. The increased cGMP level by halothane was inhibited by both alpha-1 and alpha-2 adrenergic antagonists. The stimulation of alpha adrenergic receptors in the heart increases cGMP and this effect is inhibited by alpha adrenergic antagonists whereas alpha stimulation can cause a reduction in cAMP levels. It is possible that stimulation of the alpha receptor activates Gi protein,
which inhibits adenyl cyclase and thus reduces cAMP level.\textsuperscript{50} Since cAMP and cGMP possess antagonistic effects, the decreased cAMP may facilitate the formation of cGMP.\textsuperscript{48-50} It is proposed that Gi protein may be involved in the halothane induced increase in cGMP because pertussis toxin, an inhibitor of Gi protein, reduced the myocardial cGMP response to halothane.\textsuperscript{51} In contrast, pertussis toxin does not inhibit the inhibitory effects of halothane on cAMP, suggesting the cAMP response to halothane is independent of the Gi protein.\textsuperscript{51} On the other hand, the decreased cAMP induced by halothane may be due to interfering with the beta-stimulatory action of catecholamines because beta adrenergic antagonist propranolol diminishes the halothane decreased cAMP level. It is also supported by the fact that halothane inhibits the isoproterenol-induced increase in cAMP formation. Therefore, halothane may increase cGMP by stimulating Gi protein and decrease cAMP via inhibiting Gs protein.
MATERIALS and METHODS

Adult mongrel dogs (15-35 kg) were anesthetized with sodium pentobarbital, 30 mg/kg. The hearts were removed rapidly via a lateral thoracotomy and placed in carbogenated (95% O₂, 5% CO₂, Liquid Carbonic, Chicago, IL) Tyrode’s solution at room temperature to let them pump free of blood. The Tyrode’s solution contained the following composition in millimoles per liter: NaCl, 137.0; NaHCO₃, 12.0; CaCl₂ 1.8; MgCl₂, 0.5; Na₂HPO₄, 1.8; KCl, 4.0; and glucose, 5.5.

Left ventricular Purkinje fibers were uncovered by cutting the left ventricle from base to apex along the interpapillary free wall. Purkinje fiber and a myocardial pad as a marker were carefully cut and placed in a beaker of Tyrode’s solution bubbled with carbogen (95% O₂ and 5% CO₂) by a glass dispersion tube.

Right ventricular Purkinje fibers were uncovered after cutting the right ventricle from base to apex along the anterior interventricular sulcus. Purkinje fiber and a myocardial pad as a marker were carefully dissected free and placed in a beaker of Tyrode’s solution bubbled with carbogen (95% O₂ and 5% CO₂) by a glass dispersion tube.
At least four Purkinje fibers were obtained from each heart. Four Purkinje fibers were mounted in two separate tissue baths, control and drug-exposed (Figure 24). Therefore, two Purkinje fibers of the four were mounted in control tissue bath while the other two in drug-exposed tissue bath. In the drug-exposed tissue bath, only one of the two Purkinje fibers was used to record electrophysiological characteristics, and both of the fibers were analyzed for cyclic AMP concentration. In the control tissue bath, both of the Purkinje fibers were used to determine cyclic AMP concentration in comparison with the treatment. In order to avoid the possible alterations in Purkinje fiber cyclic AMP concentrations related to time elapsed, both treatment and control Purkinje fiber from the two tissue baths were collected for analysis of cyclic AMP concentration at the same time. All four Purkinje fibers were mounted on molded acrylic tissue holders with the anatomical proximal ends of the fibers overlying bipolar platinum extracellular electrodes. The holders were fixed on a lucite tissue bath by nylon screws. The baths were perfused with carbogenated (95% O₂ and 5% CO₂) Tyrode's solution. The perfused solution was temperature (37°C) and pH (7.4) controlled. The preparations were stimulated at a constant rate (60 times/minutes) through the stimulating electrodes by rectangular pulses of 2-msec duration at twice the threshold voltage.
Figure 24
Schematic Diagram of Electronic Equipment
Figure 24
The Purkinje fiber from which was recorded electrophysiological characteristics was impaled with two microelectrodes at proximal and distal recording sites. The microelectrodes with resistances ranging from 10 to 30 MΩ were prepared on a horizontal magnetic electrode puller (Narishige Scientific Instrument Laboratories, Tokyo, Japan). Microelectrode were filled with 2.5 M KCl and then coupled by an Ag-AgCl cell to an amplifier (S-7071A, WP Instruments, Inc., New Haven CT) with high input impedance and input capacity compensation. The output of the amplifier was connected to an oscilloscope (Model 5111A with one 5A18N four channel and 5B12N dual time base, Tektronix, Inc., Beaverton, OR) with storage capabilities. The first derivatives of the action potentials were obtained electronically using a differentiator. The measurement is linear from 0 to 1000 volts/second. The action potentials and their derivatives were displayed on the first and second channels of the oscilloscope, respectively. Records of Purkinje fiber membrane potential and their derivatives were calibrated by passing 100 mV and 600 volts/second signals, respectively, between the microelectrodes and the Ag-AgCl ground reference cell. The membrane potentials and their derivatives were also recorded on a VCR (General Electric) and Polaroid film for analysis.
The basic protocol was to record normal membrane characteristics and electrophysiological parameters after an equilibration period of 30 minutes. During a test period, a drug was introduced and the same membrane characteristics were determined for comparison to controls. The membrane characteristics which were determined during the control and test periods were as follows:

**Action Potential Characteristics (Figure 25):**

1. Resting Membrane Potential, RMP (mV): the potential just before rapid depolarization phase.

2. Maximum Diastolic Potential, MDP (mV): the most negative potential measured at the beginning of phase 4.

3. Action Potential Amplitude, APA (mV): the height of rapid depolarization phase from RMP to peak potential.

4. Overshoot, OS (mV): the peak positive potential from zero potential to peak potential.

5. Action Potential Duration, APD (msec): time from the beginning of rapid depolarization to 50% (APD50), and 90% (APD90) of the action potential repolarization.
Figure 25

Action potentials of paced and unpaced Purkinje fibers.

A: Action potential of paced Purkinje fiber
B: Action potential of unpaced Purkinje fiber
Figure 25

A. PACED PURKINJE FIBER

- APD50
- APD90
- RMP
- OS
- PEAK POTENTIAL
- GROUND ELECTRODE POTENTIAL
- 50% REPOLARIZATION

B. UNPACED PURKINJE FIBER

- MDP
- PEAK POTENTIAL
- GROUND ELECTRODE POTENTIAL
6. Maximum rate of rise of rapid depolarization phase, \( V_{\text{max}} \) or \( \frac{dV}{dt} \) (V/sec): the derivative of rapid depolarization phase.

Electrophysiological Parameters:

1. Effective Refractory period, ERP (msec): ERP was defined as the period during which a second stimulus cannot elicit a propagated action potential. This time was measured by delivering a second stimulus, identical to the basic stimulus at progressively earlier times after every seventh basic stimulus until the cell no longer responded to the second stimulus.

2. Automaticity:

1). Escape Time, ET (second): the time from termination of stimulus to the first spontaneous action potential.

2). Spontaneous Rate, SR (beats/min): the rate of action potentials of Purkinje fiber at its own inherent rhythm in absence of external stimulus.

After the control period, the following tests were made:

1. Isoproterenol (Sigma Chemical Company)
1.0 \times 10^{-8} \text{ M}, 1.0 \times 10^{-7} \text{ M} \text{ and } 1.0 \times 10^{-6} \text{ M alone}

2. Halothane (Halocarbon Laboratories, INC.)
   1\%, 2\% \text{ and } 4\% alone

3. Halothane (2\%), followed by isoproterenol
   1.0 \times 10^{-8} \text{ M}, 1.0 \times 10^{-7} \text{ M} \text{ and } 1.0 \times 10^{-6} \text{ M}

4. Aminophylline (Sigma Chemical Company)
   5.0 \times 10^{-6} \text{ M}, 5.0 \times 10^{-5} \text{ M} \text{ and } 5.0 \times 10^{-4} \text{ M alone}

5. Halothane (2\%), followed by Aminophylline
   5.0 \times 10^{-6} \text{ M}, 5.0 \times 10^{-5} \text{ M} \text{ and } 5.0 \times 10^{-4} \text{ M}

6. Aminophylline 5.0 \times 10^{-5} \text{ M}, followed by isoproterenol
   1.0 \times 10^{-8} \text{ M}, 1.0 \times 10^{-7} \text{ M} \text{ and } 1.0 \times 10^{-6} \text{ M}

7. Halothane (2\%), followed by aminophylline 5.0 \times 10^{-5} \text{ M},
   then isoproterenol 1.0 \times 10^{-7} \text{ M}

Each drug was made up freshly every day in Tyrode's solution and added to the perfusate reservoir.

In all experiments, halothane was carried by O_2 via a calibrated vaporizer (FLUOTEC 3, CYPRANE Keighley, England) and introduced to the perfusate reservoir through
a glass dispersion tube. Monitoring of $P_{O_2}$, $P_{CO_2}$ and pH was performed so as to maintain them at predetermined levels. Two 1.0 ml bath samples were drawn and injected into sealed vials at the conclusion of each anesthetic exposure for gas chromatographic analysis (Gas Chromatograph, Model 370, Varian Instrument Group, Sunnyvale, CA). Standard curves were constructed daily from known amounts of vapor. The chromatographic peaks of halothane from the drawn sample were compared with peaks from standard curve. A scavengering system for limiting exposure of personnel to anesthetics was employed.

After each treatment, two pieces of Purkinje fibers were carefully removed from the control and drug-exposed tissue bath. Each fiber was placed in 1.0 ml of cold 5% trichlororacetic acid (TCA) and immediately homogenized using a 1.0 ml ground glass pestle and tube grinder (Kontes Scientific Glassware/Instruments) and a homogenizer motor (Stirrer type RZ RL-46, Caframo, Wiarton, Ontario, Canada). Following homogenization, 1.0 ml of cold TCA was used to rinse the grinder tube and then added to the first 1.0 ml. The sample was then frozen at -80°C until cyclic AMP concentration was determined.

**Cyclic AMP Assay**

Each preparation was assayed in duplicate for cAMP.
concentration using the radioimmunoassay (RIA) procedure of Steiner et al.\(^5\) and for protein level using the Lowry procedure.\(^7\)

1. Reagents

Antisera: cAMP antisera were developed in the Department of Pharmacology, Ohio State University. 1.0 ml antisera were diluted in 9.6 ml 0.05 M sodium acetate buffer and 0.4 ml 5% bovine serum albumin.

Radiolabeled cAMP: \(^{125}\)I-cAMP were labeled in the Department of Pharmacology, Ohio State University. 0.5 ml \(^{125}\)I-cAMP was diluted in 10 ml 0.25% bovine globulin.

60% saturated ammonium sulfate (Mallinckrodt, INC.)

Water saturated ethyl ether (J.T. Baker Chemical CO.)

Acetic anhydride (Fisher Scientific) and triethylamine (Mallinckrodt, INC.): 2 ml acetic anhydride was mixed with 5 ml triethylamine.

2. Procedure:

The TCA treated homogenate was centrifuged for 10 minutes at 2,000 \(\times\) g. The supernatant was used for cAMP
assay. The precipitate was collected for protein assay. The supernatant was extracted three times with 5 ml water-saturated ethyl ether. The bulk of the ether was removed by suction and the remaining ether was evaporated in a 56°C water bath under a hood for 30 minutes. The pH of each tube was adjusted to about 6.5 by addition of 200 ul 1.0 M sodium acetate buffer. A standard curve which provided a sensitivity of about 10 to 10,000 femtomoles cAMP per tube was constructed daily. 100 ul of standard and samples were aliquoted into 12 mm X 75 mm glass tubes in duplicate. 100% binding (B₀) tubes, nonspecific binding (blank or B₁) tubes and total radioactivity tubes were also set up (Table 11). Acetylation of the standards and samples was performed by adding 10 ul of a 2:5 (v:v) mixture of acetic anhydride and triethylamine to increase the sensitivity of RIA. 50 ul of diluted antiserum was added to each tube of standards and samples. 50 ul of the diluted radiolabeled cAMP was then added to each tube of standards and samples. The tubes were vortexed and incubated for 24 hours at 4°C. The bound and free fractions of the antibody-cAMP complexes were separated by addition of 60% saturated ammonium sulfate at room temperature and incubated for 20 minutes. After a 20 minute centrifugation at 2,000 X g, the supernatant was decanted and the remaining precipitate in the tubes was counted for radioactivity in a Beckman Gamma 7000 spectrometer to determine the percentage of bound ^{125}I
Table 11

Determination of Absolute Binding Percentage (AB%)

<table>
<thead>
<tr>
<th>tubes</th>
<th>buffer</th>
<th>acetylation</th>
<th>$^{125}$I</th>
<th>antisera</th>
<th>other*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$B_1$</td>
<td>100ul</td>
<td>10ul</td>
<td>50ul</td>
<td>----</td>
<td>50ul</td>
</tr>
<tr>
<td>$B_0$</td>
<td>100ul</td>
<td>10ul</td>
<td>50ul</td>
<td>50ul</td>
<td>----</td>
</tr>
<tr>
<td>T</td>
<td>----</td>
<td>----</td>
<td>50ul</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

* = 9.6 ml buffer + 0.4 ml 5% BSA
buffer = 0.05 M sodium acetate buffer
acetylation = a mixture 2 ml acetic anhydride and 5 ml triethylamine
$B_1$ = nonspecific binding
$B_0$ = 100% binding
T = total radioactivity (total counts)

$AB\% = \frac{(B_0 - B_1)}{(T - B_1)} \times 100\%$
cAMP. The cAMP concentrations were determined using a computer program written by this author (see Appendix).

The statistical significance of differences in mean values for both electrophysiological and cyclic AMP data was assessed using analysis of variance and Duncan's multiple range test. Correlation coefficients were determined by linear regression. Differences of \( P < 0.05 \) were considered significant.
RESULTS

Effects of Halothane and the Combination of Halothane and Aminophylline and/or Isoproterenol on Action Potential Parameters of Purkinje Fibers

Means and standard errors of the means of action potential durations at 50% and 90% repolarization and effective refractory periods of Purkinje fibers after each treatment are listed in Tables 13 and 15. The effects of halothane (2% and 4%) on the action potentials and effective refractory periods of Purkinje fibers are compared in Figure 26. Panel a shows a control action potential. Panel b and c are the action potentials after halothane (2% and 4%), respectively. Panel d is measurement of effective refractory period without halothane. Panels e and f are the effective refractory periods after halothane (2% and 4%), respectively. Halothane (2% and 4%) decreases the effective refractory period significantly (P < 0.05). The percentage changes of action potential duration at 50% and 90% repolarization and effective refractory period of Purkinje fibers after halothane (1% to 4%) are presented graphically in Figure 31. The figure shows that the effects of halothane are the greatest on action potential duration at 50% repolarization, less on the effective refractory period, and the least on the duration at 90% repolarization.
The effects of aminophylline (5 \times 10^{-6} to 5 \times 10^{-4} \text{ M}) on the action potential of Purkinje fiber are compared in Figure 27. Panel a shows a control action potential. Panels b, c, and d are the action potentials after aminophylline 5 \times 10^{-6} to 5 \times 10^{-4} \text{ M}. Aminophylline decreases the action potential durations and effective refractory period in a dose-dependent manner (Figure 27 and Table 13).

The effects of isoproterenol (10^{-8} to 10^{-6} \text{ M}) on the action potentials of Purkinje fiber are compared in Figure 28. Panel a shows a control action potential. Panels b, c, and d are the action potentials after isoproterenol 10^{-8} to 10^{-6} \text{ M}. Isoproterenol shortens the action potential durations and effective refractory periods in a dose-dependent manner (Figure 28 and Table 13).

The effects of 2% halothane and the combination of 2% halothane and aminophylline (5 \times 10^{-6} to 5 \times 10^{-4} \text{ M}) on the action potentials of Purkinje fiber are compared in Figure 29. Panel a shows a control action potential. Panel b is the action potential after 2% halothane. Panels c, d, and e are the action potentials after the combination of 2% halothane and aminophylline 5 \times 10^{-6} to 5 \times 10^{-4} \text{ M}. Two percent halothane decreases the action potential durations and effective refractory periods. The
combination of halothane (2\%) and aminophylline (5 \times 10^{-6}

\text{to} \ 5 \times 10^{-4} \text{ M}) further decreases the durations and
refractory periods significantly (p < 0.05) (Figure 29 and
Table 15).

The effects of 2\% halothane and the combination of
2\% halothane and isoproterenol (10^{-8} \text{ to} \ 10^{-6} \text{ M}) on the
action potentials of Purkinje fiber are compared in Figure
30. Panel a shows a normal action potential. Panel b is
the action potential after 2\% halothane. Panels c, d and e
are the action potentials after the combination of 2\%
halothane and isoproterenol 10^{-8} \text{ to} \ 10^{-6} \text{ M}. Two percent
halothane decreases the action potential durations and
effective refractory periods significantly. The combination
of halothane (2\%) and isoproterenol (10^{-8} \text{ to} \ 10^{-6} \text{ M})
further decreases the durations and refractory periods
significantly (Figure 30 and Table 15).

Aminophylline 5 \times 10^{-5} \text{ M decreases the action
potential durations and effective refractory periods. The
combination of aminophylline (5 \times 10^{-5} \text{ M}) and isoproterenol
(10^{-8} \text{ M}) further decreases the duration and refractory
periods (Table 15).

Two percent halothane deceases the action potential
durations and effective refractory periods. The combination of halothane (2%) and aminophylline (5 X 10^{-6} to 5 X 10^{-4} M) further decreases the durations and refractory periods. However, the combination of halothane (2%), aminophylline (5 X 10^{-5} M) and isoproterenol (10^{-7} M) produces greater effect on these parameters (Table 15).

Means and standard errors of the means of the maximum rate of rise of phase 0 are listed in Tables 17 and 18. Means and standard errors of the means of other electrophysiological parameters, such as action potential amplitude, overshoot, resting membrane potential and maximum diastolic potential after each treatment are listed in Tables 12 and 14. Action potential amplitude and overshoot are decreased by halothane. Resting membrane potential and maximum diastolic potential are also decreased by halothane. However, the decreases are not significant. The effects of aminophylline and isoproterenol, although they vary, generally are opposite from the effects of halothane (Table 12).

Effects of Halothane and the Combination of Halothane and Aminophylline and/or Isoproterenol on Cyclic AMP Concentration of Purkinje Fibers
Means and standard errors of the means of cyclic AMP (cAMP) concentration of Purkinje fiber after each treatment are listed in Table 16. The percentages of change of cyclic AMP concentration after each treatment are graphically presented in Figure 33. Halothane (1% and 2%) increases the cAMP level. Aminophylline (5 X 10^{-6} and 5 X 10^{-5} M) and isoproterenol (10^{-8} and 10^{-7} M) increase the cAMP level. The percentages of increase in the cAMP level are greater in the following combinations: 2% halothane and aminophylline 5 X 10^{-5} M, 2% halothane and isoproterenol 10^{-7} M, aminophylline 5 X 10^{-5} M and isoproterenol 10^{-7} M. However, the greatest increase in the cAMP level is found with the combination of 2% halothane, aminophylline 5 X 10^{-5} M and isoproterenol 10^{-7} M.

Effects of Halothane and the Combination of Halothane and Aminophylline and/or Isoproterenol on Automaticity of Purkinje Fibers

To study the effects on automaticity, escape time and spontaneous rate are determined. Means and standard errors of the means of escape time and spontaneous rate after each treatment are listed in Tables 17 and 18. Figure 34 shows the action potentials of Purkinje fiber after halothane (2% and 4%). Panel a shows control escape time
and spontaneous rate. Panels b, c and d are the escape time and spontaneous rate after 2% and 4% halothane, respectively. The percentage changes of the escape time and spontaneous rate from control and after halothane (1%, 2% and 4%) are presented graphically in Figure 35. Figure 36 summaries the percentage changes of the escape time and spontaneous rate after 2% halothane and the combination of 2% halothane and aminophylline $5 \times 10^{-5}$ M and/or isoproterenol $10^{-7}$ M. Halothane increases the escape time and decreases the spontaneous rate in a dose-dependent manner (Figure 35 and Table 17) whereas aminophylline and isoproterenol decrease the escape time and increase the spontaneous rate (Table 17). Aminophylline and isoproterenol decrease the halothane prolonged escape time and increase the halothane-decreased spontaneous rate (Figure 36 and Table 18). The greater effects are found in the combination of aminophylline and isoproterenol (Figure 36 and Table 18).
Table 12

Means and Standard Errors of the Means of Action Potential Amplitude (APA), Overshoot (OS), Resting Membrane Potential (RMP), and Maximum Diastolic Potential (MDP) for Each Treatment Administered to Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APA (mV)</th>
<th>OS (mV)</th>
<th>RMP (-mV)</th>
<th>MDP (-mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>122.5±5.01</td>
<td>30.6±5.19</td>
<td>91.9±0.81</td>
<td>93.2±2.19</td>
</tr>
<tr>
<td>1% HAL (N=5)</td>
<td>119.9±3.44</td>
<td>32.1±3.31</td>
<td>87.8±0.85</td>
<td>93.8±1.03</td>
</tr>
<tr>
<td>2% HAL (N=9)</td>
<td>119.8±2.07</td>
<td>39.9±2.80</td>
<td>88.8±1.80</td>
<td>92.9±1.57</td>
</tr>
<tr>
<td>4% HAL (N=6)</td>
<td>113.9±2.49</td>
<td>28.2±1.39</td>
<td>85.6±3.22</td>
<td>89.7±2.89</td>
</tr>
<tr>
<td>Control</td>
<td>128.0±2.77</td>
<td>38.1±1.73</td>
<td>89.8±1.33</td>
<td>92.3±2.02</td>
</tr>
<tr>
<td>AMI1</td>
<td>128.3±2.56</td>
<td>38.6±1.16</td>
<td>89.6±0.87</td>
<td>94.1±2.02</td>
</tr>
<tr>
<td>AMI2</td>
<td>127.1±0.98</td>
<td>36.8±0.92</td>
<td>90.3±1.15</td>
<td>93.0±1.73</td>
</tr>
<tr>
<td>AMI3</td>
<td>129.5±2.14</td>
<td>35.5±2.71</td>
<td>94.0±1.50</td>
<td>95.6±2.09</td>
</tr>
</tbody>
</table>

(N=3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APA (mV)</th>
<th>OS (mV)</th>
<th>RMP (-mV)</th>
<th>MDP (-mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127.2±2.06</td>
<td>35.2±1.21</td>
<td>92.0±1.92</td>
<td>93.3±2.01</td>
</tr>
<tr>
<td>ISO1</td>
<td>129.1±1.97</td>
<td>35.2±1.25</td>
<td>93.9±2.46</td>
<td>95.3±3.46</td>
</tr>
<tr>
<td>ISO2</td>
<td>129.8±2.42</td>
<td>35.7±2.19</td>
<td>94.0±2.99</td>
<td>95.1±2.77</td>
</tr>
<tr>
<td>ISO3</td>
<td>132.1±2.46</td>
<td>37.1±2.50</td>
<td>95.0±3.44</td>
<td>96.7±3.26</td>
</tr>
</tbody>
</table>

(N=5)

* = P < 0.05, ** = P < 0.01 compared with control
HAL = Halothane,
AMI1 = Aminophylline 5X10^-6 M,
AMI2 = Aminophylline 5X10^-5 M,
AMI3 = Aminophylline 5X10^-4 M,
ISO1 = Isoproterenol 10^-8 M,
ISO2 = Isoproterenol 10^-7 M,
ISO3 = Isoproterenol 10^-6 M

Table 13

Means and Standard Errors of the Means Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90), Effective Refractory Period (ERP) for Each Treatment Administered to Canine Purkinje Fiber

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50 (mSec)</th>
<th>APD90 (mSec)</th>
<th>ERP (MSec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>269.4±15.16</td>
<td>357.8±25.31</td>
<td>376.6±28.76</td>
</tr>
<tr>
<td>1% HAL (N=5)</td>
<td>248.2±22.96</td>
<td>357.4±22.06</td>
<td>369.2±29.03</td>
</tr>
<tr>
<td>2% HAL (N=9)</td>
<td>222.8±11.00*</td>
<td>325.8±15.83*</td>
<td>311.4±13.27*</td>
</tr>
<tr>
<td>4% HAL (N=6)</td>
<td>174.6±14.49*</td>
<td>287.3±15.92*</td>
<td>271.6±12.89*</td>
</tr>
<tr>
<td>Control</td>
<td>219.0±18.30</td>
<td>357.3±23.96</td>
<td>351.0±42.50</td>
</tr>
<tr>
<td>AMI1</td>
<td>193.3±21.81</td>
<td>272.3±17.55</td>
<td>294.3±32.66</td>
</tr>
<tr>
<td>AMI2</td>
<td>155.0±18.94</td>
<td>242.0±29.39</td>
<td>299.3±26.77</td>
</tr>
<tr>
<td>AMI3</td>
<td>156.0±10.16</td>
<td>239.3±12.36</td>
<td>238.0±13.32</td>
</tr>
</tbody>
</table>

(N=3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50 (mSec)</th>
<th>APD90 (mSec)</th>
<th>ERP (MSec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>307.0±34.93</td>
<td>400.1±30.32</td>
<td>393.8±29.03</td>
</tr>
<tr>
<td>ISO1</td>
<td>279.1±21.74</td>
<td>365.8±21.38</td>
<td>361.0±22.63</td>
</tr>
<tr>
<td>ISO2</td>
<td>255.3±23.23</td>
<td>328.2±19.90</td>
<td>318.4±17.17*</td>
</tr>
<tr>
<td>ISO3</td>
<td>269.2±23.57</td>
<td>342.1±19.68</td>
<td>332.6±20.79</td>
</tr>
</tbody>
</table>

(N=5)

* = P < 0.05 compared with control
For HAL, AMI1-3 and ISO1-3 see Table 12
Table 14
Means and Standard Errors of the Means of Action Potential Amplitude (APA), Overshoot (OS), Resting Membrane Potential (RMP), and Maximum Diastolic Potential (MDP) for Each Treatment Administered Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APA (mV)</th>
<th>OS (mV)</th>
<th>RMP (-mV)</th>
<th>MDP (-mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>124.2±3.80</td>
<td>31.5±3.98</td>
<td>92.7±1.43</td>
<td>94.2±0.89</td>
</tr>
<tr>
<td>2% HAL</td>
<td>119.3±5.23</td>
<td>28.0±4.61</td>
<td>91.3±2.24</td>
<td>93.3±1.39</td>
</tr>
<tr>
<td>2% HAL+AMI1</td>
<td>121.2±4.56</td>
<td>29.2±4.11</td>
<td>91.8±3.18</td>
<td>92.9±2.64</td>
</tr>
<tr>
<td>2% HAL+AMI2</td>
<td>120.5±5.01</td>
<td>30.1±4.83</td>
<td>90.2±2.50</td>
<td>91.4±1.83</td>
</tr>
<tr>
<td>2% HAL+AMI3</td>
<td>119.8±4.52</td>
<td>28.6±4.92</td>
<td>90.8±2.86</td>
<td>93.2±2.24</td>
</tr>
<tr>
<td></td>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>124.2±3.50</td>
<td>36.2±2.65</td>
<td>88.0±2.00</td>
<td>91.0±1.90</td>
</tr>
<tr>
<td>2% HAL</td>
<td>122.1±5.20</td>
<td>36.2±3.75</td>
<td>85.8±2.55</td>
<td>90.5±3.10</td>
</tr>
<tr>
<td>2% HAL+ISO1</td>
<td>115.2±5.05</td>
<td>31.3±2.95</td>
<td>84.8±1.65</td>
<td>85.5±2.15</td>
</tr>
<tr>
<td>2% HAL+ISO2</td>
<td>117.6±6.05</td>
<td>33.5±3.25</td>
<td>84.0±2.80</td>
<td>86.6±2.40</td>
</tr>
<tr>
<td>2% HAL+ISO3</td>
<td>116.0±6.85</td>
<td>29.8±5.40</td>
<td>86.1±2.90</td>
<td>88.3±1.85</td>
</tr>
<tr>
<td></td>
<td>(N=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>123.5±2.83</td>
<td>35.8±3.06</td>
<td>87.6±3.23</td>
<td>92.6±3.29</td>
</tr>
<tr>
<td>AMI2</td>
<td>127.0±1.83</td>
<td>36.1±0.58</td>
<td>90.8±1.73</td>
<td>95.3±2.14</td>
</tr>
<tr>
<td>AMI2+ISO1</td>
<td>130.6±0.29</td>
<td>35.1±2.59</td>
<td>95.5±2.89</td>
<td>97.5±2.71</td>
</tr>
<tr>
<td>AMI2+ISO2</td>
<td>126.8±2.05</td>
<td>33.8±2.37</td>
<td>93.0±4.04</td>
<td>96.6±1.73</td>
</tr>
<tr>
<td>AMI2+ISO3</td>
<td>130.5±0.75</td>
<td>36.1±3.58</td>
<td>94.3±2.83</td>
<td>95.1±2.38</td>
</tr>
<tr>
<td></td>
<td>(N=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>121.0±6.93</td>
<td>29.6±6.76</td>
<td>91.4±0.69</td>
<td>93.7±1.50</td>
</tr>
<tr>
<td>2% HAL</td>
<td>120.2±1.91</td>
<td>32.0±2.31</td>
<td>88.2±0.69</td>
<td>90.3±1.56</td>
</tr>
<tr>
<td>2% HAL+AMI1</td>
<td>117.3±4.50</td>
<td>26.6±3.41</td>
<td>89.3±4.39</td>
<td>88.6±4.33</td>
</tr>
<tr>
<td>2% HAL+AMI2</td>
<td>117.3±4.06</td>
<td>30.1±2.60</td>
<td>87.2±1.96</td>
<td>97.6±3.12</td>
</tr>
<tr>
<td></td>
<td>(N=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For HAL, AMI1-3 and ISO1-3 see Table 12.
Table 15
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90), Effective Refractory Period (ERP) for Each Treatment Administered to Canine Purkinje Fiber

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>ERP(MSec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>273.0±16.88</td>
<td>375.2±24.78</td>
<td>363.8±10.94</td>
</tr>
<tr>
<td>2% HAL</td>
<td>242.2±18.83</td>
<td>354.8±24.06</td>
<td>345.6±12.79</td>
</tr>
<tr>
<td>2% HAL+AMI1</td>
<td>213.2±36.17</td>
<td>335.6±20.57</td>
<td>325.2±14.76</td>
</tr>
<tr>
<td>2% HAL+AMI2</td>
<td>199.2±20.53*</td>
<td>324.2±21.47</td>
<td>304.8±14.45*</td>
</tr>
<tr>
<td>2% HAL+AMI3</td>
<td>182.8±26.79*</td>
<td>275.8±34.88*</td>
<td>265.0±29.96**</td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>229.5±7.35</td>
<td>313.5±16.65</td>
<td>292.7±13.65</td>
</tr>
<tr>
<td>2% HAL</td>
<td>212.5±5.95*</td>
<td>302.5±14.80</td>
<td>279.0±13.65</td>
</tr>
<tr>
<td>2% HAL+ISO1</td>
<td>195.0±5.00**</td>
<td>286.2±11.45</td>
<td>262.0±15.05</td>
</tr>
<tr>
<td>2% HAL+ISO2</td>
<td>162.5±6.29**</td>
<td>251.2±22.00*</td>
<td>219.7±18.50**</td>
</tr>
<tr>
<td>2% HAL+ISO3</td>
<td>146.6±1.45**</td>
<td>251.6±5.20*</td>
<td>211.5±3.82**</td>
</tr>
<tr>
<td>(N=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>224.3±19.98</td>
<td>298.7±15.70</td>
<td>289.3±25.46</td>
</tr>
<tr>
<td>AMI2</td>
<td>195.0±22.92</td>
<td>255.0±30.43</td>
<td>256.0±39.95</td>
</tr>
<tr>
<td>AMI2+ISO1</td>
<td>175.0±29.27</td>
<td>238.3±37.89</td>
<td>238.0±47.92</td>
</tr>
<tr>
<td>AMI2+ISO2</td>
<td>202.0±14.55</td>
<td>253.7±23.79</td>
<td>258.7±28.18</td>
</tr>
<tr>
<td>AMI2+ISO3</td>
<td>196.7±16.40</td>
<td>243.3±14.26</td>
<td>239.3±13.80</td>
</tr>
<tr>
<td>(N=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>295.0±12.76</td>
<td>369.7±28.87</td>
<td>393.0±45.90</td>
</tr>
<tr>
<td>2% HAL</td>
<td>239.7±18.88</td>
<td>353.3±10.39</td>
<td>332.7±12.64</td>
</tr>
<tr>
<td>2% HAL+AMI1</td>
<td>235.0±39.09</td>
<td>288.0±39.84</td>
<td>281.0±27.08*</td>
</tr>
<tr>
<td>2% HAL+AMI1+ISO2</td>
<td>165.7±56.24</td>
<td>244.0±48.79*</td>
<td>265.7±36.72*</td>
</tr>
<tr>
<td>(N=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = P < 0.05, ** = P < 0.01 compared with control
For HAL, AMI1-3 AND ISO1-3 see Table 12
Figure 26

Action potentials recorded from Purkinje fibers before and after halothane 2% and 4%

a: Control
b: Halothane 2%
c: Halothane 4%
d: Control for effective refractory period (ERP)
e: ERP after halothane 2%
f: ERP after halothane 4%
Figure 27

Action potentials recorded from Purkinje fibers before and after aminophylline $5 \times 10^{-6}$ to $5 \times 10^{-4}$ M

a: Control

b: Aminophylline $5 \times 10^{-6}$ M

c: Aminophylline $5 \times 10^{-5}$ M

d: Aminophylline $5 \times 10^{-4}$ M
Figure 27
Figure 28

Action potentials recorded from Purkinje fibers before and after isoproterenol $10^{-8}$ to $10^{-6}$ M.

a. Control
b. Isoproterenol $10^{-8}$ M
c. Isoproterenol $10^{-7}$ M
d. Isoproterenol $10^{-6}$ M
Figure 28
Figure 29

Action potentials recorded from Purkinje fibers before and after halothane 2% and the combination of halothane 2% and aminophylline 5 x 10^{-6} to 10^{-4} M.

a. Control
b. Halothane 2%
c. Halothane 2% + Aminophylline 5 x 10^{-6} M
d. Halothane 2% + Aminophylline 5 x 10^{-5} M
e. Halothane 2% + Aminophylline 5 x 10^{-4} M
Figure 29
Figure 30

Action potentials recorded from Purkinje fibers before and after halothane 2% and combination of halothane and isoproterenol 10^{-8} to 10^{-6} M.

a: Control
b: Halothane 2%
c: Halothane 2% + Isoproterenol 10^{-8} M
d: Halothane 2% + Isoproterenol 10^{-7} M
e: Halothane 2% + Isoproterenol 10^{-6} M
Figure 31

Percentage changes of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) from control after halothane 1%, 2% and 4%.

Figure 32

Percentage changes of action potential duration at 50% and 90% repolarization (APD50 and APD90) and effective refractory period (ERP) from control after the following treatments:

Halothane 2% (2% HAL)
Halothane 2% + Aminophylline 5 X 10^-5 (AMI2)
Halothane 2% + Aminophylline 5 X 10^-5 + Isoproterenol 10^-8 M (ISO2)
Figure 31

Figure 32
<table>
<thead>
<tr>
<th>Treatment</th>
<th>cAMP (pM/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.93±0.34</td>
</tr>
<tr>
<td>1% HAL (N=5)</td>
<td>3.06±0.53</td>
</tr>
<tr>
<td>2% HAL (N=9)</td>
<td>3.73±0.43*</td>
</tr>
<tr>
<td>Control</td>
<td>1.46±0.14</td>
</tr>
<tr>
<td>AMI1</td>
<td>1.87±0.17</td>
</tr>
<tr>
<td>AMI2</td>
<td>2.72±0.64*</td>
</tr>
<tr>
<td>(N=3)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.94±0.33</td>
</tr>
<tr>
<td>ISO1</td>
<td>4.18±0.39</td>
</tr>
<tr>
<td>ISO2</td>
<td>4.44±0.50</td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.11±0.27</td>
</tr>
<tr>
<td>2% HAL+ISO2 (N=4)</td>
<td>5.45±1.22</td>
</tr>
<tr>
<td>Control</td>
<td>1.86±0.35</td>
</tr>
<tr>
<td>AMI2+ISO2 (N=3)</td>
<td>4.79±0.76</td>
</tr>
<tr>
<td>Control</td>
<td>1.82±0.18</td>
</tr>
<tr>
<td>2% HAL+AMI2 (N=3)</td>
<td>5.36±0.44**</td>
</tr>
<tr>
<td>2% HAL+AMI2+ISO2 (N=3)</td>
<td>8.34±0.72**</td>
</tr>
</tbody>
</table>

* = P < 0.05, ** = P < 0.01 compared with control

For HAL, AMI1-3 and ISO1-3 see Table 12

* = r = -0.98 between cAMP and APD50
  r = -0.79 between cAMP and APD90
  r = -0.84 between cAMP and ERP
Figure 33

Percentage changes of cyclic AMP (cAMP) level from control after the following treatments:

Halothane 2% (2% HAL)
Aminophylline 5 X 10^-7 M (AMI2)
Isoproterenol 10^-7 M (ISO2)
Halothane 2% + Aminophylline 5 X 10^-5 M
Halothane 2% + Isoproterenol 10^-7 M
Aminophylline 5 X 10^-5 M + Isoproterenol 10^-7 M
Halothane 2% + Aminophylline 5 X 10^-5 M + Isoproterenol 10^-7 M
Table 17
Means and Standard Errors of the Means of Escape Time (ET), spontaneous Rate (SR), and Maximum Rate of Rise of Phase 4 (Vmax) for Each Treatment Administered to Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ET (mSec)</th>
<th>SR (Beats/Sec)</th>
<th>Vmax (v/Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.10±0.75</td>
<td>29.25±2.32</td>
<td>594.0±59.48</td>
</tr>
<tr>
<td>1% HAL (N=5)</td>
<td>2.24±0.42</td>
<td>27.48±1.14</td>
<td>572.0±54.29</td>
</tr>
<tr>
<td>2% HAL (N=9)</td>
<td>2.63±0.60*</td>
<td>25.57±2.54*</td>
<td>465.5±41.37</td>
</tr>
<tr>
<td>4% HAL (N=6)</td>
<td>6.22±1.92*</td>
<td>16.86±3.65*</td>
<td>373.0±37.10</td>
</tr>
<tr>
<td>Control</td>
<td>22.8±10.68</td>
<td>18.3±11.89</td>
<td>541.0±44.86</td>
</tr>
<tr>
<td>AMI1</td>
<td>14.7±8.235</td>
<td>42.5±32.79</td>
<td>535.0±47.69</td>
</tr>
<tr>
<td>AMI2</td>
<td>5.03±2.98</td>
<td>29.4±15.36</td>
<td>546.3±59.47</td>
</tr>
<tr>
<td>AMI3 (N=3)</td>
<td>2.03±0.57</td>
<td>47.8±18.19</td>
<td>538.7±37.53</td>
</tr>
<tr>
<td>Control</td>
<td>5.90±1.83</td>
<td>19.29±5.59</td>
<td>521.0±43.25</td>
</tr>
<tr>
<td>ISO1</td>
<td>3.95±0.77</td>
<td>23.30±5.35</td>
<td>565.0±22.09</td>
</tr>
<tr>
<td>ISO2</td>
<td>2.90±0.81</td>
<td>32.20±8.83</td>
<td>574.0±53.35</td>
</tr>
<tr>
<td>ISO3 (N=5)</td>
<td>3.03±0.59</td>
<td>35.72±8.58</td>
<td>595.6±56.93</td>
</tr>
</tbody>
</table>

* = P < 0.05 compared with control
For HAL, AMI1-3 and ISO1-3 see Table 12
Means and Standard Errors of the Means of Escape Time (ET), Spontaneous Rate (SR), and Maximum Rate of Rise of Phase 4 (Vmax) for Each Treatment Administered to Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ET (mSec)</th>
<th>SR (Beats/Sec)</th>
<th>Vmax (v/Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.83±0.42</td>
<td>28.53±6.55</td>
<td>547.3±62.21</td>
</tr>
<tr>
<td>2% HAL</td>
<td>2.63±0.54</td>
<td>21.64±3.89</td>
<td>475.1±74.91</td>
</tr>
<tr>
<td>2% HAL+AMI1</td>
<td>2.07±0.43</td>
<td>27.06±5.99</td>
<td>466.1±71.51</td>
</tr>
<tr>
<td>2% HAL+AMI2</td>
<td>2.07±0.36</td>
<td>34.18±8.32</td>
<td>479.0±82.78</td>
</tr>
<tr>
<td>2% HAL+AMI3</td>
<td>1.75±0.17</td>
<td>37.1±5.84</td>
<td>496.2±85.69</td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.43±0.29</td>
<td>53.92±7.81</td>
<td>517.8±47.60</td>
</tr>
<tr>
<td>2% HAL</td>
<td>1.90±0.37</td>
<td>42.30±4.96</td>
<td>456.3±43.65</td>
</tr>
<tr>
<td>2% HAL+ISO1</td>
<td>1.15±0.05</td>
<td>62.89±9.04</td>
<td>477.5±33.05</td>
</tr>
<tr>
<td>2% HAL+ISO2</td>
<td>0.88±0.13</td>
<td>76.30±10.45</td>
<td>473.0±28.40</td>
</tr>
<tr>
<td>2% HAL+ISO3</td>
<td>0.60±0.14*</td>
<td>104.0±22.50*</td>
<td>395.0±13.90</td>
</tr>
<tr>
<td>(N=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.53±2.06</td>
<td>23.92±2.63</td>
<td>535.3±60.62</td>
</tr>
<tr>
<td>AMI2</td>
<td>1.60±0.50</td>
<td>70.10±21.0</td>
<td>558.3±54.62</td>
</tr>
<tr>
<td>AMI2+ISO1</td>
<td>1.37±0.49</td>
<td>67.8±20.09</td>
<td>613.3±81.93</td>
</tr>
<tr>
<td>AMI2+ISO2</td>
<td>1.00±0.20</td>
<td>78.0±12.01</td>
<td>571.7±56.00</td>
</tr>
<tr>
<td>AMI2+ISO3</td>
<td>0.73±0.19</td>
<td>98.0±19.69*</td>
<td>630.3±83.78</td>
</tr>
<tr>
<td>(N=3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.38±1.09</td>
<td>28.40±0.32</td>
<td>686.7±69.63</td>
</tr>
<tr>
<td>2% HAL</td>
<td>5.88±2.54</td>
<td>20.46±3.79</td>
<td>623.3±41.28</td>
</tr>
<tr>
<td>2% HAL+AMI2</td>
<td>5.42±1.65</td>
<td>23.30±5.35</td>
<td>456.7±54.56</td>
</tr>
<tr>
<td>2% HAL+AMI2+ISO2</td>
<td>3.82±1.99</td>
<td>35.2±7.49</td>
<td>643.3±71.71</td>
</tr>
</tbody>
</table>

* = P < 0.05 compared with control
For HAL, AMI1-3 and ISO1-3 see Table 12.
Figure 34

Action potentials recorded from Purkinje fibers before and after halothane 2% and 4%.

a: Control for escape time (ET) and spontaneous rate (SR)
b: ET and SR after Halothane 2%
c: ET and SR after Halothane 4%
Figure 34
Figure 35

Percentage changes of escape time (ET) and spontaneous rate (SR) from control after halothane 1%, 2% and 4%.

Figure 36

Percentage changes of escape time (ET) and spontaneous rate (SR) from control after the following treatments:

- Halothane 2% (2% HAL)
- Halothane 2% + Aminophylline 5 X 10^-5 (AMI2)
- Halothane 2% + Aminophylline 5 X 10^-5 + Isoproterenol 10^-8 M (ISO2)
Figure 35

Figure 36
DISCUSSION

The purposes of this present study are to study the effects of halothane on canine cardiac Purkinje fiber electrophysiology and cyclic AMP concentration and to determine whether or not the changes in electrophysiology correlate with the changes of cyclic AMP. The results show that halothane does produce some effects on electrophysiology and cyclic AMP concentration. These changes are very important for its arrhythmogenic effects.

As reported previously by other investigators,\textsuperscript{5,9,35} Table 13 and Figure 26 show that halothane depresses the action potential plateau, decreases the action potential duration and decreases effective refractory period of Purkinje fibers. Figure 31 compares the percentage changes of the action potential duration at 50\% and 90\% repolarization and effective refractory period. The results show that halothane has a greater effect on the action potential duration at 50\% repolarization than at 90\% repolarization. Furthermore, the effects of halothane on effective refractory period are more than on the duration at 90\% repolarization. There are at least three ionic currents \textsuperscript{58-59, 60-61} responsible for the formation of the Purkinje fiber action potential duration, i.e. slow inward
Ca++ current, $I_{Si}$, delayed outward potassium current, $I_x$, and TTX-sensitive inward sodium current, Na+ window current. The two inward currents, $I_{Si}$ and Na+ window current, maintaining the action potential duration and plateau, are overcome by the outward current, $I_x$. Increasing the inward currents increases the duration and increasing the outward current decreases the duration and hence completes the repolarization. Therefore, depending on the relative effects on the inward or outward currents, either a longer or a shorter duration will result. Consequently, the decrease in action potential duration by halothane may be due to either a decrease in the inward currents or an increase in the outward current or both.

Lynch et al.\textsuperscript{35} reported that halothane depresses the slow action potential and decreases the slow inward Ca++ current, $I_{Si}$ in guinea pig papillary muscle and thus decreases the action potential duration. Therefore, the depression of plateau phase in the Purkinje fibers by halothane may result from its inhibitory effect on the $I_{Si}$ and the decrease in the action potential duration may be mainly due to the inhibition of $I_{Si}$ and/or increase in $I_x$. Our results are consistent with the one reported by Hauswirth in which he found that halothane decreases the action potential duration and refractory period in sheep Purkinje fibers.\textsuperscript{5}
Table 13 and Figure 28 show that beta-adrenergic agonist, isoproterenol, elevates the plateau, shortens the action potential duration at 50% and 90% repolarization and effective refractory period at the concentration of $10^{-8}$ to $10^{-7}$ M. Increasing the concentration to $10^{-6}$ M, however, does not decrease the duration and refractory period further (Table 13). Two currents, $I_{si}$ and $I_{x}$ are responsible for the effects of catecholamines on the action potential duration.\textsuperscript{62} Catecholamines are believed to increase the $I_{si}$ and thus produce a positive inotropic effect.\textsuperscript{63,64} Increasing $I_{si}$, however, will prolong the action potential duration and elevate the plateau.\textsuperscript{63} Therefore, an increase in $I_{si}$ by isoproterenol may be responsible for the elevation of plateau phase. It is reported that catecholamines also increases the magnitude of $I_{x}$.\textsuperscript{65} Therefore, depending on the relative effects on $I_{si}$ and $I_{x}$, either a longer or a shorter duration of action potential will prevail. The effect of catecholamines on the action potential duration also depends on the concentration.\textsuperscript{65} A larger responsiveness of $I_{x}$ as compared to $I_{si}$ at the concentration of $10^{-8}$ to $10^{-7}$ M may explain our result in which isoproterenol shortens the duration whereas at the concentration of $10^{-6}$ M isoproterenol does not further decreases the duration and refractory period.
possibly due to a saturating effect of isoproterenol on $I_x$.

Aminophylline is an inhibitor of the enzyme phosphodiesterase which breaks down cyclic AMP. The effects of aminophylline on the action potential duration and effective refractory period are listed in Table 13 and Figure 27. Aminophylline, like isoproterenol, elevates the plateau phase of action potential, decreases the duration and decreases refractory period. These results agree with previous findings obtained with theophylline, and caffeine.

The combined effects of 2% halothane and aminophylline $5 \times 10^{-6}$ to $5 \times 10^{-4}$ and/or isoproterenol $10^{-8}$ to $10^{-6}$ M are tested and the results are listed in Table 15 and Figures 29, 30 and 32. The results show that aminophylline and isoproterenol further shorten the action potential duration and effective refractory period decreased by halothane. Isoproterenol, a beta-adrenergic agonist, increases cyclic AMP level by activating adenylate cyclase whereas aminophylline increases cyclic AMP by inhibiting an enzyme phosphodiesterase which breaks down cyclic AMP. Electrophysiologically, aminophylline and membrane-
permeable derivatives of cyclic AMP produce adrenaline-like effects in Purkinje fibers. Therefore, this suggests that the effects of isoproterenol and aminophylline are through a final common mechanism, cAMP. Iontophoretic injection of cyclic AMP into cardiac cells initially elevates the plateau of the action potential and prolongs the action potential duration and thereafter shortens the action potential. The alteration in action potential duration suggests that at least two conductances are increased: $I_{si}$, which elevates the plateau and prolongs the action potential, and $I_X$, which shortens the action potential. It is thought that the increase in $I_{si}$ depends on activation of cyclic AMP dependent protein kinase. The latter phosphorylates membrane proteins associated with the Ca++ channel, and thus opens the channels.

As previously reported by others, our results show that halothane depresses the plateau, decreases the action potential duration and decreases effective refractory period possibly due to decrease in $I_{si}$ and increase in $I_X$. Isoproterenol and aminophylline, however, elevate the plateau by an increase in the $I_{si}$. Figures 29 and 30 show that aminophylline and isoproterenol elevate the depressed
plateau by halothane. In addition, the effects of halothane, aminophylline and isoproterenol on $I_x$ are additive, which leads to further decreasing the action potential duration.

The effective refractory period is the period during which a second normal stimulus can not give rise to an action potential because the fast sodium channels are completely closed and there is little inward sodium diffusion. Therefore, the effective refractory period does not necessarily depend on the action potential duration. Halothane, aminophylline and isoproterenol decrease the effective refractory period (Table 13). The combination produces a greater effect on the refractory period than any of these three compounds alone. Therefore, these compounds reduce the cardiac refractoriness and lead to arrhythmias.

Halothane decreases action potential amplitude and overshoot, resting membrane potential and maximum diastolic potential (Table 12) and the maximum rate of rise of phase 0 (Table 17). Resting membrane potential is primarily maintained by the electrochemical gradient of potassium across the sarcolema. Because of the concentration gradient, outward movement of the potassium creates a net
negative charge inside the cell and an outward current, named background outward current, $I_{k1}$. The decreased potassium permeability and thus the $I_{k1}$ may explain the depressant effect of halothane on resting membrane potential. The depolarization of phase 0 is related to the influx of Na+ through its channel by virtue of a sudden increase in Na+ conductance. The maximum rate of rise of phase 0 depends on the percentage of available Na+ channels and the sodium electrochemical potential and thus the Na+ current. The decreased maximum rate of rise of phase 0 may result from its inhibition of the Na+ current due to either a decrease in the number of channels available or in the opening time of the channels (kinetics). Bean and Rupperberg reported separately that halothane inhibits the Na+ current in crayfish giant axon and human skeletal muscle. The decrease in maximum rate of rise of phase 0 leads to decrease in the action potential amplitude and overshoot.

Conflicting results have been reported, in which beta agonists produce an increase, a decrease or no change in resting membrane potential and action potential amplitude as well as maximum rate of rise of phase 0. In this study, aminophylline and isoproterenol increase the
resting membrane potential, maximum diastolic potential and action potential amplitude and the maximum rate of rise of phase 0. The increase in maximum diastolic potential by isoproterenol and aminophylline may be explained by the fact that they stimulate the electrogenic sodium-potassium exchange pump. This pump produces an outward current by pumping three sodium ions for two potassium ions. Epinephrine was reported \(^{70}\) to stimulate this pump. Therefore, stimulating this pump causes hyperpolarization.

The results of halothane, aminophylline and isoproterenol on cyclic AMP are listed in Table 16 and Figure 33. Halothane (1% and 2%) increases the cAMP level. As expected isoproterenol and aminophylline increase the cAMP concentration. The combination of the three compounds causes greatest increase in the cAMP concentration.

It has been reported that halothane decreases cAMP in cardiac muscle \(^{44-46}\) and increases cAMP in Purkinje fibers.\(^ {47}\) As mentioned above, halothane decreases markedly action potential duration and effective refractory period of Purkinje fibers whereas it has little effect on the action potential duration and refractoriness of
papillary muscle.\textsuperscript{5,9} It may be a plausible explanation that the decrease in action potential duration and effective refractory period in Purkinje fiber may be due to the increase in cAMP level by halothane. In contrast, the little effect of halothane on action potential and the depressant effect of halothane on cardiac muscle may be exerted by its inhibition of adenylate cyclase and hence decrease in cAMP level. Therefore, halothane may have different effect on cardiac muscle and Purkinje fiber in terms of cAMP concentration and electrophysiological properties.

A good correlation is found between cAMP and action potential duration and effective refractory period, particularly the action potential duration at 50\% repolarization (Table 16). It is believed that the effect of halothane on the plateau phase of Purkinje fiber action potential and cAMP concentration could be an important factor in induction of arrhythmias.

Table 17 show the effects of halothane, aminophylline and isoproterenol on escape time and spontaneous rate. The combined effects of halothane, aminophylline and/or isoproterenol on escape time and spontaneous rate are
listed in Table 18. Figure 34 show that halothane increases the escape time and decreases the spontaneous rate in a dose-dependent manner. The percentage changes of escape time and spontaneous rate after halothane and combinations of halothane and aminophylline and/or isoproterenol are compared in Figures 35 and 36. Aminophylline and isoproterenol decrease the escape time and increase the spontaneous rate. Aminophylline and isoproterenol decrease the halothane-increased escape time and increase the halothane-decreased spontaneous rate.

Since Purkinje fibers have the property of automaticity, they can reach their threshold and fire spontaneously when the external stimulation is interrupted. The frequency of firing of the automatic Purkinje fibers is varied by changes in the rate of diastolic depolarization (slope of phase 4), the threshold potential and maximum diastolic potential. The decrease in the spontaneous rate or increase in the escape time may result from a decrease in the rate of diastolic depolarization (slope of phase 4), and an increase in the threshold and the maximum diastolic potential (more negative). In this study, however, halothane decreases the maximum diastolic potential (less negative). Therefore, the decreased
automaticity by halothane may be from either a decrease in the diastolic depolarization, which is consistent with the previous reports, \(^5,^9\) or, an increase in the threshold potential or both. In Purkinje fibers, the diastolic depolarization is ascribed to at least two ionic currents, \(^81\) the hyperpolarization-induced inward current, \(I_f\) and the outward \(K^+\) current, \(I_{K2}\). Therefore, a decrease in the \(I_f\) or an increase in the \(I_{K2}\) may be responsible for the halothane-decreased automaticity. Reuter \(^82\) reported that adrenaline shifts the steady-state activation voltage of \(I_{K2}\) and its time constant along the voltage axis towards less negative potentials. As result of this shift, \(I_{K2}\) decays more rapidly and thus automaticity increases. Phosphodiesterase inhibitors mimic the effect of adrenaline on this current.\(^82\) The adrenergic transmitters also increase the \(I_f\).\(^81\)

Clinical Significance

It has been reported that the combination of catecholamines and aminophylline results in a greater incidence of arrhythmias during halothane anesthesia.\(^25-30\) In this study, halothane itself decreases the action potential duration and effective refractory period and the
combination of halothane and aminophylline and/or isoproterenol causes a further decrease in the duration and refractory period. Premature beats from ectopic foci may encounter the early excitable tissue. Therefore, this facilitates formation of reentry arrhythmias.

However, the decrease in action potential duration and effective refractory period is the only action shared by halothane and catecholamines. Basically, halothane and catecholamines are mutually antagonistic in most of their cardiovascular effects. Our results show that halothane and isoproterenol or aminophylline have opposite effects on automaticity of the Purkinje fibers. While halothane decreases the automaticity, isoproterenol and aminophylline increase the automaticity. It is believed that halothane possesses both arrhythmic and antiarrhythmic properties.\(^1\)\(^,\)\(^2\)\(^1\) Halothane-decreased automaticity of Purkinje fibers showed in this present study and others \(^5\)\(^,\)\(^9\) may be antiarrhythmic whereas halothane-decreased refractory period may be arrhythmogenic. Nevertheless, abbreviation of the refractory period by halothane and isoproterenol as well as aminophylline is very important in the induction of arrhythmias and is responsible, at least in part, for the cardiac sensitization.


11. Sigurdsson, G.H., O. Werner, and T. Fahraeus: Ventricular Arrhythmias or Supraventricular Arrhythmia with Aberrant Conduction? An Electrocardiographic Study


22. Reynolds, A.K.: On the Mechanism of Myocardial Sensitization to catecholamines by hydrocarbon


CHAPTER III

THE EFFECTS OF CALCIUM AND HALOTHANE ON ELECTROPHYSIOLOGICAL PROPERTIES OF HYPERTROPHIED VENTRICULAR PAPILLARY MUSCLE OF CONGESTIVE HEART FAILURE RATS

INTRODUCTION

The transmembrane action potential plays a key role in the initiation of the excitation-contraction coupling in heart muscle. It has been reported that the depression of contractility in hypertrophied myocardium is associated with some alteration of the transmembrane action potential, such as depression of action potential plateau and prolongation of action potential.\textsuperscript{1-5} There is a direct relation between the duration of the plateau phase of the action potential, the amount of Ca++ influx, and developed tension.\textsuperscript{6-10} Ca++ is a key factor in the process of excitation-contraction coupling in the heart muscle. The abnormal subcellular handling of Ca++ may provide the basis for cardiac contractile failure.

The cardiac electrophysiological properties have been
widely studied in a variety of hypertrophied animal heart models: for example, spontaneously hypertensive rat (SHR),\textsuperscript{11,13} renal hypertensive rat \textsuperscript{3,8} and cat with chronic right ventricular hypertension.\textsuperscript{1-2,4} Recently, a congestive heart failure model was derived from SHR and a Koletsky rat.\textsuperscript{14} This colony is characterized by hypertension, ventricular hypertrophy and spontaneous cardiomyopathic-congestive heart failure.\textsuperscript{14} However, the electrophysiological properties in this heart failure model have not been studied nor have the effects of changing Ca++ concentration on the cardiac electrophysiological properties been reported.

Halothane is known to sensitize the heart to the arrhythmogenic effects of catecholamines and to depress contractility in cardiac muscle.\textsuperscript{15-26} Halothane may act on different sites of the excitation-contraction coupling to inhibit cardiac contractility, including the slow channel Ca++ influx, the function of sarcoplasmic reticulum (SR) and Ca++ pump at the sarcolemma. The effects of halothane on the electrophysiological properties in this hypertrophied myocardium have not been studied.

The purposes of the present study are to characterize the electrophysiological properties of hypertrophied ventricular papillary muscle in this heart failure model and to examine the effects of halothane on the electrophysiological properties.
LITERATURE REVIEW

Excitation-contraction coupling is referred to as the process in which cardiac systole is set into motion by a series of steps which begin when the action potential depolarizes the sarcolemma, and end when Ca++ binds to troponin C of the cardiac contractile proteins and contraction occurs. Transition from diastole to systole is initiated by an influx of Ca++ ions through Ca++ channels during the plateau phase of action potential, which causes a release of Ca++ ions from sarcoplasmic reticulum, a process called Ca++ triggered Ca++ release from intracellular Ca++ stores. Therefore, the action potential duration and plateau voltage can modulate the amount of Ca++ influx and thus the force of contraction.

Cardiac hypertrophy results in some electrocardiographic, electrophysiological and contractile changes. However, in those studies, controversial results have been reported. Uhley in 1961 investigated the electrical performance of hypertrophied heart in rats and reported no differences in the transmembrane action potential between normal and hypertrophied hearts. In 1973, Bassett et al used a different model in which right ventricular hypertrophy was induced in cats by surgically placing a coarcting band around the root of the pulmonary artery. They found a depression of the action potential
plateau phase, prolongation of action potential duration, and a decrease in resting membrane potential and maximum rate of rise of action potential. Ten Eick et al., using the same cat model, demonstrated that the action potential duration was prolonged and the plateau voltage was depressed in hypertrophied myocardium. Decrease in slow inward Ca++ current and outward K+ current may be responsible for the depression of the plateau phase and the prolongation of the action potential, respectively. It has been reported that in spontaneously hypertensive rats (SHR) and rats made hypertensive with deoxycorticosterone acetate (DOCA), the action potential duration is longer than in controls. More recently, Keung in 1989, however, reported that calcium current is increased in isolated adult myocytes from hypertrophied rat myocardium. He proposed that the prolongation of the action potential is due to an increase in peak current density and to the slower inactivation of the maximal calcium current.

An abnormal response to catecholamines was found in congestive heart failure in humans. Human hearts, at the end-stage of congestive failure, have diminished inotropic responsiveness to beta-adrenergic agonists. This may result from a decreased number of beta₁-adrenergic receptors and depressed transmembrane signal via the beta-adrenergic receptor-stimulatory guanine nucleotide regulatory
protein(Gs)-adenylate cyclase pathway or increased levels of inhibitory guanine nucleotide regulatory protein (Gi).

It is well known that halothane, isoflurane and enfurane depress cardiac contractility in a dose-dependent manner. Although the mechanism(s) responsible for the depressant effects is not yet certain, several sites critical to the regulation and binding of calcium in the myocardial cell are probably involved. These may include contractile proteins, the sarcolemma, and the sarcoplasmic reticulum. A number of electrophysiological studies have demonstrated that halothane depresses the slow action potential and decreases the slow inward Ca++ currents via the Ca++ channels. Wheeler et al. reported that halothane increases calcium release from sarcoplasmic reticulum and produces a transient increase in free intracellular calcium concentration, which finally depletes the calcium from sarcoplasmic reticulum and thus depresses the cardiac contraction. Housmans et al. reported that halothane also decreases the myofibrillar responsiveness to Ca++ and/or Ca++ sensitivity of the contractile protein. In intact animal studies, halothane decreases the maximal rate of pressure development (dP/dt), the ratio of dP/dt to pressure at its maximum point [(dP/dt)], left ventricular work,
and left ventricular velocity of shortening.\textsuperscript{52} It increases left ventricular end-diastolic pressure \textsuperscript{55} and shifts the left ventricular function curve down and to the right, indicating depression of cardiac performance.

However, the effects of halothane on electrophysiological properties of congestive failure heart have not widely been studied. In 1982, Reiz \textit{et al.} reported that halothane can unload the failing left ventricle via its systemic vasodilatory effect although it has direct depressant effect on heart.\textsuperscript{56} The peripheral vasodilatory action is predominant over the direct cardiodepressant effect of this agent.
MATERIALS AND METHODS

A total of 35 rats of either sex were included in this study. The rats were classified into three groups: normal rats, slight heart failure rats and severe heart failure rats, according to their clinical signs and heart sizes. The heart failure rats were derived from spontaneous hypertensive rats and Koletsky rats. These rats developed heart failure as early as 11 months up to 22 months of age. They showed clinical signs of heart failure similar to those seen in human patients, such as generalized spontaneous edema, hydrothorax, ascites and dyspnea. Control rats are normal age-matched Wistar rats.

The rats were anesthetized with sodium pentobarbital, 30 mg/kg, and the hearts were rapidly removed and placed in oxygenated Tyrode's solution of the following composition (mM): NaCl, 137.0; NaHCO₃, 12.0; CaCl₂, 1.8; MgCl₂, 0.5; NaH₂PO₄, 1.8; KCl, 4.0; and glucose, 5.5.

Ventricular papillary muscles were removed from the right ventricle and placed in a tissue superfusion bath containing oxygenated Tyrode's solution and maintained at 35°C and 7.4 pH. Action potentials were recorded from the papillary muscles using standard microelectrode recording techniques. The muscle was stimulated at the rate of 60 times per minute with a 2-msec duration at twice the threshold voltage. Microelectrodes, with resistances
ranging from 10 to 30 MΩ, were made on a horizontal magnetic electrode puller (Narishige Scientific Instrument Laboratories, Tokyo, Japan). The microelectrodes were filled with 2.5 M KCl and then coupled by an Ag-AgCl cell to an amplifier (S-7071A, WP Instruments, Inc., New Haven CT). The output of the amplifier was connected to an oscilloscope (Model 5111A with one 5A18N four channel and 5B12N dual time base, Tektronix, Inc., Beaverton, OR). The first derivatives of the action potentials were obtained electronically using a differentiator. The measurement is linear from 0 to 1000 volts/sec. The action potentials and their derivatives were displayed on the first and second channels of the oscilloscope, respectively. The data were recorded on a VCR (General Electric) and on Polaroid film for analysis.

The basic protocol was to record normal membrane characteristics after an equilibration period of 30 minute. During a test period, a drug was introduced and the same membrane characteristics were determined for comparison to controls. The membrane characteristics which were determined during the control and test periods were as follows:

Action Potential Characteristics (Figure 37):

1. Resting Membrane Potential, RMP (mV): the potential just
Figure 37

Action potentials of ventricular papillary muscle.
Figure 37
before rapid depolarization phase.

2. Action Potential Amplitude, APA (mV): the height of rapid depolarization phase from RMP to peak potential.

3. Action Potential Duration, APD (msec): time from the beginning of rapid depolarization to 50% (APD$_{50}$), and 90% (APD$_{90}$) of the action potential repolarization.

4. Maximum rate of rise of rapid depolarization phase, $V_{\text{max}}$ or $dV/dt$ (V/sec): the derivative of rapid depolarization phase.

After the control period, the following tests were made:

1. Increasing Ca++ concentration from 1.8 mM to 3.6 mM and then washing out 3.6 mM Ca++ to 1.8 mM for ventricular papillary muscle.

2. Halothane (Halocarbon Laboratories, Inc)
   2% and 4%
   At 4% halothane, increasing Ca++ concentration from 1.8 mM to 3.6 mM and then washing out 3.6 mM Ca++ to 1.8 mM for ventricular papillary muscle.

   In all experiments, halothane was carried by $O_2$ via a calibrated vaporizer and introduced to the perfusate
reservoir through a glass dispersion tube. Monitoring of P\textsubscript{O2}, P\textsubscript{CO2} and pH were performed so as to maintain them at predetermined levels. Two 1.0 ml bath samples were drawn and injected into sealed vials at the conclusion of each anesthetic exposure for gas chromatographic analysis. Standard curves were constructed daily from known amounts of vapor. The chromatographic peaks of halothane from the drawn sample were compared with peaks from the standard curve. A scavengering system for limiting exposure of personnel to anesthetics was employed.

The statistical significance of differences in mean values for these parameters was assessed using analysis of variance and Duncan's multiple range test. P values less than 0.05 were considered significant.
RESULTS

The Electrophysiological Properties of the Hypertrophied Ventricular Papillary Muscle of the Heart Failure Rats

Means and standard errors of the means of the heart weight and action potential parameters recorded from the hypertrophied right ventricular papillary muscle are listed in Tables 19 and 20. The action potentials from normal rats (Panel a), slight heart failure (Panel b) and severe heart failure (Panel c) rats are compared in Figure 38. The action potential durations are prolonged significantly and progressively in the heart failure rats. The action potential durations at 50% repolarization in slight heart failure and severe heart failure rats are longer by approximately 70% and 170% respectively than those of normal rats. The durations at 90% in slight and severe heart failure rats are longer by approximately 90% and 200% than the ones of normal rats. Other parameters, such as resting membrane potential, action potential amplitude and maximum rate of rise of phase 0 do not change significantly although the maximum rates of rise of phase 0 are lower in the heart failure groups than those of normal rats.

Effects of Changing Ca++ Concentration on the Action Potentials of the Hypertrophied Ventricular Papillary
Muscle of Heart Failure Rats

The effects of changing Ca++ concentration on the action potentials are listed in Tables 19 and 20. The action potentials recorded from the hypertrophied ventricular muscle before and after increasing Ca++ concentration from 1.8 mM to 3.6 mM and washing out the high Ca++ from 3.6 mM to 1.8 mM are compared in Figure 39. In this Figure, the action potentials in row A are from normal rats. Row B and C show the action potentials recorded from slight and severe heart failure rats, respectively. Column a is control in which the Ca++ concentration is 1.8 mM. The Ca++ concentration is doubled in Column b. Column c shows the action potentials 10 minutes after washing the high concentration of Ca++ out with Tyrode's solution containing 1.8 mM Ca++. Quantitative summaries of the action potential duration at 50% and 90% repolarization before and after changing the Ca++ concentration are graphically presented in Figures 40 and 41. The percentage changes of action potential duration at 50% and 90% after changing Ca++ levels are shown in Figures 42 and 43. The results show that the action potential duration at 50% repolarization is shortened after increasing Ca++ concentration from 1.8 mM to 3.6 mM in all groups. The significant levels are shown in Table 20. The decreases in the action potential duration at 50%
repolarization by increasing Ca++ concentration, however, are dependent upon the degree of heart failure; the more severe the heart failure the more decreased are action potential durations. The duration at 90% repolarization, however, is increased after increasing Ca++ level. Although the increases in the duration at 90% repolarization also depend on the degree of heart failure, the less severe the heart failure, the more the durations are increased. The constant increases of the action potential durations at 50% repolarization are found after washing the high Ca++ concentration 3.6 mM out to 1.8 mM in all groups; but the greatest increases are found in the severe heart failure group, and less in the normal group. The significance levels are shown in Table 20. The action potential durations at 90% repolarization following washout are decreased in the normal group, and increased in heart failure groups (42.0% in slight heart failure and 69.0% in severe heart failure).

The Effects of Halothane on the Action Potentials of the Hypertrophied Ventricular Papillary Muscle of the Heart Failure Rats

The means and standard errors of the means of the action potential parameters of the hypertrophied
ventricular papillary muscle before and after halothane and the combination of halothane (4%) and changing Ca++ concentration are listed in Tables 21 and 22. The effects of halothane (2% and 4%) and the combination of halothane and changing Ca++ concentration on action potential duration at 50% and 90% repolarization are summarized graphically in Figures 44 and 45. The percentage changes in action potential duration at 50% and 90% after halothane (2% and 4%) are shown in Figures 46 and 47. Although halothane does not change the action potential parameters significantly, it decreases the action potential duration at 50% and 90% repolarization in normal rats in a dose-dependent manner (Figures 46 and 47). In severe heart failure rats, however, halothane at 2% slightly decreases the duration at 50% repolarization and at 4% increases the duration (Figure 46). Halothane increases the duration at 90% repolarization in severe heart failure in a dose-dependent manner (Figure 47).

The Effects of Changing Ca++ Concentration on the Action Potentials Pretreated by Halothane

Figures 48 and 49 show the effects of changing Ca++ concentration on the action potential duration at 50% and 90% repolarization with and without pretreatment by 4% halothane. In normal rats, increasing Ca++ concentration
from 1.8 mM to 3.6 mM decreases the duration at 50% repolarization 2.0% without halothane pretreatment ($P > 0.05$) and 19.0% with 4% halothane pretreatment ($P > 0.05$). Halothane itself decreases the duration at 50% repolarization 16.0%. Therefore, in normal rats, increasing Ca++ concentration further decreases the action potential duration at 50% repolarization. In normal rats, increasing Ca++ concentration from 1.8 mM to 3.6 mM increases the duration at 90% repolarization 22.0% without halothane pretreatment ($P > 0.05$) and 7.74% with 4% halothane pretreatment ($P > 0.05$). Halothane itself decreases the duration at 90% repolarization 17.0%. Therefore, halothane prevents the effects of Ca++ on the action potential duration at 90% repolarization.

In severe heart failure rats, however, increasing Ca++ concentration from 1.8 mM to 3.6 mM decreases the duration at 50% repolarization 21.0% without halothane pretreatment and 20.0% with 4% halothane pretreatment. Therefore, Ca++ does not have different effects on the action potential duration at 50% repolarization in heart failure rats with or without pretreatments by 4% halothane. In severe heart failure rats, increasing Ca++ concentration from 1.8 mM to 3.6 mM increases the duration at 90% repolarization slightly without halothane pretreatment and 16.0% with 4% halothane pretreatment. Halothane itself increases the
duration 11.0% at 90% repolarization. Therefore, Ca++ further increases the halothane-increased action potential duration at 90% repolarization in severe heart failure rats.

No difference between action potential durations of normal and severe heart failure rats has been found after washout except that in normal rats the percentage decreases of the action potential durations at 90% repolarization are 15.0% without halothane pretreatment and 4.0% with halothane pretreatment.

The effects of Ca++ on the action potential duration of normal and severe heart failure rats with or without pretreatment with 4% halothane are also summarized in Tables 23 and 24.
Table 19
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Maximum Rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Ventricular Papillary Muscle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP(-mV)</th>
<th>APA(mV)</th>
<th>Vmax(v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Rats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>78.07±1.43</td>
<td>92.58±2.49</td>
<td>189.0±18.82</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>78.92±1.51</td>
<td>92.32±4.06</td>
<td>164.0±17.51</td>
</tr>
<tr>
<td>Washout</td>
<td>81.17±0.64</td>
<td>94.83±1.95</td>
<td>168.7±10.16</td>
</tr>
<tr>
<td><strong>Slight Heart Failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80.75±1.59</td>
<td>90.83±3.90</td>
<td>132.5±19.39</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>82.17±1.64</td>
<td>99.08±2.85</td>
<td>143.7±15.47</td>
</tr>
<tr>
<td>Washout</td>
<td>81.83±1.08</td>
<td>98.00±1.18</td>
<td>125.0±11.18</td>
</tr>
<tr>
<td><strong>Severe Heart Failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>78.63±0.80</td>
<td>92.62±3.70</td>
<td>132.8±31.71</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>78.66±1.03</td>
<td>98.75±2.89</td>
<td>146.7±27.88</td>
</tr>
<tr>
<td>Washout</td>
<td>82.08±1.33</td>
<td>104.17±2.39</td>
<td>158.8±27.14</td>
</tr>
</tbody>
</table>

N = 6
Control = 1.8 mM Ca++
2 X Ca++ = 3.6 mM Ca++
Washout = 1.8 mM Ca++
Table 20
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) and heart weight (HW), for Each Treatment Administered to Ventricular Papillary Muscle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
<th>HW(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.50±1.50</td>
<td>39.33±5.14</td>
<td>1.15±0.06</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>14.17±1.68</td>
<td>48.00±9.96</td>
<td></td>
</tr>
<tr>
<td>Washout</td>
<td>15.67±1.65</td>
<td>41.00±4.90</td>
<td></td>
</tr>
<tr>
<td>Slight Heart Failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.67±3.46*</td>
<td>74.33±9.42*</td>
<td>1.72±0.07**</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>22.33±2.40*</td>
<td>79.83±9.11</td>
<td></td>
</tr>
<tr>
<td>Washout</td>
<td>33.17±5.19*</td>
<td>112.92±8.65**</td>
<td></td>
</tr>
<tr>
<td>Severe Heart Failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39.67±3.66**</td>
<td>116.08±7.09**</td>
<td>2.58±0.15**</td>
</tr>
<tr>
<td>2 X Ca++</td>
<td>31.50±2.98</td>
<td>116.33±6.93</td>
<td></td>
</tr>
<tr>
<td>Washout</td>
<td>65.00±11.47*</td>
<td>197.00±14.65**</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01 compared with control except *
*: Compared with normal rats, N = 6
Control = 1.8 mM Ca++
2 X Ca++ = 3.6 mM Ca++
Washout = 1.8 mM Ca++
Figure 38

Action potentials recorded from ventricular papillary muscle.

a: Normal Heart
b: slight Failure Heart
c: Severe Failure Heart
Figure 38
Figure 39

Action potentials recorded from ventricular papillary muscle.

Row A: Normal Heart
a: Control
b: Ca++ 3.6 mM
c: Washout with Tyrode’s solution containing 1.8 mM Ca++

Row B: Slight Failure Heart
a: Control
b: Ca++ 3.6 mM
c: Washout with Tyrode’s solution containing 1.8 mM Ca++

Row C: Severe Failure Heart
a: Control
b: Ca++ 3.6 mM
c: Washout with Tyrode’s solution containing 1.8 mM Ca++
Figure 39
Figure 40

Effects of increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ on action potential durations at 50% repolarization (APD50) of ventricular papillary muscle. * compared with control

Figure 41

Effects of increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ on action potential durations at 90% repolarization (APD90) of ventricular papillary muscle. * and ** compared with control
Figure 40

Figure 41
Figure 42

Percentage changes of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode’s solution containing 1.8 mM Ca++ from control.

Figure 43

Percentage changes of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode’s solution containing 1.8 mM Ca++ from control.
Figure 42

Figure 43
Table 21
Means and Standard Errors of the Means of Resting Membrane Potential (RMP), Action Potential Amplitude (APA), and Maximum Rate of Rise of Phase 0 (Vmax) for Each Treatment Administered to Ventricular Papillary Muscle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RMP (-mV)</th>
<th>APA (mV)</th>
<th>Vmax (v/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Rats (N=6)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>75.93±3.26</td>
<td>90.80±4.18</td>
<td>189.0±19.39</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>76.92±2.96</td>
<td>98.27±2.98</td>
<td>187.4±17.67</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>77.50±2.65</td>
<td>98.93±3.62</td>
<td>180.0±17.06</td>
</tr>
<tr>
<td>3.6 mM Ca++</td>
<td>77.30±2.38</td>
<td>101.23±3.25</td>
<td>172.0±10.94</td>
</tr>
<tr>
<td>Washout</td>
<td>78.23±3.07</td>
<td>103.73±1.75</td>
<td>166.4±10.12</td>
</tr>
<tr>
<td><strong>Severe heart failure (N=7)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>74.36±3.54</td>
<td>84.14±5.94</td>
<td>118.3±29.70</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>74.27±2.78</td>
<td>83.44±5.01</td>
<td>118.8±29.25</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>73.97±3.94</td>
<td>83.59±5.55</td>
<td>120.2±27.32</td>
</tr>
<tr>
<td>3.6 mM Ca++</td>
<td>79.86±3.28</td>
<td>97.30±4.99</td>
<td>160.8±29.43</td>
</tr>
<tr>
<td>Washout</td>
<td>74.87±5.18</td>
<td>84.57±7.37</td>
<td>130.3±26.53</td>
</tr>
</tbody>
</table>

The treatments of 3.6 mM Ca++ and washout are with pretreatment of halothane 4%
Table 22
Means and Standard Errors of the Means of Action Potential Duration at 50% and 90% Repolarization (APD50 and APD90) for Each Treatment Administered to Ventricular Papillary Muscle

<table>
<thead>
<tr>
<th>Treatment</th>
<th>APD50(mSec)</th>
<th>APD90(mSec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal Rats (N=6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.67±3.26</td>
<td>49.50±9.31</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>12.00±2.67</td>
<td>44.50±8.15</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>10.67±2.29</td>
<td>41.17±7.47</td>
</tr>
<tr>
<td>3.6 mM Ca++</td>
<td>10.25±2.11</td>
<td>53.33±9.40</td>
</tr>
<tr>
<td>Washout</td>
<td>11.83±2.65</td>
<td>51.33±9.01</td>
</tr>
<tr>
<td><strong>Severe Heart Failure (N=7)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31.86±4.80</td>
<td>87.21±6.23</td>
</tr>
<tr>
<td>2% Halothane</td>
<td>30.00±4.54</td>
<td>89.00±7.18</td>
</tr>
<tr>
<td>4% Halothane</td>
<td>33.71±5.96</td>
<td>96.57±9.66</td>
</tr>
<tr>
<td>3.6 mM Ca++</td>
<td>25.64±4.01</td>
<td>101.3±11.28</td>
</tr>
<tr>
<td>Washout</td>
<td>52.20±11.59</td>
<td>168.6±18.94**</td>
</tr>
</tbody>
</table>

** = P < 0.01 compared with control

The treatments of 3.6 mM Ca++ and washout are with pretreatment of halothane 4%
Figure 44

Effects of halothane 2% and 4% and increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ after halothane 4% on action potential durations at 50% repolarization (APD50) of ventricular papillary muscle.

Figure 45

Effects of halothane 2% and 4% and increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ after halothane 4% on action potential durations at 90% repolarization (APD90) of ventricular papillary muscle. ** compared with control
Figure 44

Figure 45
Figure 46

Percentage changes of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle after halothane 2% and 4% from control.

Figure 47

Percentage changes of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle after halothane 2% and 4% from control.
Figure 46

Figure 47
Figure 48

Percentage changes of action potential duration at 50% repolarization (APD50) of ventricular papillary muscle with and without pretreatment by halothane 4% after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode's solution containing 1.8 mM Ca++ from control.

Figure 49

Percentage changes of action potential duration at 90% repolarization (APD90) of ventricular papillary muscle with and without pretreatment by halothane 4% after increasing Ca++ from 1.8 mM to 3.6 mM and washout with Tyrode’s solution containing 1.8 mM Ca++ from control.
Figure 48

Figure 49
Table 23
Effects of Ca++ on Action Potential Durations at 50% Repolarization (APD50) at Different Conditions*

<table>
<thead>
<tr>
<th></th>
<th>Normal Rats</th>
<th>Severe Heart Failure Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>↓</td>
<td>♦</td>
</tr>
<tr>
<td></td>
<td>2.28%</td>
<td>20.6%</td>
</tr>
<tr>
<td>HAL 4%</td>
<td>↑↑↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>19.1%</td>
<td>19.5%</td>
</tr>
</tbody>
</table>

* = Different conditions include the followings:
Normal rats + Control (without halothane)
Normal rats + halothane 4%
Severe heart failure rats + Control
Severe heart failure rats + halothane 4%
HAL = Halothane
♦ - ↑↑↑ = the degree of decrease in APD50 from the least to the most
Values in percentage = percentage decreases in APD50

Table 24
Effects of Ca++ on Action Potential Durations at 90% Repolarization (APD90) at Different Conditions*

<table>
<thead>
<tr>
<th></th>
<th>Normal Rats</th>
<th>Severe Heart Failure Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>↑↑↑↑</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>22.04%</td>
<td>0.22%</td>
</tr>
<tr>
<td>HAL 4%</td>
<td>↑↑</td>
<td>♦</td>
</tr>
<tr>
<td></td>
<td>7.74%</td>
<td>16.16%</td>
</tr>
</tbody>
</table>

* = Different conditions include the followings:
Normal rats + Control (without halothane)
Normal rats + halothane 4%
Severe heart failure rats + Control
Severe heart failure rats + halothane 4%
HAL = Halothane
♦ - ↑↑↑ = the degree of increase in APD90 from the least to the most
Values in percentage = percentage decreases in APD90
DISCUSSION

The purposes of this present study are to characterize the electrophysiological properties of the hypertrophied ventricular papillary muscle in congestive heart failure rats and to investigate the effects of changing extracellular Ca++ concentration on the action potentials with and without halothane pretreatment and the effects of halothane alone on the action potentials. The results from this study, consistent with others 1-5, show some alterations which are very important for understanding the mechanisms of the electrophysiological changes of hypertrophied ventricular muscle.

As previously reported by other investigators 1-5, Figure 38 shows that the action potential durations are prolonged in heart failure groups significantly and progressively, despite the fact that they do not show any significant changes in resting membrane potential, action potential amplitude and maximum rates of rise of phase 0 although the maximum rates of rise of phase 0 are lower in heart failure groups. It has been reported that in hypertrophied ventricles, the action potential durations are prolonged and the plateau are depressed in various animal models, such as cat 1-2, rabbit 57, and rat 3, 58 as well as human patients. 59 Gulch et al. 60 found that the
prolongation of action potential duration becomes more marked as the degree of hypertrophy becomes more severe. Our results confirm this finding (Figure 38 and Table 20).

Although the ionic basis for the prolongation of action potential duration in hypertrophied ventricle is yet to be clarified, explanations have been proposed. One is proposed by Aronson. He studied the effects of exposure of high-Ca++ or low-Na+ Tyrode's solution on the action potential duration in hypertrophied myocardium and found that the high Ca++ or low Na+ produced greater decreases in the action potential duration in the hypertrophied myocardium than in normal myocardium. One explanation for this result is that a slowing of the inactivation of a Ca++ inactivated inward current may be responsible for the prolongation of the action potential duration in the hypertrophied ventricle. The high Ca++ could accelerate the inactivation of the Ca++-inactivated inward current and thus decreases the duration. Our study shows similar results in which increasing Ca++ concentration from 1.8 to 3.6 mM decreases the action potential duration at 50% repolarization the greatest in severe heart failure rats (P < 0.01) and the least in normal rats (P > 0.05) (Table 20 and Figure 39). Therefore, our results concur with this hypothesis. This hypothesis is indirectly supported by the fact that in our study, washing out the high concentration
of Ca++ from 3.6 to 1.8 mM increases the duration at 50% repolarization the greatest in severe heart failure rats and the least in normal rats. If it is the case that high Ca++ accelerates the inactivation of the Ca++-inactivated inward current, decreasing Ca++ concentration by washout (from 3.6 to 1.8 mM) should produce an opposite effect on the durations. Therefore, the durations at 50% repolarization are prolonged after washout more markedly in heart failure groups. The percentage changes in the action potential durations at 50% repolarization by increasing Ca++ concentration and washout are shown in Figure 42. However, these changes are only limited to the duration at 50% repolarization.

Increasing Ca++ concentration from 1.8 to 3.6 mM decreases the action potential duration at 50% repolarization and increases the duration at 90% repolarization in normal rats (P > 0.05) (Table 20 and Figures 39 to 43). It is known that elevated Ca++ concentrations shorten the action potential and reduced concentrations lengthen it. The explanation, however, still remains unclear. It is generally accepted that increasing Ca++ concentration enhances the slow inward current and thus elevates the plateau. This increased slow inward current, however, should also prolong the action potential duration, which cannot explain the fact that elevated Ca++
concentrations shorten the duration. It has been demonstrated that Ca++ entry inactivates the slow inward calcium current and activates the slow outward potassium current, which terminates the plateau and completes the repolarization.61 This explains the high Ca++ decreased action potential duration at 50% repolarization, but not the duration at 90% repolarization because in our study, the duration at 90% repolarization in normal rats is prolonged after high Ca++. Kass reported 61 that when Ca++ entry inactivates the \( I_{si} \), it slows the activation of the slow outward potassium current, \( I_x \). In addition, increase in slow inward current, if any, should increase the duration. Therefore, the net change of the action potential duration depends upon the balance between the changes of the two currents, i.e. \( I_{si} \) and \( I_x \). In our study, the high Ca++ induced decrease in action potential duration at 50% repolarization may be due to the Ca++ inactivation of the slow inward calcium current or increase in Ca++-dependent slow outward potassium current and the increased duration at 90% repolarization may be due to the increase in the slow inward current or slowing of activation of the slow outward potassium current. Decreasing the extracellular Ca++ concentration (washout) should produce an opposite effect on the durations (Figures 42 and 43).

Table 20 and Figure 43 show that increasing Ca++ concentration from 1.8 to 3.6 mM increases the action
potential duration at 90% repolarization the greatest in normal rats and the least in severe heart failure rats. If this is the case in which the Ca++-increased duration at 90% repolarization in normal rats is due to the increase in the slow inward current or slowing of activation of the slow outward potassium current, the changes of the action potential duration at 90% repolarization in hypertrophied myocardium could result from alteration of either the slow inward current or the slow outward potassium current. The alteration of slow inward current in the hypertrophied myocardium is still unclear and has been the subject of a number of recent studies. Conflicting results, however, have been reported. While Keung 8 found that the Ca++ current is increased in isolated adult myocytes from hypertrophied rat myocardium, Ten Eick 62 reported that the slow-inward current amplitude or the density of the Ca++ current is decreased in hypertrophied myocardium. The decrease in the slow-inward current may be responsible for the depressed voltage during the plateau phase and decreased contraction. The decreased Ca++ current in hypertrophied myocardium may explain why high Ca++ increases the duration at 90% repolarization less in heart failure rats than in normal rats. The Ca++-dependent outward potassium current is reduced in hypertrophied myocardium,3, 63-64 which is responsible for the prolongation of action potentials in hypertrophied
myocardium. It is reported that high Ca++ restores the decreased Ca++-dependent outward potassium current,¹ which should decrease the duration (This is true at 50% repolarization). However, the effects of altering the Ca++-dependent outward potassium current on the duration at 90% repolarization needs further investigation.

In normal rats, increasing extracellular Ca++ concentration increases the slow inward current and thus increases the action potential duration at 90% repolarization. Vice versa, decreasing the extracellular Ca++ concentration (washout) should decrease the slow inward current and the duration at 90% repolarization (Table 20 and Figure 43). In the heart failure rats, however, decreasing the extracellular Ca++ concentration increases the duration at 90% repolarization progressively, possibly due to further decreasing the Ca++-dependent outward potassium current.

The effects of halothane on action potentials from both normal and heart failure rats are shown in Tables 21, 22 and Figures 44 to 47. Halothane decreases the action potential duration at 50% and 90% repolarization of normal rats in a dose-dependent manner (Figures 46 and 47). Hauswirth ⁵⁵ found that halothane decreases the action potential duration in sheep Purkinje fibers and ventricular
muscle. Lynch et al.\textsuperscript{19} reported that halothane depresses the slow action potential and decreases the slow inward Ca++ current, $I_{sl}$ in guinea pig papillary muscle and thus decreases the action potential duration and contraction. Therefore, the decrease in the action potential duration by halothane may be, at least in part, due to decrease in the $I_{sl}$. A decrease in the $I_{sl}$ is not the only reason for a decrease in action potential and contraction. Wheeler et al.\textsuperscript{47} reported that halothane produces a transient increase in free intracellular calcium concentration. This may result from a direct effect of halothane on the sarcoplasmic reticulum, possibly an enhancement of calcium release, which finally leads to depleting the calcium from sarcoplasmic reticulum and depressing the cardiac contraction. The transient increase in free intracellular calcium concentration further inactivates the Ca++ inactivated slow inward current. A greater decrease in action potential duration, therefore, results. In heart failure rats, halothane at 2\% decreases the duration at 50\% repolarization and at 4\% increases the duration (Figure 46). Figure 47 shows that halothane increases the duration at 90\% repolarization in a dose-dependent manner. As discussed above, the slowing of the inactivation of a Ca++ inactivated inward current or a decrease in the Ca++-dependent outward potassium current may be the cause of the prolongation of the action potential duration in
hypertrophied myocardium. Halothane may either further slow the slowed Ca++ inactivated inward current or decrease the reduced Ca++-dependent outward potassium current and thus further increase the duration. Also the function of the sarcoplasmic reticulum in hypertrophied myocardium may be impaired. Halothane may further depress the impaired function of sarcoplasmic reticulum. Therefore, less free intracellular calcium is available for contractile protein, leading to further depression of cardiac contraction and prolongation of action potential.

Tables 23, 24 and Figures 48, 49 summarize the effects of increasing Ca++ concentration on the action potential pretreated by 4% halothane. High Ca++ decreases the action potential duration at 50% repolarization in all groups. In normal rats without halothane pretreatment, increasing Ca++ decreases the duration only about 2.0%. Increasing Ca++ concentration in normal rats pretreated with halothane and in heart failure rats without halothane pretreatment, the duration decreases 19.0% and 21.0%, respectively. Therefore, the papillary muscle from both 4% halothane pretreated and hypertrophied hearts have same response to increasing Ca++ concentration, possibly due to inhibition of the slow inward current. Increasing Ca++ in the heart failure, halothane pretreated muscles, however, does not produce additive effects on the duration (decrease 20.0%).
suggesting that there must be other mechanisms involved. However, further studies are needed to investigate the possibility.

Increasing Ca++ increases the action potential duration at 90% repolarization in all groups. In normal rats without halothane pretreatment, increasing Ca++ increases the duration 22.0% whereas high Ca++ increases the duration in normal rats with halothane pretreatment about 8.0% and heart failure rats without halothane pretreatment slightly, possibly resulting from a reduction of the Ca++-dependent outward potassium current. In the heart failure group with halothane pretreatment, high Ca++ increases the duration 16.0%. Again, there may be other mechanisms involved in the hypertrophied myocardium or responsible for the effects of halothane.


22. Lynch C.: Differential Depression of Myocardial


APPENDIX
Figure 50

Flow chart of computer program for analysis of cyclic AMP concentration.
Input values for cAMP
Input values for protein
Calculation
Output

start
Output heading

cAMP concentration in pmol/mg protein
Table 25

Computer program for analysis of cyclic AMP concentration

1 SCREEN 1
2 COLOR 9,0
3 I PRINT CHR$(14);"RIA Computer Analysis System"
4 I PRINT CHR$(14); "" (ZHANG 1998)"
5 I PRINT CHR$(18); ""
6 X=0
7 X=3
8 X=10
9 X=20
10 X=40
11 PRINT "Input Your Y-Values of Standard Curve for Protein";Y1,Y2,Y3,Y4,Y5,Y6
12 LET A= N* (X1*Y1+X2*Y2+X3*Y3+X4*Y4+X5*Y5+X6*Y6)
13 LET F=(X1+X2+X3+X4+X5+X6) * (Y1+Y2+Y3+Y4+Y5+Y6)
14 LET C=N* (X1*X1+X2*X2+X3*X3+X4*X4+X5*X5+X6*X6)
15 LET D=(X1+X2+X3+X4+X5+X6) * (X1+X2+X3+X4+X5+X6)
16 LET H=(A-F)/(C-D)
17 LET XM=(X1+X2+X3+X4+X5+X6)/N
18 LET YM=(Y1+Y2+Y3+Y4+Y5+Y6)/N
19 LET B=-M*XM*YM+Y
20 PRINT "SLOPE OF PROTEIN ASSAY STANDARD CURVE";M
21 PRINT "INTERCEPT OF PROTEIN ASSAY STANDARD CURVE";B
22 INPUT "Input dilution factor for protein assay"; DF
23 INPUT "Input number of unknown sample"; I
24 FOR J=1 TO I
25 INPUT "Input Your Y-Value for Protein Unknown Sample"; Y
26 X=((Y-B)/M)*DF
27 PRINT "Unknown concentration of protein"; X
28 IF J>1, GOTO 438
29 XN1=3.39794
30 XN2=3.09691
31 XN3=2.79508
32 XN4=2.49485
33 XN5=2.19382
34 XN6=1.89279
35 XN7=1.59176
36 XN8=1.29073
37 XN9=.9097
38 XN10=.68867
39 PRINT "%Binding for Nucleotide Standard Curve"
40 LET X1=LOG(L1)
41 LET X2=LOG(L2)
42 LET X3=LOG(L3)
43 LET X4=LOG(L4)
44 LET X5=LOG(L5)
45 LET X6=LOG(L6)
46 LET X7=LOG(L7)
47 LET X8=LOG(L8)
48 LET X9=LOG(L9)
49 LET X10=LOG(L10)
Table 25 (continue),

<table>
<thead>
<tr>
<th>Line</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>401</td>
<td>LET L0=BS0/(1-BS0)</td>
</tr>
<tr>
<td>402</td>
<td>LET YS0=LOG(L0)</td>
</tr>
<tr>
<td>404</td>
<td>LET L9=BS9/(1-BS9)</td>
</tr>
<tr>
<td>405</td>
<td>LET YS9=LOG(L9)</td>
</tr>
<tr>
<td>407</td>
<td>LET L10=BS10/(1-BS10)</td>
</tr>
<tr>
<td>412</td>
<td>LET P=H1*(XH1<em>YH1+XH2</em>YH2+XH3<em>YH3+XH4</em>YH4+XH5<em>YH5+XH6</em>YH6+XH7<em>YH7+XH8</em>YH8+XH9+YH9)/H1</td>
</tr>
<tr>
<td>414</td>
<td>LET P=(XH1+XH2+XH3+XH4+XH5+XH6+XH7+XH8+XH9+YH9)</td>
</tr>
<tr>
<td>416</td>
<td>LET Q=H1*(XH1<em>YH1+XH2</em>YH2+XH3<em>YH3+XH4</em>YH4+XH5<em>YH5+XH6</em>YH6+XH7<em>YH7+XH8</em>YH8+XH9+YH9)</td>
</tr>
<tr>
<td>418</td>
<td>LET R=(XH1+XH2+XH3+XH4+XH5+XH6+XH7+XH8+XH9+YH8+YH9+YH10)</td>
</tr>
<tr>
<td>420</td>
<td>LET N1=P/(Q-R)</td>
</tr>
<tr>
<td>422</td>
<td>LET YS=(Y1+Y2+Y3+Y4+Y5+Y6+Y7+Y8+Y9+Y10)</td>
</tr>
<tr>
<td>424</td>
<td>LET N2=1-YS</td>
</tr>
<tr>
<td>426</td>
<td>LET M1=(P+Q)/Q</td>
</tr>
</tbody>
</table>
| 428 | LPRINT USING "SLOPE of cyclic nucleotide standard curve \\
| | \\
| | "; M1 |
| 429 | LPRINT USING "INTERCEPT of cyclic nucleotide standard curve \\
| | \\
| | "; N1 |
| 430 | INPUT "Input dilution factor for cyclic nucleotide assay"; DF1 |
| 432 | PRINT USING "Intercept of standard curve for nucleotide \\
| | \\
| | "; N1 |
| 433 | PRINT USING "Input "binding of nucleotide unknown sample"; B1 |
| 434 | S=DS/(1-B1) |
| 436 | LET YS=(Y1+Y2+Y3+Y4+Y5+Y6+Y7+Y8+Y9+Y10)/H1 |
| 438 | PRINT USING "Slopes of cyclic nucleotide standard curve \\
| | \\
| | "; M1 |
| 439 | PRINT USING "Absorbance of Unknown Protein Sample \\
| | \\
| | "; Y |
| 441 | PRINT USING "Input dilution factor for cyclic nucleotide assay"; DF1 |
| 442 | PRINT USING "Unknown Concentration of Nucleotide f mol/2ml \\
| | \\
| | "; XHP |
| 444 | PRINT USING "Unknown Concentration of Protein ug/2ml \\
| | \\
| | "; XF |
| 446 | PRINT USING "unknown concentration of nucleotide f mol/2ml \\
| | \\
| | "; XHP |
| 448 | PRINT USING "unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; XH |
| 450 | PRINT USING "Unknown Concentration of Nucleotide f mol/mg protein \\
| | \\
| | "; X |
| 452 | PRINT USING "Input dilution factor for cyclic nucleotide assay"; DF1 |
| 454 | PRINT USING "Input "binding of nucleotide unknown sample"; B1 |
| 456 | PRINT USING "binding of Unknown Nucleotide \\
| | \\
| | "; BS |
| 458 | PRINT USING "Input dilution factor for cyclic nucleotide assay"; DF1 |
| 460 | PRINT USING "Unknown Concentration of Nucleotide f mol/2ml \\
| | \\
| | "; XHP |
| 462 | PRINT USING "Input Unknown Concentration of Nucleotide f mol/2ml \\
| | \\
| | "; XHP |
| 464 | PRINT USING "Input Unknown Concentration of Protein ug/2ml \\
| | \\
| | "; XF |
| 466 | PRINT USING "Input Unknown Concentration of Protein ug/mg protein \\
| | \\
| | "; XF |
| 468 | PRINT USING "Input Unknown Concentration of Nucleotide f mol/mg protein \\
| | \\
| | "; X |
| 470 | PRINT USING "Input Unknown Concentration of Nucleotide f mol/mg protein \\
| | \\
| | "; X |
| 472 | PRINT USING "Input "binding of nucleotide unknown sample"; B1 |
| 474 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 476 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 478 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 480 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 482 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 484 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 486 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 488 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 490 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 492 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 494 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 496 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 498 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 500 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 502 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 504 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 506 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 508 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 510 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 512 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 514 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 516 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 518 | PRINT USING "Input "binding of unknown concentration of nucleotide f mol/mg protein \\
| | \\
| | "; B1 |
| 520 | NEXT J |
| 522 | END |
 liste de référence


42. England, P.J., H.T. Pask, and D. Mills: Cyclic-AMP-Dependent Phosphorylation of Cardiac Contractile


52. Gelband H., and A. Bassett: Depressed Transmembrane Potentials during Experimentally Induced Ventricular
Failure in Cats. Circ Res. 32:625-634, 1973


75. Isenberg, G: Is Potassium Conductance of Cardiac


86. Komai H. and B. Rusy: Negative Inotropic Effects of
Isoflurane and Halothane in Rabbit Papillary Muscle. 

87. Konishi T: Electrophysiological Study on the 
Hypertrophied Cardiac Muscle Experimentally Produced in 

88. Kroll, D.A. and P.R. Knight: Antifibrillatory Effects 
of Volatile Anesthetics in Acute Occlusion/Reperfusion 

89. Luk, H.N., C.I. Lin, J. Wei, and C.L. Chang: 
Depressant Effects of Isoflurane and Halothane on 

90. Lynch C.: Differential Depression of Myocardial 
Contractility by halothane and Isoflurane in vivo. 

91. Lynch III, C., S. Vogel, and N. Sperelakis: Halothane 
Depression of Myocardial slow Action Potentials. 
Anesthesiology 55:360-368, 1981.

92. Malinconico, S.M., C.R. Hartzell, and R.L. McCarl: 
Effect of Calcium on Halothane-Depressed Beating in 
Heart Cells in Culture. Mol. Pharmacol. 23:417-424, 
1983.

on Cardiac Sarcoplasmic Reticulum Ca2+-ATPase at Low 

94. Maze, M. and C.M. Smith: Identification of Receptor 
Mechanism Mediating Epinephrine-induced Arrhythmias 
during Halothane Anesthesia in the Dog. Anesthesiology. 

Chedid, E. Fox, B. Summers and T.T. Wei: SHR:N-Mcc-cp 
Rat Substrain: General Characteristics and Congestive 

96. McDonald, T.F. and W. Trautwein: The Potassium Current 
Underlying Delayed Rectification in Cat Ventricular 

97. Merin, R., T. Kumazawa and C. Hoing: Reversible 
Interaction between halothane and Ca++ on Cardiac 
Actomyosin Adenosin Triphosphatase: Mechanism and 


120. Reynolds, A.K., J.F. Chiz, and T. Tanikella: On the


131. Stanfield, P.R: Tetraethylammonium Ions and the


141. Ten Eick, R. E., H. Gelband, M. Goode, A. Bassett: Increased Inward Rectifying Potassium Current in Cat Ventricle Subjected to Chronic Pressure Overload


