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OCCURRENCE, MECHANISMS AND DETERMINANTS OF PROARRHYTHMIA ASSOCIATED WITH CLASS I ANTIARRHYTHMIC AGENTS

by

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A thesis submitted to the Faculty of Graduate Studies and Research

Mc Gill University, Montreal

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for the degree of Doctor in Philosophy

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DEDICATION

I would like to dedicate my thesis

to my friend Richard, my mother, and my sisters Louise and Céline.

ACKNOWLEDGMENTS

I would like to express my gratitude to the following persons:

Dr. Stanley Nattel, my thesis supervisor, who has given me the opportunity to complete post-graduate studies in his laboratory. He has provided me with an intellectually stimulating miiieu and inspired me with standards of excellence for my research projects. Dr. Nattel has patiently encouraged me to think out scientific ideas and to address them thoroughly with the most pertinent experimental approaches. He has carefully reviewed and guided my work.

Dr. A. Claudio Cuello, the Chairman of the Department of Pharmacology and Therapeutics, who has followed my scientific progress and demonstrated interest in my work.

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I owe a great deal to some of the people in my laboratory at the Institut de Cardiologie de Montréal, not only for their valuable professional assistance but as friends: **Carol Matthews, Emma De Blasio and Christine Villemaire.**

PREFACE

Candidates have the option of including, as part of their thesis, the text of a paper(s) submitted or to be submitted for publication, or the clearly-duplicated text of a published paper(s). These texts must be bound as an integral part of the thesis.

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This thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation". **The thesis must include:** A Table of Contents, an abstract in English and French, an introduction which clearly states the rationale and objectives of the study, a comprehensive review of the literature, a final conclusion and summary, and a thorough bibliography or reference list.

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STATEMENT OF AUTHORSHIP

This thesis is composed of the following published papers:

Publication 1

Suzanne Ranger, Mario Talajic, Robert Lemery, Denis Roy, Stanley Nattel. Amplification of Flecainide-Induced Ventricular Conduction Slowing by Exercise. A Potentially Significant Clinical Consequence of Use-Dependent Sodium Channel Blockade.
Circulation 1989; 79:1000-1006.

Dr. Stanley Nattel proposed the original hypothesis and suggested the experimental approach. Dr. Mario Talajic, Dr. Robert Lemery and Dr. Denis Roy accepted to carry out a short pacing protocol during electrophysiologic testing of patients as part of the clinical evaluation of drug efficacy in treating ventricular tachycardia. Electrophysiologists also requested threadmill exercise testing for these patients. I developed the experimental design with Dr. Nattel, took the electrocardiographic recordings at fast paper speed during both procedures, reviewed patient file, collected clinical information and plasma drug levels, performed data tabulation and data analysis, and wrote a draft manuscript. Dr. Talajic participated in the discussion of the results and read the manuscript. Dr. Stanley Nattel helped in clarifying the mechanisms of action involved, suggested graphic representation of data and helped me to complete the final manuscript.

Publication 2

Suzanne Ranger, Mario Talajic, Robert Lemery, Denis Roy, Christine Villemaire, Stanley Nattel.
Kinetics of Use-Dependent Ventricular Conduction Slowing by Antiarrhythmic Drugs in Humans.
Circulation 1991; 83: 1987-1994.

Experimental design and protocols were similar to ones used for the first publication. Christine Villemaire assisted me in analyzing some of the data. My contribution and responsibilities were similar to those in the first paper, but I contributed more to hypothesis generation and study design because of a better understanding of the concepts and underlying mechanisms of action of antiarrhythmic drugs.

Publication 3

Suzanne Ranger, Stanley Nattel.
Determinants and Mechanisms of Flecainide-induced Promotion of Ventricular Tachycardia in Anesthetized Dogs.
Circulation 1995; 92: 1300-1311.

The initial ideas, design of studies and analysis of data were almost all my own, although Dr. Nattel's suggestions improved the quality and thoroughness of my work. I developed the necessary techniques and performed all experiments in a largely independent fashion. Results were discussed with Dr. Nattel who improved the writing of the discussion and conclusion of the paper.

Publication 4

Suzanne Ranger, Robert Sheldon, Bernard Fermini, Stanley Nattel.
Modulation of Flecainide's Cardiac Sodium Channel Blocking Actions by Extracellular Sodium: A Possible Cellular Mechanism for the Action of Sodium Salts in Flecainide Toxicity.
J Pharmacol Exp Ther 1992; 264: 1160-1167.

Having reported proarrhythmic effects in our first publication, I chose to investigate the mechanism of action of sodium salts associated with flecainide's toxicity using both electrophysiological and biochemical approaches. Dr. Nattel introduced me to the microelectrodes technique; I submitted hypotheses to test and experimental protocols to Dr. Nattel who made suggestions that helped in refining the questions I was addressing. I completed all experimental work with microelectrode techniques, data tabulation, analysis, graphic representation and Dr. Nattel help me with both the discussion and interpretation of the results. Dr. Nattel suggested that I pursue my investigations with a biochemical approach and arranged for a stay in Dr. Robert Sheldon's laboratory. I learned to isolate rat myocytes and to perform radioligand binding assays. I designed the protocols to assess the interaction between extracellular sodium concentration and flecainide's effect on [^{3}H]-batrachotoxinin 20α-benzoate, performed all the experimental work and data analysis. Dr. Paul Clark suggested a method of analysis for the radioligand binding data. In addition, I studied the effects of flecainide directly on the sodium current. Dr. Bernard Fermini introduced me to the whole-cell voltage-clamp technique. Dr. Nattel gave me some advice on the limitations of this method and on the possible experimental design. I performed all experimental work, data analysis, graphic representations and manuscript preparation. Dr. Sheldon and Fermini helped in reading the manuscript, Dr. Nattel finalized the manuscript.

Publication 5

Stanley Nattel, Suzanne Ranger, Mario Talajic, Robert Lemery, Denis Roy. Erythromycin-Induced Long QT Syndrome: Concordance with Quinidine and Underlying Cellular Electrophysiologic Mechanism.
Am J Med 1990; 89: 235-238.

I performed all experimental work using the microelectrode technique. Dr. Nattel suggested the protocol, provided the clinical data and prepared the final manuscript.

ABSTRACT

Antiarrhythmic drugs, designed to prevent or suppress cardiac arrhythmias, may cause the worsening of an arrhythmia already present in a patient or provoke new and qualitatively different arrhythmias. Cardiotoxic effects of antiarrhythmic drugs may be rate-related or due to intoxication, and may lead to serious and potentially lethal ventricular arrhythmias. The goals of my research were (1) to study the mechanisms by which class IC antiarrhythmic drugs cause ventricular arrhythmias and (2) to explain the mechanisms of action of sodium salts in the reversal of class IC cardiotoxicity.

We used flecainide (F) as a prototype of its class to study the mechanism of action of class IC antiarrhythmic agents (AA). Flecainide is a potent sodium channel blocker producing a major effect on conduction velocity and a minor effect on refractoriness. *In vitro* studies have shown that F causes frequency-dependent reduction of phase 0 upstroke of cardiac action potential (V_{max}) in ventricular tissue. Flecainide, in the physiologic range of heart rates and at clinically relevant concentrations, may produce rate-dependent effects because of its relatively slow binding and unbinding kinetics.

We studied the effects of F in humans during exercise and showed that F produces an enhanced slowing of conduction when heart rate is increased, because of use-dependent sodium channel blockade. We demonstrated that a variety of class I AA produce use-dependent QRS prolongation in man with characteristic kinetics, which are similar to the kinetics of V_{max} depression *in vitro*. We and others have reported proarrhythmic events associated with the rate-related cardiotoxicity of flecainide. Using a canine model of myocardial infarction, we showed the importance of previous myocardial infarction in flecainide-induced proarrhythmia. With epicardial mapping, we identified anisotropic reentry as the mechanism for the ventricular arrhythmias. We reported that reentry occurred in the infarct zone around an arc of conduction block in the transverse direction.

Cardiotoxicity associated with F includes severe conduction slowing and life-threatening ventricular arrhythmias. Sodium salts have been found to reverse the effects caused by some class I antiarrhythmic agents (AA), but the mechanism of action is unknown. Using electrophysiological and biochemical studies, we investigated the role of extracellular sodium concentration ($[Na^+_o]$) in modulating F's actions. In order to isolate the role of $[Na^+_o]$, we used a range of $[Na^+_o]$ and equimolar substitution with choline chloride. Our microelectrode experiments showed the ability of $[Na^+_o]$ to modulate directly F's effects on the phase 0 upstroke (V_{max}). Our radioligand studies of displacement of [^{3}H]-batrachotoxinin A 20α-benzoate ([^{3}H]-BTXB) binding showed that this interaction was due to an effect of $[Na^+_o]$ on the binding of F to its receptor. We found that EC_{50} values for depression of V_{max} in electrophysiologic experiments and IC_{50} values for flecainide displacement of [^{3}H]-BTXB in biochemical studies were highly correlated (r=0.99). A limitation of our electrophysiologic study was the use of V_{max} as an index of sodium current (I_{Na}). Using the whole-cell voltage clamp technique we found that increasing $[Na^+_o]$ opposed F's blocking effect on I_{Na}, confirming the role of $[Na^+_o]$.

These studies showed that the use-dependent blocking actions of class I AA are responsible for drug-induced conduction slowing *in vivo*. We demonstrated that F-induced proarrhythmia is due to an interaction determined by rate-dependency, underlying substrate and drug-induced slowing of conduction. Our findings of the modulation of F's cardiac sodium channel blocking actions by extracellular sodium give insight into the mechanisms by which sodium salts may reverse the toxic effects of sodium channel blockers.

FRENCH ABSTRACT

Les agents antiarythmiques (AA) destinés à prévenir ou à supprimer les arythmies cardiaques, peuvent aggraver une arythmie déja présente chez le patient ou provoquer une arythmie nouvelle et qualitativement différente. La carditoxicité associée aux AA peut être liée à la fréquence ou être due à l'intoxication. Les buts de ma recherche étaient (1) d'étudier les mécanismes par lesquels les AA de classe IC peuvent provoquer des arythmies ventriculaires et (2) expliquer les mécanismes d'action des sels sodiques dans le renversement de la toxicité causée par les agents antiarythmiques de classe IC.

Nous avons utilisé la flécainide (F) comme prototype de sa classe afin d'étudier le mécanisme d'action des AA de classe IC. La F est un puissant bloqueur du canal sodique; son effet est marqué sur la vitesse de conduction, et mineur sur la période refractaire. Des études *in vitro* ont démontré que la F cause une réduction liée à la fréquence de la phase 0 du potentiel d'action cardiaque (V_{max}) du tissu ventriculaire. A cause de la cinétique relativement lente de liaison et de libération à son récepteur, la F produit des effets liés à la fréquence dans l'écart physiologique de la fréquence cardiaque et à des concentrations cliniquement pertinentes.

Nous avons étudié chez l'homme les effets de la F cours de l'exercice et nous avons démontré que cet agent accroît le ralentissement de la conduction lorsque la fréquence cardiaque est augmentée, ceci étant dû au bloc occupation-dépendant du canal sodique. Nous avons démontré que qu'une variété d'AA de classe I produisent une prolongation occupation-dépendante du complexe QRS chez l'homme, avec des cinétiques caractéristiques, semblables à celles de la depression de V_{max} *in vitro*. Nous, ainsi que d'autres, avons rapporté des effets proarythmiques associés à la cardiotoxicité occupation-dépendante de la F. Utilisant un modèle d'infarcisation chez le chien, nous avons démontré l'importance de la présence de l'infarctus du myocarde dans la proarythmie causée par la F. A l'aide d'un système de cartographie épicardique, nous avons identifié la reentrée comme étant le mécanisme responsable des arythmies ventriculaires, et nous avons montré que la reentrée s'effectue dans la zone infarcisée autour d'un arc de bloc de conduction dans la direction transverse.

La cardiotoxicité associée avec la F comprend un ralentissement sévère de la conduction et la présence d'arythmies ventriculaires potentiellement létales. Les sels sodiques peuvent renverser les effets causés par certains AA de classe I, toutefois leur mécanisme d'action est jusqu'à maintenant inconnu. Nous avons étudié le rôle de la concentration extracellulaire de sodium ($[Na^+]_o$) sur la modulation des effets de la F à l'aide d'une approche biochimique et électrophysiologique. Afin d'isoler le rôle du $[Na^+]_o$, nous avons utilisé un écart de $[Na^+]_o$, ainsi qu'une substitution isosmotique du chlorure de sodium. Nos expériences utilisant des microélectrodes ont montré que la $[Na^+]_o$ produit une modulation directe des effets de la F sur la phase 0 (V_{max}) du potential d'action cardiaque. Nos études du déplacement de la liaison du [^{3}H]-20α-benzoate de batrachotoxin A ([^{3}H]-BTXB) montre que cette interaction est due à un effet de la $[Na^+]_o$ sur la liaison de la F à son récepteur. Nous avons trouvé une forte corrélation (r=0.99) entre les valeurs CE_{30} de la depression de V_{max} des études électrophysiologiques et les valeurs IC_{30} du déplacement du [^{3}H]-BTXB de nos études biochimiques. L'utilisation de V_{max} comme index du courant sodique (I_{Na}) représente une limitation de nos études électrophysiologiques. Nos expériences utilisant la technique de voltage imposé nous ont permis de montré que l'augmentation de $[Na^+]_o$ contraient le bloc causé par la F sur I_{Na} et confirmait le rôle de la $[Na^+]_o$.

Ces études apportent une meilleure compréhension des effets des AA de classe IC dus au bloc occupation-dépendant, des mécanismes de proarythmie et du renversement des effets toxiques par les sels sodiques associés à ces agents.

LIST OF ABBREVIATIONS

AA:	antiarrhythmic agent
SA node:	sinoatrial node (SAN)
AV node:	atrioventricular node (AVN)
K^+_e:	extracellular potassium
K^+_i:	intracellular potassium
Na^+_e:	extracellular sodium
Na^+_i:	intracellular sodium
Cl^-_e:	extracellular chloride
Cl^-_i:	intracellular chloride
Ca^{2+}_e:	extracellular calcium
Ca^{2+}_i:	intracellular calcium
V_{max}:	maximum rate of rise of phase 0, upstroke of the cardiac action potential
ECG:	electrocardiogram
EPS:	electrophysiological study
AP:	action potential
APD:	action potential duration
CV:	conduction velocity
ERP:	effective refractory period
MI:	myocardial infarction
LAD:	left anterior descending coronary artery
VT:	ventricular tachycardia
SVT:	sustained ventricular tachycardia
NSVT:	nonsustained ventricular tachycardia
VF:	ventricular fibrillation
EAD:	early afterdepolarization
DAD:	delayed afterdepolarization
I_{Na}:	transient inward sodium current
I_s:	slow inward current
$I_{Ca,T}$:	voltage-gated, short-lasting, transient inward calcium current
$I_{Ca,L}$:	voltage-gated, long-lasting, transient inward calcium current
I_K:	outward delayed rectifier potassium current
I_{Kr}:	fast component of delayed rectifier potassium current
I_{Ks}:	slow component of delayed rectifier potasssium current

I_{K1}:	inward rectifier background current
I_{Kur}:	ultra-rapidly activating delayed rectifier outward potassium current
4-AP:	4-aminopyridine
I_{to1}:	4-AP-sensitive, transient outward potassium current
I_{to2}:	Ca^{2+}-activated, transient outward current
I_f:	hyperpolarization-activated inward sodium and potassium current
I_{Cl}:	time-independent chloride current
$I_{Cl,cAMP}$:	cyclic AMP-regulated chloride current
$I_{Ca,Cl}$:	calcium-activated chloride current
$I_{Cl,swell}$:	swelling-induced chloride current
$I_{Cl,PKC}$:	protein kinase C-induced chloride current
$I_{Cl,purinergic}$:	purinergic ATP-activated chloride current
I_{KATP}:	ATP-regulated potassium current
I_{KACh}:	acetylcholine-regulated potassium current
I_{KNa}:	sodium-regulated potassium current
I_p:	Na^+-K^+ pump current
EC_{50}:	effective concentration for half-maximal response, *in vivo*
IC_{50}:	effective concentration for half-maximal response, *in vitro*
[^{3}H]-BTXB:	tritiated-batrachotoxin A 20α-benzoate

CONTENTS

CHAPTER I

INTRODUCTION

1. ELECTROPHYSIOLOGY OF NORMAL CARDIAC RHYTHM

1.1 Normal sequence of activation

The normal cardiac cycle includes both electrical and mechanical events but the electrical events precede and initiate the corresponding mechanical events. The electrical impulse that triggers a normal cardiac contraction originates at regular intervals in the sinoatrial node (SA node) which constitutes the normal pacemaker. This impulse propagates rapidly through the atria and enters the atrioventricular node (AV node), which is normally the only conduction pathway between the atria and the ventricles, and where a conduction delay of the electrical impulse is observed. The impulse then propagates via the His-Purkinje system to all parts of the ventricles. Conduction velocity in the AV node is estimated to be 0.02 to 0.05 m/sec in isolated rabbit and dog hearts (Hoffman et al, 1959; Spach et al, 1971), much less than in the atria where conduction velocity is estimated to be 0.9 to 1 m/sec (Wang et al, 1992), in the ventricles where values of 0.4 to 0.9 m/sec have been reported and in Purkinje fibers with values of 1 to 4 m/sec (Dominguez and Fozzard, 1970). A coordinated and hemodynamically effective contraction of the atria and ventricles requires a specialized electrical system which distributes the electrical impulse to the atrial and ventricular fibers in the proper sequence and at the proper time.

1.2 Ionic basis of membrane activity

1.2.1 Membrane potential

Cardiac cells have an intracellular composition that differs from the extracellular milieu. Each ion has a characteristic equilibrium potential (E) determined by its concentration gradient across the membrane.

$$E = \frac{RT}{zF} \ln \frac{[C]_o}{[C]_i}$$

where R = universal gas constant
T = absolute temperature
z = valence of the ion
F = Faraday's constant
$[C]_o$ and $[C]_i$ = extracellular and intracellular ion concentration

The transmembrane potential of cardiac cells at any time is determined by the concentration of several ions across the membrane and by the

permeability of the membrane to various ions and their equilibrium potentials. The Goldman-Hodgkin-Katz equation may be used to calculate the membrane potential:

$$E_{m} = \frac{RT}{zF} \ln \frac{(P_K \times K_o)+(P_{Na} \times Na_o)+(P_{Cl} \times Cl_i)+(P_{Ca} \times Ca_i)}{(P_K \times K_i)+(P_{Na} \times Na_i)+(P_{Cl} \times Cl_o)+(P_{Ca} \times Ca_o)}$$

where P_K, P_{Na}, P_{Cl}, P_{Ca} = membrane permeability for the respective ion

K_o, Na_o, Cl_o, Ca_o = extracellular potassium, sodium, chloride, calcium concentrations

K_i, Na_i, Cl_i, Ca_i = intracellular potassium, sodium, chloride, calcium concentrations

1.2.2 Regulation of ionic permeability

1.2.2.1 Ion channels

Ion channels are the main route by which ions diffuse through the membrane and represent the most important mechanism for the cardiac action potential. Most channels are relatively ion-specific and the flux of ions through them is thought to be controlled by gates (details in sections 1.4.2 and 3.4.2).

1.2.2.2 Pumps/carriers

Other mechanisms are present to maintain the ionic concentrations inside the cell at appropriate levels. There are at least two sarcolemmal ATP-dependent pumps: the Na^+-K^+ pump and the Na^+-Ca^{2+} exchanger. The Na^+-K^+ pump contributes indirectly to the transmembrane potential by maintaining the gradients necessary for diffusion through the channels; it generates a small outward current because during each cycle it transports three Na^+ ions out and two K^+ ions into the cell (Gadsby, 1984). Carriers, for their part, facilitate the exchange of ions or substrates, or pump them using energy. An example is the Na^+-Ca^{2+} exchanger system which exchanges one Ca^{2+} for 3 Na^+ (Mechmann and Pott, 1986; Sheu et al, 1986).

1.3 Cardiac action potentials

Cardiac cells of various parts of the heart possess unique electrical properties which may be best understood by studying transmembrane potentials. Transmembrane action potentials recorded from cells from

different parts of the heart possess a characteristic morphology, reflecting the role assumed by different parts of the heart, but also the varying presence and/or contribution of ion channels and pump/carriers involved in the generation of the cardiac action potentials. Action potential configurations within the atria, the ventricles, the sino-atrial node, the atrioventricular node, the His-Purkinje fibers each have their own characteristics (Figure 1). Experimental studies using standard microelectrode techniques suggest that different action potential configurations from different sites may reflect a varying contribution of specific ionic currents (Litovsky and Antzelelvitch, 1988 and 1989). For example, results of electrocardiographic, action potential, and whole-cell voltage-clamp recording from single epicardial cells correlated developmental changes in the 4-aminopyridine-sensitive transient outward current with modifications of the action potential and the QT interval (Jeck and Boyden, 1992). In 1993 Wang et al characterized in human atrial myocytes, different outward current patterns which corresponded to, and possibly accounted for, differences in action potential morphology (Wang et al, 1993).

1.3.1 Fast-channel tissues

Myocardial cells are frequently divided into slow- and fast-response fibers according to their action potential configuration and the propagation velocity of the action potential. Fast-response fibers are encountered in the normal working myocardium (atria and ventricles), as well as in the specialized conducting system of the heart (His-Purkinje fibers). Fast-channel tissue refers to tissue whose phase 0 characteristics result from the presence of sodium channels.

The relationship between the active membrane properties (the source) and the passive membrane properties (the sink) are the major determinants of conduction. In cardiac events, the safety factor may be defined as the difference between the source current needed to elicit successful propagation (the excess of source over sink). Fast-channel tissues, where response is an all-or-none phenomenon, possess a high safety factor, whereas slow-channel tissues have a low safety factor. Successful propagation in cardiac tissue depends on the rapid inward transient sodium current (I_{Na}) reflected by its rapid maximum rate of rise of phase 0. The movement of the ionic current through the sodium channel produces a local circuit current which may result in propagation of the action potential depending on the magnitude of the source and the characteristics of the sink. When this local circuit current is above threshold, propagation of

Figure 1

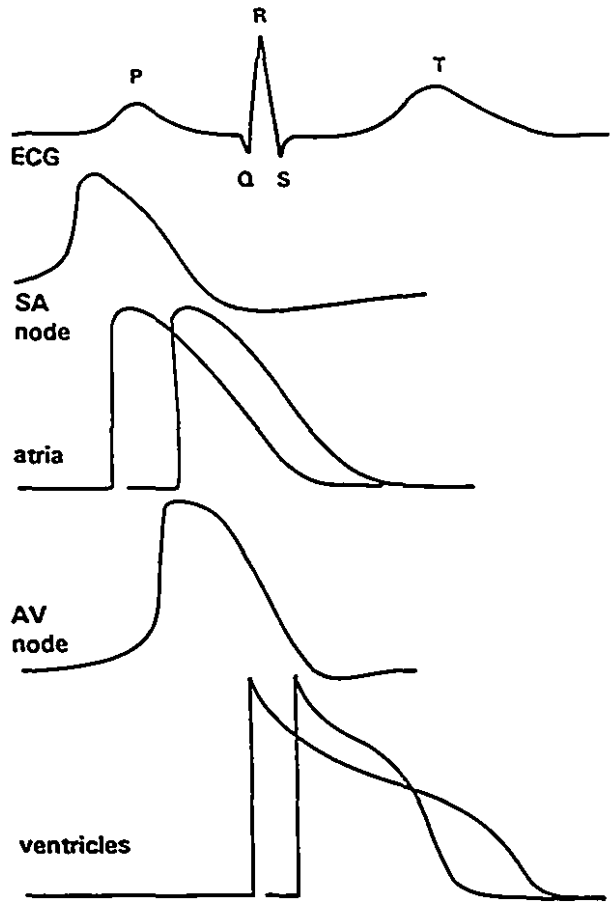


Figure 1: **Examples of action potentials from various parts of the heart.**
The upper trace is a standard lead II electrocardiogram (ECG) for time reference. The first action potential of the cardiac cycle is that of the sinoatrial node (SA). The next action potentials are those of the atria, generating the P wave of the ECG. Next is the atrioventricular node (AV) action potential. Finally, the ventricular action potentials produce the QRS on depolarization and the T wave on repolarization. The action potential duration of the ventricular cells is nonuniform.

Adapted from:

Fozzard HA, Ansdorf MF. Cardiac Electrophysiology. In The Heart and Cardiovascular System, Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE. (1986), Raven Press, New York.

Fozzard HA. Electrophysiological basis of arrhythmias. In Acute Cardiac Care, edited by D.S.DasGupta, Yearbook, Chicago.

the action potential occurs. Source may be measured indirectly by the maximum rate of rise of phase 0 of the fast-response action potential (V_{max}). The relationship between V_{max} and conduction velocity will be discussed later (section 1.4.1). The source may also be measured directly with the use of the voltage clamp technique, although non-physiological conditions (eg. reduced temperature and altered ionic compositions) must be used in order to adequately control I_{Na}.

Driving force and resultant current is greater in fast- than in slow-response cells, and the reactivation process is also faster in the former. Upon depolarization of fast-response tissue the sodium channels become inactivated, making the channels unavailable for reactivation during a period of time called the refractory period. In fast-response tissues, recovery from inactivation is faster than in slow-response tissues, and action potential duration (APD) is the major determinant of refractoriness (since duration of the action potential plateau is the main determinant of the time required for the transmembrane voltage to return to the level at which I_{Na} can be reactivated).

1.3.2 Slow-channel tissues

Slow-response cells are normally present only in the SA and AV nodes. Slow-response cells are activated via a current which has been called slow inward current (I_{si}), generated through calcium channels (probably I_{Ca-L}). As opposed to fast-response cells, slow-response cells have a low safety factor. Consequently, conduction is not an all-or-none phenomenon and impulses with reduced strength may slow down and eventually fail to propagate (decremental conduction). In slow-response tissues, recovery from inactivation is slow, and unlike fast-response tissues, slow recovery from inactivation of the calcium channels represents the main determinant of refractory period. When the action potential is shortened, the refractory period is less affected and because it outlasts the duration of the action potential, post-repolarization refractoriness may be observed.

1.3.3 Phases of the cardiac action potential

Cardiac cells undergo an orderly sequence of depolarization and repolarization: 5 phases of the action potential have been described (Figure 2).

Figure 2

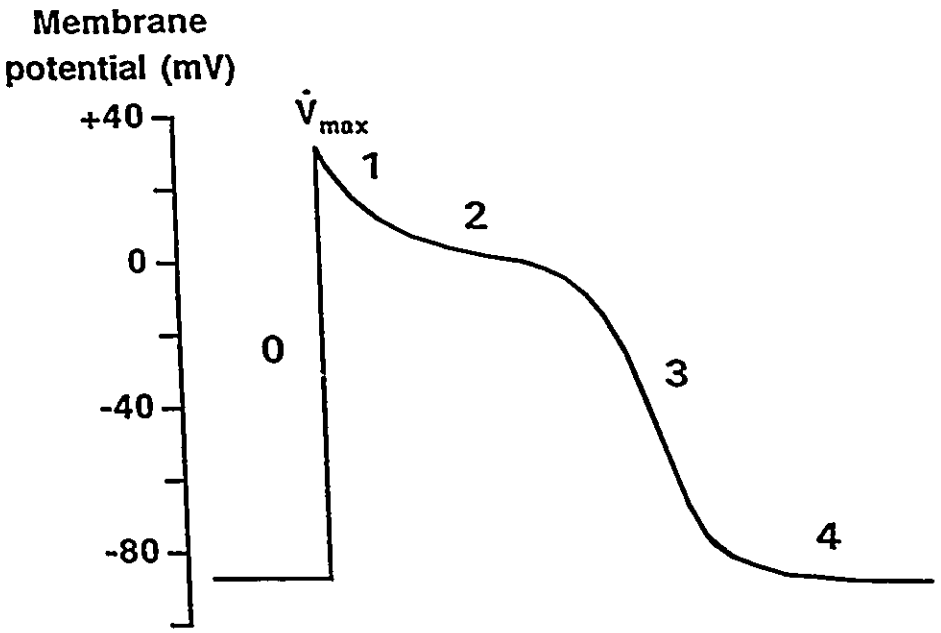


Figure 2: Schematic representation of cardiac ventricular action potential. Phase 0, upstroke; phase 1, a brief period of repolarization; phase 2, the plateau phase; phase 3, a rapid repolarization; phase 4, diastolic period. (refer to text for more details).

1.3.3.1 Phase 4

During electrical diastole (phase 4), the membrane is much more permeable to potassium than to any other ions. Resting membrane potential is caused by the difference in membrane permeabilities to sodium and potassium and to the difference in intracellular and extracellular concentrations of these ions. In normal Purkinje and ventricular cells, resting membrane potential is around -90 mV, approaching the potassium equilibrium potential (E_K = - 96 mV, for $[K^+]_o$ = 4 mM and $[K^+]_i$ = 150 mM). In the cells of the SA node, spontaneous diastolic depolarization occurs during phase 4 and is responsible for initiating the impulse that propagates through the heart. Diastolic depolarization is generated by a time-dependent process incorporating an increase in inward current(s), a decrease in outward current(s), or both. The ionic currents that appear to contribute to phase 4 depolarization are 1) an inward current through a relatively non-selective cation channel (I_f) which is activated by hyperpolarization and found in the sinoatrial node (SA node) and in Purkinje fibers (Brown and DiFrancesco, 1980; DiFrancesco and Ojeda, 1980; DiFrancesco, 1981), and 2) a background, inwardly rectifying potassium current (I_{K1}) (DiFrancesco, 1981). $I_{Ca,T}$ is a voltage-gated Ca^{2+} current that is activated at relatively negative potentials (eg. -80 mV). The T-type calcium current is relatively short-lasting and contributes to impulse initiation (Tseng and Boyden, 1989). $I_{Ca,L}$ is relatively large and long-lasting and flows through a different voltage-dependent calcium channel (Tsien et al, 1987). $I_{Ca,L}$ can be activated from a relatively depolarized threshold potential (Hirano et al, 1989) and produces depolarization and propagation in SA and AV node. When the cell reaches its threshold potential a spontaneous action potential is generated. The current through the L-type channel inactivates slowly and the time course of its inactivation is a major determinant of the rate of repolarization during the plateau. This rate of inactivation is voltage-dependent and depends on Ca^{2+}_i concentration (Tsien et al, 1987).

1.3.3.2 Phase 0

In fast-channel tissues, the phase 0 of the cardiac action potential is due to the rapid entry of sodium ions from the extracellular space into the cell (Hille, 1986). The sudden influx of positive charges into the cell brings the transmembrane potential to positive values (+20 to +40 mV). This represents the upstroke of the cardiac action potential. The large underlying inward current (mostly I_{Na}) results in depolarization of

adjacent tissue and is responsible for the fast propagation of the cardiac impulse in the longitudinal direction. In the cells of the atrial and ventricular myocardium and the His-Purkinje system, the sodium current is responsible for the action potential upstroke, while the calcium current, which is turned on by sodium-dependent depolarization, contributes to the amplitude of the upstroke. The sodium channel is critical to the genesis of action potentials in working myocardium.

1.3.3.3 Phase 1

During phase 1, the sodium conductance rapidly decreases due to the inactivation of sodium and calcium channels and K^+ conductance increases. There are two components of the cardiac transient outward current (I_{to}), the 4-aminopyridine sensitive current (I_{to1}) (Giles and Imaizumi, 1988; Tseng and Hoffman, 1989; Hiraoka and Kawano, 1989; Zygmunt and Gibbons, 1991) and Ca^{2+}-activated transient outward current (I_{to2}), carried by chloride (Zygmunt and Gibbons, 1991). Recently, I_{to2} has been shown to be responsible for the initial rapid phase 1 repolarization at physiologic heart rates in the rabbit (Wang et al, 1995).

1.3.3.4 Phase 2

Phase 2 represents the plateau of the action potential in fast channel tissue. During the plateau phase there exists a fine balance between individual currents. An increase in the membrane conductance for Ca^{2+} keeps inward and outward currents relatively equal during the plateau. This phase is important in the excitation-contraction process; it also determines the refractory period. The Purkinje fibers possess the longest phase 2 among cardiac cells. Under physiologic conditions, inward currents during phase 2 may be carried by sodium through a sodium window current (Attwell et al, 1979) or a slowly inactivating sodium current (Gintant et al, 1984), by calcium through the L-type Ca^{2+} channel (Kass and Tsien, 1976) or a calcium window current (Cohen and Lederer, 1987; January and Riddle, 1989) and by electrogenic Na^+-Ca^{2+} exchange (Glitsch et al, 1970 and 1982; Kass et al, 1978a and 1978b). The L-type channel is postulated to participate in the maintenance of the action potential plateau (Kass and Tsien, 1976). During the plateau, some outward current may be carried by a slowly inactivated component of I_{to}.

1.3.3.5 Phase 3

Phase 3 corresponds to the rapid repolarization caused by a decrease in the inward Na^+ and Ca^{2+} currents and an increase in outward K^+ current. Repolarization occurs as the net current becomes more outward during phase 3, until membrane resting potential is reached. The currents involved in the transition between phase 2 and 3 include the time- and voltage-dependent decay of $I_{Ca\text{-}L}$ (Hirano et al, 1989), the Na^+-K^+ pump current (I_p), the time-dependent increase of the outward delayed rectifier potassium current (I_K) (Wang et al, 1993), and an increase in the conductance of the inward rectifier potassium current (I_{K1}).

1.4 Electrophysiology of the sodium channel

1.4.1 V_{max} measurement

Some properties of the sodium channel may be deduced from the action potential. The maximum rate of rise of phase 0 (V_{max}) of the fast-response action potential has been used as an index of sodium current in cardiac cells, where the total ionic current across the cell membrane at the time of V_{max} consists mainly of sodium current. Important concepts of the interaction of antiarrhythmic drugs with the sodium channel have been studied using V_{max} as an index of I_{Na} (Hondeghem and Katzung, 1977). V_{max} accurately reflects the net maximal inward current contributing to the depolarization of a given action potential, in uniformly depolarized tissues or in single cells in appropriate ionic and physiologic conditions (Cohen et al, 1984). However, the interpretation of V_{max} measured in multicellular preparations may be more complex. Under certain conditions, a nonlinear relation exists between V_{max} and I_{Na}, and then the measurement of the maximum rate of rise of phase 0 of action potential may not be used as a reliable method for quantitative studies of sodium channel behavior (Sheets et al, 1988).

1.4.2 Measurement of sodium current

I_{Na} may be measured directly with the voltage-clamp technique. A current is applied across the cell membrane to clamp its potential at a desired level. This current is equal and opposite to the instantaneous membrane current, and can be used to infer the membrane current. The development of techniques for enzymatic dissociation of adult cardiac tissue to obtain single cells has allowed the application of new approaches to voltage-

clamp studies of single cells (Hamill et al, 1981).

Hodgkin and Huxley were the first ones to demonstrate that a transient inward current was responsible for action potential propagation (Hodgkin and Huxley, 1952). They demonstrated that the sodium channel in squid giant axons can be characterized by three states during the action potential: the rested state, during which the channel is closed but available for activation; the activated state, during which the channel is open and sodium ions move inward; and the inactivated state, during which the channel is closed and unavailable for activation. The sodium channel cycles from rested, to activated and to inactivated states. Under normal conditions, the membrane is fully polarized and the sodium channels are predominantly in the rested state. During the upstroke of the action potential, the membrane becomes depolarized and most of the channels move to the activated state. Sodium ions are allowed to flow rapidly across sodium channels. The current then rapidly decays as most of the sodium channels move to the inactivated state. The concept of a channel "gate" was also introduced by Hodgkin and Huxley. Their mathematical formulation describing the behavior of the sodium channel involved a gating mechanism resulting in a time- and voltage-dependent behavior of ionic current. They defined sodium conductance (g_{Na}) as follows:

$$g_{Na}(V, t) = \sigma \overline{g_{Na}}$$

where g_{Na} = sodium conductance

$\overline{g_{Na}}$ = maximal value of g_{Na} achievable

σ = fraction of the sodium channels open, determined by the probability that a channel is open.

During the resting state, very few of the sodium channels conduct. As the membrane depolarizes the fraction of channels which are open increases. The response to an instantaneous voltage change does not occur instantaneously. A certain period of time is needed for the mechanism to change to a new steady state. The gating mechanism thus introduces both voltage- and time-dependence into the ionic current.

In the Hodgkin-Huxley model the opening process results from a movement of three independent channel-blocking elements, each having the probability

m to be in the position for the channel to be open. The closing process results from a single blocking element, having the probability h to be in the unblocked position. The variable h describes the inactivation process. The probability that the three m gates and one h gate are in a position for a channel to be open is m^3h, so the above equation becomes:

$$g_{Na} = \overline{g_{Na}}\ m^3h$$

Provided that the kinetics describe adequately the voltage- and time-dependence of the ionic current, the model may be used to understand how the ionic current changes during normal activity.

The Hodgkin-Huxley model resulted from the analysis of kinetics of the gating mechanisms in the squid nerve membrane. There is evidence that the functional behavior of the sodium channel in the heart is not completely described by this model. The behavior of the sodium channel has been reviewed by Hille in 1986, and Fozzard et al in 1983, and is based on the rates of activation and inactivation of sodium channels. In nerve the recovery from inactivation occurs at a speed similar to that of the inactivation process; recovery occurs within a few milliseconds. In cardiac tissue, inactivation of sodium current occurs in a few milliseconds at voltages corresponding to the action potential plateau. However, there may be some overlap of the activation and inactivation curves under certain circumstances, so that inactivation may be incomplete and the steady state sodium conductance may remain higher at some depolarized potentials than at resting potential (Gettes and Reuter, 1974; Brown et al, 1981). The time course for development of and recovery process occurs with two time constants in single rat myocytes, a fast (a few milliseconds), and a slow component (Brown et al, 1981; Makielsky, 1987). The slow component of inactivation is thought to be due to the reopening of inactivated channels (Kunze et al, 1985).

The single channel behavior of sodium channels accounts for the properties of macroscopic I_{Na}. The time course of I_{Na} is determined primarily by the product of the time-to-opening (latency) of the channels and the mean open time. The magnitude of I_{Na} is the result of four factors: 1) the fraction of channels available for opening at the onset of the voltage step 2) the fraction of those channels that open versus those inactivating without opening 3) the synchrony of opening and 4) the single channel current (Makielski et al, 1987).

2. CARDIAC ARRHYTHMIAS

Although the incidence of sudden cardiac death has decreased in parallel with an overall reduction in cardiovascular mortality, it remains a major cause of mortality in the United States (Gillem, 1989). The most common arrhythmias causing sudden death are ventricular tachycardia and ventricular fibrillation, and these are likely to occur in the setting of ischemia and infarction.

2.1 Mechanisms of cardiac arrhythmias

A general classification of mechanisms of cardiac arrhythmias was formulated in the sixties and seventies, and suggested that cardiac arrhythmias result from abnormal impulse generation, abnormal impulse conduction, or a combination of both mechanisms (Hoffman, 1960; Hoffman and Cranefield, 1964; Wit et al, 1974a and 1974b). This simple scheme was developed further with the concept of triggered arrhythmias, resulting from studies performed on abnormal electrical activity of Purkinje fibers in the AV valves and coronary sinus (Wit and Cranefield, 1976; Wit and Cranefield, 1977). Arrhythmias due to abnormal impulse generation include rhythms resulting from either normal or abnormal automatic mechanisms and from triggered activity. Reentry and reflection are mechanisms of cardiac arrhythmia involving abnormal impulse conduction. Finally parasystole is subclassified in the category of mechanisms due to both abnormalities of impulse generation and conduction (Zipes, 1983).

2.1.1 Automaticity

Automaticity is the property resulting in regular, spontaneous activity of the heart. The normal pacemaker impulse originates from automaticity in the SA node, whose rapid intrinsic rate is dominant over the other regions of the heart (Purkinje system, and other specialized conduction systems in the atria and atrioventricular junction) which also have the ability to sustain an automatic rhythm. Automaticity depends on spontaneous phase 4 depolarization (see above). The functional determinants of spontaneous automaticity are the maximum diastolic potential, the rate of phase 4 depolarization, the threshold potential and the action potential duration. In the category of arrhythmias due to abnormal impulse generation, disturbances of rhythm result either from a normal mechanism for impulse generation such as the one normally present in the His-Purkinje system, or from some abnormal mechanism: the former are referred to as "enhanced automaticity", and the latter as "abnormal automaticity".

2.1.1.1 Enhanced automaticity

Normal automaticity in Purkinje fibers may contribute to ventricular arrhythmias when the spontaneous rate is enhanced by catecholamines (eg. during acute ischemia) (Gilmour et al, 1986; Friedman et al, 1973a and 1973b; Lazzara et al, 1973 and 1974). When a reduction of membrane potential brings this value closer to threshold potential (eg depolarization from -90 mV to -75 mV) this may increase the spontaneous rate of depolarization. *In vivo* studies have suggested that some forms of premature ventricular beats and ventricular tachycardia following myocardial infarction may be due to enhanced automaticity in subendocardial Purkinje fibers (Hope et al, 1976; Horowitz et al, 1976; Scherlag et al, 1974).

2.1.1.2 Abnormal automaticity

Abnormal automaticity may be present in diseased cardiac tissues such as in the early phase of acute ischemia (Euler et al, 1981). In 1975, Katzung et al demonstrated that the flow of current across the border between ischemic and normal myocardium might contribute to the genesis of ectopic activity (Katzung et al, 1975). The idea that ectopic activity is caused by injury current is supported by studies on arrhythmias caused by embolization of a coronary artery with latex (Euler et al, 1981). Injury currents may flow through an inexcitable segment or depolarized cells interposed between ischemic cells and normal cells which have repolarized, and reexcite the normal cells to cause premature depolarizations. When the injury current is of sufficient magnitude it may result in single or multiple premature beats (Janse et al, 1982). Evidence from studies in the canine heart one to four days after myocardial occlusion suggests that although delayed afterdepolarizations occur at 24 hr after infarction, the predominant rhythm appears to be abnormal automaticity (LeMarec et al, 1985).

2.1.2 Triggered activity

Triggered rhythms are thought to result from either early or delayed afterdepolarizations (Cranefield, 1977). Triggered activity requires an impulse to initiate the abnormal activity. The basis for triggered activity is an afterdepolarization or an oscillation of the membrane potential that occurs near the time that the cell is repolarizing. The oscillations occurring during phase 2 or 3 of the cardiac action potential are known as early afterdepolarizations (EADs) (Roden and Hoffman, 1985)

and those occurring just after repolarization or during phase 4 of the cardiac action potential are known as delayed afterdepolarizations (DADs) (Ferrier et al, 1973; Henning and Wit, 1984; January and Fozzard, 1988) (Figure 3). When EADs or DADs are sufficiently large to depolarize cells to their threshold potential, they result in spontaneous action potentials referred to as triggered activity (Wit and Rosen, 1986). EADs, DADs and triggered activity are thought to be responsible for a variety of tachyarrhythmias occurring in different conditions.

2.1.2.1 Early afterdepolarizations

EAD-induced triggered activity is generally cycle length dependent (Damiano and Rosen, 1984) and associated with slow pacing or a long pause (Cranefield and Aronson, 1988; Rosen, 1990). Drug-induced increases of action potential and EAD's are favored by low $[K^+]_o$ and slow rate (Dangman and Hoffman, 1981; Roden and Hoffman, 1985). Drugs, such as quinidine (Roden and Hoffman, 1985; Roden et al, 1986; Davidenko et al, 1989), N-acetyl procainamide (Dangman and Hoffman, 1981), cesium (Brachmann et al, 1983; Damiano and Rosen, 1986) and type III antiarrhythmic agents (Gough and El-Sherif, 1989), increase action potential duration and may cause EAD activity. Drug-induced APD prolongation may result from I_K inhibition (Carmeliet, 1985; Colatsky, 1982; Salata and Wasserstrom, 1988; Roden et al, 1988). Slow rate may act by reducing the Na^+-K^+ pump current (I_p) (Gadsby and Cranefield, 1977) and the low $[K^+]_o$ reduces the conductance of specific currents such as the inward rectifier (I_{K1}) and the delayed rectifier (I_{Kr}) (Zeng and Rudy, 1995).

Requirements for EAD-induced activity include: first, a critical prolongation of the repolarization phase, implying a reduction of the net outward current which may result from a decrease in outward current(s), an increase of inward current(s) or a combination of both; second, a net depolarizing current carrying the charge for the EAD; and finally sufficiently large EAD(s) to cause propagation to excitable tissue and causing an extrasystole or a tachyarrhythmia.

The depolarizing current underlying EAD results from an imbalance between the inward and the outward currents during phase 3. Three different channels may be involved: the Na^+ channel, the Ca^{2+} channel or Na^+-Ca^{2+} exchange. The specific ionic current responsible for depolarization during triggered activity due to EAD depends on the level of membrane potential at the onset of the EAD.

Figure 3

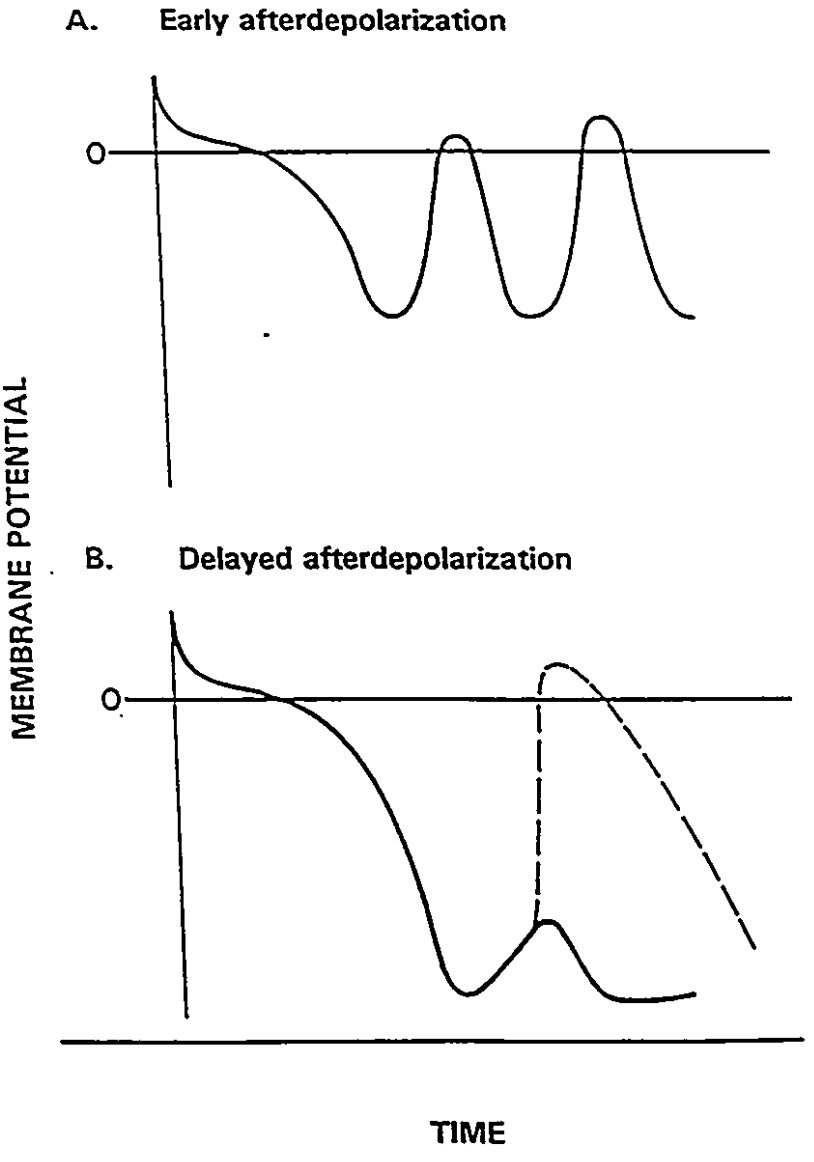


Figure 3: Schematic representation of early (A) and delayed afterdepolarization (B).

During the plateau phase and early phase 3, most Na^+ channels are inactivated and Ca^+ channels are more likely to generate slow inward current responsible for secondary depolarization. EAD's may thus be due to a time- and voltage-dependent recovery from L-type calcium channel from inactivation or reactivation (Nattel and Quantz, 1988; January and Riddle, 1989; Zeng and Rudy, 1995).

At more repolarized membrane potential, Na^+ channels are partially reactivated and may generate EAD's. The electronic Na^+-Ca^{2+} exchange may also induce oscillations at the plateau level (Szabo et al, 1987).
Another mechanism of action involves an impairment in I_{Na} inactivation, as in the case of aconitine (Peper and Trautwein, 1967), batrachotoxin (Brown, 1983) and the sea anemone toxins anthopleurin-A and ATX-II (El-Sherif, 1988). These drugs delay I_{Na} inactivation and prolong repolarization by increasing the Na^+ window current (Attwell, 1979) or a slowly inactivating Na^+ current (Gintant et al, 1984). An inward shift in the current-voltage relationship during the plateau phase results in delaying repolarization and provides favourable conditions for a secondary depolarization.

2.1.2.2 Delayed afterdepolarizations

Delayed afterdepolarizations (DADs) usually occur in the presence of conditions which increase $[Ca^{2+}]_i$. Agents which increase directly, or lead to increases in intracellular $[Ca^{2+}]$, may cause DAD's. Cardiac glycosides may cause DADs by inhibiting the Na^+-K^+ pump (Ferrier et al, 1973; Lee and D'Agostino, 1982). Kline and Kupersmith (1982) showed that during triggered activity in atrial fibers of the canine coronary sinus, changes in membrane potential paralleled extracellular potassium activity recorded with a K^+-selective microelectrode. As triggered activity is initiated, they reported an initial phase during which the rate is increased, in association with membrane depolarization and an increase in $[K^+]_o$. Depolarization is most likely caused by an accumulation of potassium in the extracellular space resulting from fast pacing or rapid rate which exceeds the pumping capacity of the Na^+-K^+-ATPase (Kline and Kupersmith, 1982; Kline et al, 1982). Triggering is facilitated by exposure to low level of norepinephrine (Wit and Cranefield, 1977).

DADs can occur in surviving Purkinje fibers of transmural infarcts (El-Sherif et al, 1983), and elevated intracellular calcium concentration resulting from ischemia appears to play a role in DAD generation (Kimura

et al, 1984). One to four days post-infarct, automatic rhythms are not usually observed but DADs and triggered activity can be induced by pacing (LeMarec, et al, 1985). The occurrence of DADs appears to be dependent on the size of infarct, the membrane potential of the surviving Purkinje fibers and temperature of the superfusate.

The oscillatory membrane current underlying DADs is referred to as the transient inward (TI) current (Lederer and Tsien, 1976). TI current is distinct from the pacemaker current and other membrane currents occurring during the action potential. Simultaneous recordings of action potential and TI current during delayed afterdepolarization has shown that both the time course and magnitude suggests TI current as a cause for DAD's (Lederer and Tsien, 1976). It is still debated whether the TI current flows through gated membrane channels. Kass et al found a reversal potential for the TI current which was shifted as the concentration of a potential charge carrier (Na^+) was changed (Kass et al, 1978b). In other experiments on atrial fibers and isolated myocytes, a clear reversal potential was not seen (Tseng and Wit, 1984; Lipp and Pott, 1987), suggesting that the membrane current is rather caused by electrogenic Na^+-Ca^{2+} exchange, carrying Ca^{2+} into the cell (Mechmann and Pott, 1986; Lipp and Pott, 1987).

2.1.3 Parasystole

Abnormal automaticity may also occur in a region of tissue that is protected from being discharged by a zone of unidirectional block; such a region is referred to as a parasystolic focus (Langendorf and Pick, 1967). Impulses propagate out from this site to other regions of the heart, but do not propagate back into the site. Langendorf and Pick (1967) showed that the ectopic beats resulting from this type of abnormal automaticity occur at regular intervals which would be some multiple of a common denominator. Jalife and Moe (1976 and 1979) varied the coupling interval between automatic foci and demonstrated that electrotonic currents may prolong or shorten the period of a spontaneously beating pacemaker depending on its timing with respect to the period of the pacemaker. They concluded that the periodicity of a parasystolic pacemaker may be strongly influenced by the electrical events occurring in the surrounding tissue. Moe et al (1977) demonstrated that it is possible for the coupling to be constant between normal and ectopic beats, and for the cycle length between ectopic beats to be variable. For this reason it may be difficult to distinguish on the body surface electrocardiogram (ECG) an arrhythmia

resulting from parasystolic, triggered or reentrant activity.

2.1.4 Reentry

Reentry is a mechanism responsible for a variety of cardiac arrhythmias that can occur in all parts of the heart: the SA node, the atria, the atrioventricular junction, the AV node, the Purkinje system and the ventricles. In the context of this work, reentry will be reviewed principally in the ventricles and in conditions of ischemia and infarction of the myocardium. Reentry occurs when an impulse reenters and excites an area of the heart more than once (circus movement).

2.1.4.1 Model of circus movement reentry around an anatomic obstacle

Several variations of reentry around an anatomically defined circuit have been presented, but the traditional concept of circus movement reentry is based on experiments conducted by George Mines (Mines, 1913 and 1914). He formulated three criteria for determining the presence of reentry: evidence of unidirectional block, circus movement and termination by disruption of the reentrant circuit. He also hypothesized that conditions for an impulse to circulate in a reentrant circuit were probably met in certain circumstances and that reentry was most likely responsible for various arrhythmias in man.

2.1.4.1.1 Unidirectional block

One of the main elements associated with circus movement reentry is a region of functionally determined unidirectional block, necessary for the initiation of reentry. In his experiments using a ring made out of atrial and ventricular tissue from a turtle heart, Mines (Mines, 1914) introduced a premature electrical stimulus so that it would encounter some tissue still in its refractory period. The presence of an area of unidirectional block allows the impulse to propagate in only one direction.

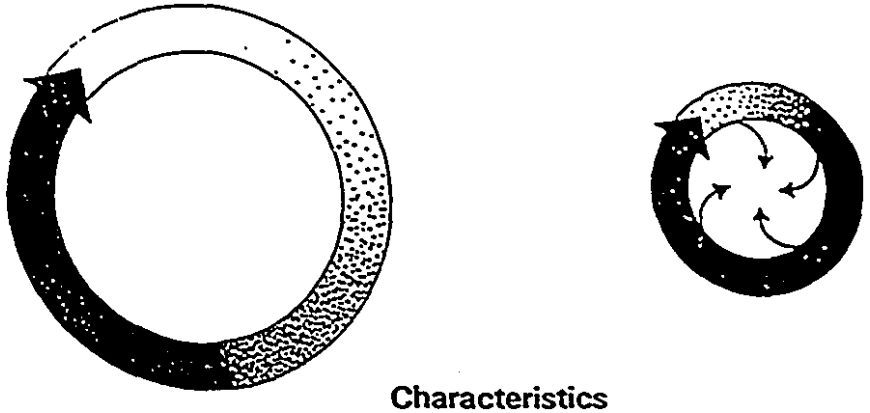
2.1.4.1.2 Reentrant circuit

Block in the ventricles may result from the large morphological heterogeneity present in the cardiac tissue. In atria, the impulse may find an anatomical obstacle in its path, possibly the orifice of the vena cava or a pulmonary vein. The anatomical obstacle then defines a pathway allowing the propagation of the excitation wave in a circus movement fashion (Figure 4 A). In this model the length of the reentrant circuit is

Figure 4

A. Model of circus movement reentry around an anatomical obstacle (Mines)

B. Model of the leading circle



Characteristics

	A. Model of circus movement reentry around an anatomical obstacle (Mines)	B. Model of the leading circle
1.	Anatomically defined circuit, fixed length of circuit	Functionally defined circuit, circuit length dependent on electrophysiological properties λ = CV x ERP ↓ CV → smaller circuit ↓ ERP → smaller circuit ↑ CV → larger circuit to sustain reentry
2.	Excitable gap between head and tail of propagating impulse (white part) Tail of relative refractoriness (dotted) Termination, entrainment, or resetting of reentrant rhythm is possible with impulses originating outside the reentrant circuit	No gap of full excitability between head and tail of propagating impulse Tail of relative refractoriness (dotted) Influence by impulses originating outside the reentrant circuit is not possible
3.	Revolution time is inversely related to conduction velocity Rate = $\frac{\text{conduction velocity}}{\text{length of circuit}}$ ↓ ERP does not affect the rate of the circus movement	Rate is inversely related to the ERP Rate σ $\frac{1}{\text{refractory period}}$ ↓ ERP → smaller circuit and revolution time, acceleration of the reentrant rhythm ↑ ERP → larger circuit, deceleration or termination of the reentrant circuit

Adapted from:

Allessie MA, Bonke FIM, Schopman FJG. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: A new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. Circ Res 1977; 41: 9-18.

fixed around the anatomic obstacle. Because of the technical limitations of the recording systems used by Mines, mapping of the electrical activity in the pathway was not possible.

An important feature of this model is the presence of an excitable gap (white part of the circle in figure 4 A) between the head of the excitation wave (black arrow in figure 4) and its tail of relative refractoriness (dotted region of the circle in figure 4 A). In the presence of an excitable gap, impulses originating outside the reentrant circuit may enter it and influence the reentrant rhythm, so that termination of tachycardia or resetting and entrainment may occur. In his experiments, Mines observed that a critically timed extrastimulus terminated the circus movement (Mines, 1914).

The conduction time of the impulse around the circuit must be long enough to allow each part of the circuit to regain its excitability by the time for the next impulse to reexcite the area. In this model, the rate is determined by the length of the circuit and the average conduction velocity of the circulating impulse. The rate of the reentrant rhythm is proportional to the inverse of revolution time, and therefore is equal to conduction velocity over the length of the circuit (Figure 4 A). In this model, shortening of the refractory period will not affect the rate of the circus movement. Shortening of the refractory period will result in a larger excitable gap, whereas an increase of the refractory period will reduce the excitable gap without affecting the rate of the reentrant rhythm. With a marked prolongation of the refractory period, the propagating wavefront will encounter a region of refractoriness, resulting in either a slowing of conduction or termination of the reentrant rhythm.

2.1.4.2 Model of the leading circle

Reentry may occur, not only in an anatomically defined pathway, but also when the pathway for altered propagation is defined by functional properties or changes in properties of the tissues. In 1973, Allessie and his collaborators provided the first direct experimental evidence that the presence of an anatomical obstacle is not essential for the initiation or maintenance of reentry. In this model of reentry, the pathway for the circulating impulse is not fixed but depends on the electrophysiological properties of the myocardium involved (Figure 4 B). The length of the reentrant circuit is given by the wavelength of the impulse, defined as the distance travelled during a period of time equal to the refractory period. The wavelength corresponds to the product of conduction velocity

(CV) times the effective refractory period (ERP) (λ = CV x ERP) (Wiener and Rosenblueth, 1946). In the absence of an obstacle, the dimensions of a reentrant loop will be determined by the shortest possible route in which the impulse can continue to circulate (the wavelength) (Allessie et al, 1976). The leading circle is defined as "the smallest possible pathway in which the impulse continues to circulate, and in which the stimulating efficacy of the wavefront is just enough to excite the tissue ahead which is still in its relative refractory phase" (Allessie et al, 1977).

Shortening of the functional refractory period or slowing of conduction velocity will result in a smaller reentrant circuit, whereas when the refractory period is long or if conduction velocity is rapid, the dimensions of the reentrant circuit to sustain circus movement become larger. Changes in some basic electrophysiological properties affect the revolution time of a leading circle tachycardia. For example, a decrease in the stimulating efficacy (mainly determined by the amplitude and upstroke velocity of the action potential) result in the lengthening of the revolution time of the leading circle, as experimentally demonstrated with the application of tetrodotoxin (TTX), which resulted in a decrease in the rate of the tachycardia (Allessie et al, 1977). A decrease in the refractory period caused by the addition of carbachol (carbachol shortens APD by activating I_{KAch}) resulted in a shortening of the revolution time and in an increase in the rate of tachycardia.

In this model there is no gap of full excitability because the stimulating efficacy of the propagating impulse is such that the tissue ahead is in its relative refractory period. Reentrant tachycardia based on the leading circle model is not thought to be influenced by premature impulses initiated outside the reentrant circuit. In the absence of an excitable gap, because the head of the impulse impinges on the relative refractory tail of the previous wave, the circulating impulse wave travels through partially refractory tissue, and therefore conduction velocity is reduced.

2.1.4.3 Factors promoting reentry

2.1.4.3.1 Nonuniform recovery of excitability

The induction of reentry requires the creation of a region of unidirectional block which may be due to nonuniform dispersion of recovery. Reentry can occur around lines of block in regions with nonuniform dispersion of refractoriness (Gough et al, 1985). However, recovery may be dispersed uniformly or nonuniformly (Chen et al, 1988; Han

et al, 1964), and some evidence suggest that nonuniform dispersion of recovery is not crucial for the induction or maintenance of reentry (Han et al, 1964; Chen et al, 1988; Frazier et al, 1989). On the other hand, there is evidence that nonuniformity in refractory periods is necessary for the occurrence of unidirectional block and reentry following premature stimulation (Allessie, 1976). Reentrant arrhythmias occurring on the basis of temporal differences in recovery of excitability are dependent on critical timing of the premature stimulus. As opposed to transient block, unidirectional block may be caused by differences in cellular connections or damage and depression of electrophysiological properties of myocardium (Spear et al, 1983a and 1983b; Spach et al, 1988; Dillon et al, 1988).

2.1.4.3.2 Slow conduction

An important factor which may determine the occurrence of reentry is slow conduction. Conditions that can reduce conduction velocity include membrane depolarization, sodium channel depression and cellular uncoupling. At relatively depolarized membrane potential levels, recovery from inactivation following an action potential is markedly prolonged and may extend beyond repolarization (Gettes et al, 1974). Depending on the membrane resting potential, the propagation of the action potential may be slowed or blocked, and may contribute to create conditions for reentry. In patients with prior myocardial infarction, zones of potential reentrant circuits may preexist because infarcted myocardium may have surviving cells intermingled with bundles of fibrotic fibers (Gardner et al, 1985) where conduction slowing may occur. The potential reentrant circuit(s) may not show enough conduction slowing to sustain a reentrant arrhythmia but sodium channel blocking drugs could facilitate reentry.

2.1.4.3.3 Anisotropy and cellular uncoupling

Studies have provided evidence that the anisotropic properties of cardiac muscle may provide an additional mechanism for differences in the excitability and safety of propagation of the cardiac action potential (Spach et al, 1981; Spach et al, 1982a and 1982b). Slowing of conduction may result from changes in membrane passive properties, such as increases in membrane resistance or gap junctional resistance, which will influence the propagation of the impulse.

Many electrophysiologic parameters are dependent on the direction of the propagating wavefront in the myocardium. The structure of cardiac myocytes and their electrical coupling via gap junction confer anisotropy in the

intercellular resistance to current flow (Spach et al, 1983). Anisotropy is determined by fiber orientation; faster propagation of the impulse occurs in the direction longitudinal to the fiber orientation and slower propagation occurs in the transverse direction. The axial and transverse resistances are determined by the degree of intercellular coupling; in the longitudinal direction gap junctions are present and allow faster propagation.

The anisotropic properties of myocardium may play an important role in the generation of reentrant arrhythmias. Studies suggest that the extent and packing geometry of intercellular junctions may influence the complex propagation patterns occurring at the microscopic level (Spach et al, 1982a and 1986). Spach et al showed that different safety factors exist in the longitudinal and the transverse axis of myocardial fibers, and that this difference may be lead to unidirectional block during premature stimulation (Spach et al, 1981).

Epicardial mapping of a region with myocardial infarction, showed that reentrant rhythm is due to reentry around an arc of conduction block over the infarct zone (El-Sherif et al, 1981; Wit et al, 1982), with block occurring in the direction of impulse propagation transverse to the myocardial fiber orientation (Cardinal et al, 1988; Dillon et al, 1988). Anisotropic conduction properties may sustain reentrant circuits, with the zone of block oriented in the transverse direction (Dillon et al, 1988). Based on a computer simulation of a ring-shaped one-dimensional cardiac fiber, Quan and Rudy suggested that cellular uncoupling plays an important role in the genesis and the maintenance of unidirectional block and reentry (Quan and Rudy, 1990).

2.1.5 Reflection

Reflection was initially described as a form of reentrant arrhythmia occurring in a one-dimensional structure, where the impulse is propagating in one direction and then back in the opposite direction into excitable tissue (Cranefield, 1975). Reflection differs from reentry in that the impulse does not require a circuit, but appears to propagate along the same path in both directions. Reflection may also involve electrotonic transmission across an area of inexcitability. Antzelevitch et al (1980) showed using the sucrose gap preparation that reflection may occur on the basis of electrotonic transmission across a small region of inexcitable tissue. Jalife and Moe (1981) used a similar method of the sucrose gap with isolated bovine and canine Purkinje fibers and suggested that time-

dependent changes in the passive electrical properties of the depressed segment may set the conditions for reflection. An *in vitro* study, using ventricular tissue without pacemaker properties, showed that delay produced by a nonhomogeneously depressed zone may result in reflection, causing closely coupled premature action potentials in proximal cells (Rozansky et al, 1984). In computer simulation studies it was also necessary to produce a delay in the distal element to produce reflection resulting in a premature action potential in the proximal elements (Janse and van Cappelle, 1982).

2.1.6 Spiral waves

Theoretical studies based on wave propagation in various types of excitable media (Winfree, 1972 and 1984; Pertsov, 1984) suggest that rotating waves (known as spiral waves) of chemical, physical or biological activity are commonly observed in homogeneous, continuous and isotropic excitable media. In a review article, Jalife et al (1991) noted a similarity between the reentrant spiralling waves of a chemical reaction (Belousov-Zhabotinski) and reentrant activity occurring in cardiac tissue. Similar behavioral aspects included the nonlinearity of the system undergoing self-sustaining oscillations, the presence of an excitable media, the property of refractoriness, and having similar laws governing propagation of activity. The main difference between the two reactions resides in the fact that unlike the chemical reaction, the heart is a highly inhomogeneous and discontinuous anisotropic medium.

Jalife et al (1991) and Davidenko et al (1990, 1991 and 1992) have used optical mapping techniques and voltage-sensitive dyes to produce high resolution mapping techniques and showed that self-sustained vortex-like reentry can be induced in thin slices of ventricular epicardial muscle. In their experiments, reentrant-like activity had characteristics similar to traditional observations of reentry. They reported anisotropic propagation during reentrant activity (longitudinal: 0.76 m/sec, transverse direction 0.19 m/sec), induction of reproducible sustained reentrant activity with one premature stimulus, presence of an excitable gap and ability to terminate reentrant activity by a properly timed electrical stimulus. In their experiments, a transient nonuniformity in refractoriness created by an appropriately timed voltage gradient was sufficient to establish circulating activity. Anisotropy and dispersion of action potential duration seemed to serve only to produce a nonuniform topographical distribution of conduction velocity and the excitable gap. Using similar techniques of optical mapping and mathematical modeling, Gray et al (1995)

showed that vortexlike reentrant activity was involved during polymorphic ventricular tachycardia in isolated rabbit heart.

The main differences between the traditional concept of reentry and that of spiral waves of excitation in excitable media, concern the initiation of reentry and persistence of activity. Firstly, in the traditional concept, circus movement reentry is initiated in cardiac muscle by a zone of block determined by either inhomogeneous anatomical or functional properties of the tissue (Allessie et al, 1990; Wit et al, 1990). In the case of spiral waves, they may form even in a tissue with homogeneous functional properties (Pertzov et al, 1984; Winfree, 1989), and the initiation of activity may depend only on transient local conditions created by the triggering stimulus. Secondly, in the traditional concept, the circulation of activity requires the presence of an anatomical or functional circuit where the impulse propagates, whereas, in the spiral wave concept, there is no predetermined circuit, the spiral wave occurs as a consequence of the initial "curling" of the wavefront. It is the curvature of the wave front which determines the size and shape of the core around which activity rotates.

2.2 Experimental models of ventricular arrhythmias

Animal models of ventricular arrhythmias must fullfil two important criteria: first, the tachyarrhythmias induced should possess similar characteristics to those observed in humans; second, they should bring insight into the possible mechanisms causing these arrhythmias.

2.2.1 Models of ventricular arrhythmias in the normal heart

In Mines's experimental model a single appropriately-timed electrical stimulus of sufficient intensity was able to induce ventricular fibrillation in normal heart (Mines, 1914). The period during which ventricular fibrillation could be induced by strong electrical stimulation was referred to as the vulnerable period (Wiggers and Wegria, 1940). This vulnerable period was believed to be characterized by a heterogeneous state of excitability which was enhanced by the strong electrical stimulation, thereby favoring reentry. In 1951, Hoffman et al compared the threshold for fibrillation and the strength of a single stimulus required to induce multiple responses and found that the former was only slightly higher than the "multiple response threshold" (Hoffman et al, 1951; Han and Moe, 1964). Adding a second or a third premature stimuli (S_2, S_3) to a

first premature stimuli (S_1) resulted in a reduction of the intensity of additional stimuli required for successful induction of a ventricular response (Matta et al, 1976).

In normal hearts, ventricular tachyarhythmias may be induced by strong or aggressive stimulation and last only a few beats. In general, one premature electrical stimulus of duration smaller than 5 msec and strength smaller than 10 times diastolic threshold, will rarely induce ventricular tachyarrhythmias in the normal heart (Echt et al, 1983; Garan et al, 1985; Hamer et al, 1984; Karagueuzian et al, 1979). The induction of fibrillation in a normal heart requires currents of large magnitude or very rapid burst of stimuli. Multiple premature electrical stimuli (S_1-S_4) may induce repetitive responses or ventricular fibrillation in normal hearts, but a low incidence is reported (Garan et al, 1985; Echt et al, 1983; Hamer et al, 1984). In comparison, in infarcted dogs, with similar modes of stimulation, higher incidences of ventricular arrhythmias were reported by Echt et al (1983), 83% of reproducibly inducible sustained ventricular tachycardia, and Michelson et al (1981), 61% of sustained ventricular arrhythmias. Long periods of nonsustained tachycardia or of sustained ventricular tachycardia do not seem to have been induced in a reproducible way in the normal heart.

Patients with suspected cardiac arrhythmias undergo electrophysiological studies during which programmed stimulation is used to induce arrhythmia, with protocols similar to those used in experimental animal studies. The results of experimental models in animals are in accordance with the findings of clinical studies on patients with normal hearts, which have shown that sustained ventricular arrhythmias are not inducible. On the other hand, sustained tachyarrhythmias can frequently be induced in patients with a history of such arrhythmias (Vanderpol et al, 1980).

2.2.2 Models of acute ischemic arrhythmias

A variety of methods have been used to produce experimental animal models which will mimic or simulate clinical conditions of acute myocardial ischemia. All models result from the ligation of one or more coronary arteries and the subsequent ischemia of the area supplied by the vessel implicated. The occlusion of the vessel(s) may be permanent, or temporary and followed by reperfusion (Janse and Wit, 1989).

2.2.2.1 Electrophysiological effects of acute myocardial ischemia

Myocardial ischemia produces changes in cardiac action potential characteristics such as membrane resting potential, action potential amplitude and duration. Individual current(s) and the normal balance of outward and inward currents present during the cardiac action potential may be affected during ischemic conditions. The electrophysiological effects of acute ischemia include changes in conduction velocity, refractoriness and automaticity.

2.2.2.2 Effects on action potential

Floating microelectrode recordings in the whole heart during ischemia have shown that the resting membrane potential of cells in the ischemic region shifts in the depolarizing direction (Downar et al, 1977; Kleber et al, 1978). The main cause of depolarization of the resting membrane potential is the loss of K^+ from the cell and the accumulation of extracellular K^+ resulting in the alteration of the K^+ gradient across the membrane (Hill and Gettes, 1980; Kleber, 1983). In studies in an open-chest animal model, it was found that in addition to the magnitude of extracellular K^+ accumulation, the rate of change of this variable may play a role in the genesis of ischemic ventricular arrhythmias (Pelleg et al, 1989). The net loss of K^+ during the early phase of ischemia may be explained either by a decreased influx or an increase efflux of K^+. The loss of K^+ by ischemic cells may be due to a partial inhibition of the Na^+-K^+-ATP pump, to a K^+ efflux secondary to loss of intracellular anions (Kleber, 1983) or to an activation of the I_{KATP} channel by a reduction in intracellular ATP concentration (Noma, 1983; Noma and Shibasaki, 1985). Other mechanisms that may contribute to the depolarization of the resting membrane potential include an overload in intracellular Ca^{2+} content (Clusin et al, 1984), and an accumulation of metabolites such as lysophosphoglycerides (LPG) in the cell membrane (Clarkson and Ten Eick, 1983). The changes in action potentials reported during ischemia include a reduction in amplitude, maximum rate of rise of phase 0 (upstroke velocity) and action potential duration.

After coronary occlusion the duration of the ventricular action potential shortens (Downar et al, 1977; Lazzara et al, 1974; Han et al, 1964) and this may result from multiple mechanisms. An increased $[K^+]_o$ is likely to be the most important mechanism for the shortening of the action potential (Vleugels et al, 1980; Isenberg et al, 1982). An increased conductance of

K^+ channels was attributed largely to a class of K^+ channels regulated by intracellular triphosphate nucleotides, mainly ATP (Noma et al, 1983). In addition to intracellular potassium loss during acute myocardial ischemia being mediated partly by ATP-dependent K^+ channels (Kantor et al, 1990; Wilde et al, 1990), it is suggested that the differential sensitivity of I_{KATP} to [ATP] is responsible for the differential shortening in action potentials during ischemia in epicardial compared to endocardial cells (Furukawa et al, 1991). Besides I_{KATP}, other causes of the reduction in action potential duration during acute ischemia may include a decrease in the slow inward current and the activation of a time-independent K^+ current mediated by an increase in intracellular [Ca^{2+}] (Isenberg et al, 1982; Vleugels et al, 1980; Morena et al, 1980).

2.2.2.3 Effects on refractoriness

The refractory period of ischemic myocardium is shortened (Elharrar et al, 1977a and 1977b; Han et al, 1964), in addition to an increased regional dispersion (Han and Moe, 1964; Levites et al, 1976; Naimi et al, 1977). Differences in recovery of excitability may be enhanced at rapid heart rates and local conduction block may therefore occur at rapid rates (Kleber et al, 1986). Unlike normal cells where the recovery from excitability is restored as the cell repolarizes, ischemic cells show postrepolarization (Downar et al, 1977; Lazzara et al, 1978a and 1978b) and the refractory period becomes time-dependent. In partially depolarized cells the recovery from inactivation may be delayed until after completion of repolarization (Gettes and Reuter 1974).

2.2.2.4 Effects on conduction

Following occlusion of a coronary artery, activation of the ischemic subepicardium is delayed and conduction velocity is reduced (Scherlag et al, 1974). Using epicardial mapping, it was shown that conduction velocity is reduced both in the longitudinal and in transverse direction after the onset of ischemia before fibrillation (Kleber et al, 1986). In the early phase of myocardial ischemia, the decrease in conduction velocity may be due to a reduction in phase 0 inward current and the amplitude of the action potential that results from membrane depolarization and the slow recovery of maximum upstroke velocity. Cellular uncoupling represents another factor that affects conduction velocity and an increase in the intercellular longitudinal resistance is observed during ischemic conditions (Wojtczak, 1979). However, a large increase in coupling

resistance is necessary before an appreciable decrease in conduction velocity results (Weigart, 1977). Mc Allister et al reported that during ischemia the majority of gap junctions of ventricular muscle become dissociated (McCallister et al, 1979). It has been proposed that the causes of the increase in the longitudinal resistance in ischemia may be an increase in intracellular Ca^{2+} and a decrease in intracellular pH (DeMello, 1975; Hess and Weingart, 1980).

Conditions favoring reentry such as slow conduction, short refractory periods, and inhomogeneity in recovery of excitability in adjacent areas, are therefore present in ischemic myocardium.

2.2.2.5 Mechanisms of arrhythmias during the acute phase of myocardial infarction

A decrease in conduction velocity can be associated with macroreentrant circuits of the leading circle type (Allessie et al, 1977) during acute myocardial ischemia. Nonreentrant premature beats (due to abnormal automaticity, triggered activity or electrotonic depolarization due to injury currents) may initiate the reentrant tachyarrhythmia in acute ischemia (Janse et al, 1980). The initiation of ventricular tachycardia or fibrillation may be caused by premature depolarizations originating in the normal myocardium close to the ischemic border (Janse et al, 1980). The main determinant of reentry during acute ischemia is a high degree of activation delay in the ischemic region, particularly in the epicardium (Janse and Kleber, 1981; Janse et al, 1980).

The arrhythmias observed during the sudden reperfusion of ischemic myocardium may be caused by two different mechanisms. When the ischemic period is relatively short (less than 10-15 min), there is a rapid return of electrical activity to previously inexcitable cells, increased inhomogeneity within the previously ischemic zone, and shortening the refractory period in the normal cells close to the border (Penkoske et al, 1978). Inhomogeneity in the recovery of excitability within the ischemic region and an increase in heart rate may augment the risk of reentry by enhancing the dispersion in refractoriness (Sheridan et al, 1980). In this case, multiple microreentrant circuits occur, resulting in reperfusion-induced fibrillation. When the ischemic period is prolonged (20-30 min), the mechanism for reperfusion-induced arrhythmias is more likely abnormal automaticity, possibly mediated by α-adrenergic stimulation in partially depolarized Purkinje fibers overlying the ischemic myocardium (Sheridan et al, 1980; Ferrier et al, 1985).

2.2.3 Subacute phase of myocardial infarction

The events occurring from approximately 8 to 72 hours after a sustained coronary occlusion are considered the subacute phase of myocardial infarction.

2.2.3.1 Delayed spontaneous ventricular arrhythmias

Sinus rhythm is often observed between three and six hours following LAD occlusion (Harris, 1950). This period is followed by a gradual increase in the incidence of premature ventricular beats. This phase lasts up to 72 hours post-occlusion and the associated spontaneous arrhythmias are often referred to as "delayed spontaneous ventricular arrhythmias" (El-Sherif et al, 1982; Scherlag et al, 1983). The spontaneous ventricular arrhythmia may be monomorphic or polymorphic and its rate may vary between 160 and 250 beats/min (Karagueuzian et al, 1979; Scherlag et al, 1974; Sugi et al, 1985). Slower rhythms (around 120 beats/min) may also occur between 24 and 48 hours (Sugi et al, 1985). Within 72 hours after occlusion, sinus rhythm is usually restored. The incidence of these experimental ventricular arrhythmias appears to be similar to some clinical arrhythmias observed in man during conditions of myocardial ischemia and infarction (Northover, 1982).

2.2.3.2 Mechanisms of spontaneous ventricular arrhythmias

After 15 to 30 minutes of ischemia without reperfusion, irreversible changes occur in some cells (Jennings et al, 1965; Corr and Witkowsky, 1986). Some of the epicardial surface may be spared and even in transmural infarcts some subepicardial muscle may survive (Ursell et al, 1985). During the period of delayed ventricular arrhythmias, electrical activity is not apparent in most of the infarcted ventricular wall (Horowitz et al, 1976; Lazzara et al, 1973), but electrical activity recorded in the subendocardial region confirms the presence of some viable cells on the endocardial surface of the infarct (Friedman et al, 1973a and 1973b; Horowitz et al, 1976; Scherlag et al, 1974; Sugi et al, 1985). At 24 hours after complete coronary occlusion, Purkinje fibers remaining on the endocardial surface may be responsible for the initiation of arrhythmias (Friedman et al, 1973a and 1973b; Horowitz et al, 1976; Lazzara et al, 1973; Feneglio et al, 1983).

Abnormal automaticity in surviving subendocardial Purkinje fibers is an important cause of delayed arrhythmias 24 hours after coronary occlusion

(Friedman et al, 1973a and 1973b; Lazzara et al, 1973; Sugi et al, 1985). During this period, abnormal automaticity is the mechanism responsible for most arrhythmogenesis, but increased normal automaticity and triggered activity caused by delayed afterdepolarizations may also occur (El-Sherif et al, 1982; LeMarec et al, 1985; Allen et al, 1978). Some arrhythmias may result from a combination of reentrant excitation and enhanced automaticity, where automaticity is responsible for induction of reentry.

After 72 hours there is a gradual normalization of the electrophysiologic characteristics of the surviving Purkinje cells. Action potential duration is gradually decreased and a significant increase in the resting membrane potential (ie becomes more negative), action potential amplitude, and maximum rate of rise is reported 72 hours after occlusion.

2.2.3.3 Delayed inducible ventricular arrhythmias

In subendocardial Purkinje fibers in isolated, superfused infarct preparations from hearts 24 to 72 hours after coronary occlusion, premature stimulation may produce reentrant excitation (Cardinal and Sasyniuk, 1974; Friedman et al 1973; Lazzara et al, 1973). Forty-eight hours after occlusion, there is a significant reduction in the occurrence of spontaneous depolarizations until gradual disappearance at 72 hours (Friedman et al, 1975) and induction of reentrant excitation with premature stimulation is not as successful as during the earlier period after coronary occlusion.

Ventricular arrhythmias may be induced by stimulation of the ventricles during the first two days after permanent occlusion of the left anterior descending coronary artery, at the time of occurrence of delayed spontaneous arrhythmias (Scherlag et al, 1983; El-Sherif et al, 1982).

2.2.3.4 Modulation of delayed spontaneous ventricular arrhythmias by sympathetic activity

In the infarcted canine heart, delayed ventricular arrhythmias are influenced by sympathetic activity. Sympathetic denervation suppresses ventricular arrhythmias and sympathetic stimulation causes an increase in the frequency of ectopic activity (Ebert et al, 1970). Sympathetic stimulation may also precipitate ventricular fibrillation (Harris et al, 1971). Subendocardial Purkinje fibers in the infarct zone have an increased sensitivity to catecholamines (Cameron and Han, 1982; Cameron et al, 1982).

2.2.4 Chronic phase of myocardial infarction

2.2.4.1 Inducibility of chronic arrhythmias

Ventricular arrhythmias may be induced by stimulation of the ventricles for at least seven days after permanent occlusion of the LAD in the dog (El-Sherif et al, 1977a and 1977b; Hope et al, 1980; Karagueuzian et al, 1979). Sustained ventricular tachycardia remains inducible after one year (Spear et al, 1985) and up to 4-6 years after infarction in the occlusion-reperfusion model (Hanich et al, 1988). Arrhythmias may be induced by single or multiple premature stimuli or by ventricular burst pacing (El-Sherif et al, 1977a and 1977b; Hope et al, 1980; Karagueuzian et al, 1979). The inducibility of ventricular arrhythmias by a single premature stimulus applied to the right ventricle decreases during the period 0 to 4 days (Karagueuzian et al, 1979). Using a gradually more aggressive protocol, it is possible to induce tachycardia in dogs with large infarcts over a period of 24 days following coronary occlusion (Duff et al, 1988). In the case of the occlusion-reperfusion model, arrhythmias persist and have been documented up to 5 years following occlusion and reperfusion (Hanich et al, 1988).

The site of ventricular stimulation in the ventricle may be an important determinant of induction of arrhythmia (Michelson et al, 1981). When the left ventricle is infarcted, stimulation of the left ventricle is more effective than stimulation of the right ventricle for arrhythmia induction. An even more effective induction occurs with the stimulus applied at the border of the infarcted and non-infarcted myocardium (Duff et al, 1988; Michelson et al, 1981). A transmural or a large infarct appears to lead to a higher prevalence of arrhythmia induction and sustained ventricular tachycardia occurs in 45 to 50% of dogs up to a month post occlusion (Garan et al, 1985). Overall, nonsustained ventricular tachycardia is induced in 50 to 60 % of infarcted dogs and sustained ventricular tachycardia in approximately 20% of dogs; in the remaining dogs no arrhythmia is induced (Janse and Wit, 1989).

2.2.4.2 Electrophysiological properties of the myocardial infarct

During the first two weeks after coronary occlusion, structural and physiological changes take place (Spear et al, 1983a; Ursell et al, 1985). Surviving myocardium on the epicardial and endocardial surfaces of the infarct are thought to be involved in arrhythmogenesis (Friedman et al, 1975; Karagueuzian et al, 1979; Lazzara et al, 1973; Ursell et al, 1985).

It is suggested that there may be an intermingling of normal and abnormal tissue in the infarcted zone (Spear et al, 1983a).

2.2.4.2.1 Effects on conduction

Conduction in the epicardium in the ischemic zone has been studied by El-Sherif using a composite electrogram (El-Sherif et al, 1977a and 1977b). Whereas electrograms of normal epicardium are not altered by increasing the stimulation frequency, the composite electrogram recorded from a 3- to 5-day old infarct develops a longer duration and may become fractionated at higher rates. The increased electrogram duration may result from a slowing of conduction or a change in the pathway of activation (El-Sherif et al, 1977a and 1977b). These results are consistent with the fragmented electrograms reported in humans with previous myocardial infarct (de Bakker et al, 1988). Fractionated electrograms recorded *in vivo* in these regions suggest slow and discontinuous activation (Josephson and Wit, 1984). The changes in conduction properties between the early healing phase and the fully healed infarct are related to the structural changes occurring during infarct healing (Gardner et al, 1985; Ursell et al, 1985). From experiments using high resolution mapping during sinus rhythm, the time for total activation was found to be larger in the infarct zone than in the same region in normal ventricle (Dillon et al, 1988; El-Sherif et al, 1981; Wit et al, 1982). Most of the epicardial surface is activated uniformly, but some regions show conduction block in areas of 5-15 mm (Dillon et al, 1988; El-Sherif et al, 1981) and sometimes in regions where transmural infarction reaches the epicardial surface (Cardinal et al, 1988; Dillon et al, 1988; El-Sherif et al, 1981). During electrical stimulation, Cardinal et al calculated a conduction velocity of 0.65 m/sec, a value similar to the conduction velocity found in the normal pre-occlusion epicardium (Cardinal et al, 1988). The electrophysiological characteristics of surviving epicardial border zone and infarcted myocardium varies with time after infarction, one to five days after infarction, resting potential, action potential amplitude, rate of depolarization are significantly reduced and abnormal (Spear et al, 1983a; Ursell et al, 1985). The results of Cardinal et al are consistent with the electrophysiologic characteristics of the cardiac action potential of epicardium 8 to 15 days after infarction which return to nearly normal values of resting potentials and maximum rates of rise of action potential (Spear et al, 1983; Gardner et al, 1985; Ursell et al, 1985). This reflects time-dependent recovery of electrophysiological characteristics of the cardiac action potential of surviving epicardium and infarcted myocardium after infarction.

No abnormal regional conduction slowing is reported when activation is uniform and fast during sinus rhythm, but in areas of slow conduction and block during sinus rhythm, enhanced regional conduction slowing and block is observed (Saltman et al, 1987). Mapping of the epicardial border and infarcted zones (3-5-day old) may show inhomogeneities in conduction: activation transverse to the myocardial fibers in the regions where block occurs during tachycardia is slow, whereas it is rapid in the longitudinal direction (Dillon et al, 1988). It was proposed that reentry results because of functional properties of the myocardium and differences in directional conduction slowing, rather than block *per se* (Dillon et al, 1988).

2.2.4.2.2 Effects on action potential

In 3 to 5-day infarct, the maximum diastolic potential of isolated surviving epicardial cells is reduced to between -65 mV and -70 mV compared to -85 mV and -90 mV in normal ventricular epicardium (Spear et al, 1983a; Ursell et al, 1985). An important reduction of diastolic potentials (less than -70 mV) is reported in approximately 15% of cells (El-Sherif and Lazzara, 1979; Lazzara et al, 1978b). The reduction of maximum diastolic potential has been related to the severity of the infarct, with larger changes in thinner surviving epicardium overlying the infarct (Gardner et al, 1981). The maximum rate of rise of the cardiac action potential is reduced to approximately 60 V/sec (Spear et al, 1983a; Ursell et al, 1985) compared to 100 to 120 V/sec in normal epicardium. The reduction of maximum rate of rise is attributed to the depression of sodium current since tetrodotoxin (TTX) and lidocaine abolish excitability (Lazzara et al, 1978b). The plateau phase of the cardiac action potential and consequently action potential duration is shortened (Boyden et al, 1988). This change may be the result of a decrease in I_{Ca-L} (Boyden et al, 1988). A study on myocytes isolated from 5-day old infarcts showed that the electrophysiological properties of the cells in the border zone are different from those in the noninfarcted zone. Action potential amplitude and maximum upstroke velocity are decreased, action potential duration is reduced, and there is no clear phase 1 repolarization in cells of the infarcted border zone compared to the normal zone (Lue and Boyden, 1992).

2.2.4.2.3 Changes in coupling

The structure of cardiac myocytes and their electrical coupling via gap junctions results in tissue anisotropy. Anisotropy implies that the velocity and the uniformity of impulse propagation is dependent on its

direction relative to fiber orientation. The conduction velocity in normal ventricular myocardium is approximately two to four times faster in the longitudinal than the transverse direction (Spach et al, 1982b). Cell coupling also influences refractoriness and excitability.

Cell coupling is compromised during the early stages of infarction and during the induction of reentry. Using standard microelectrode techniques, Spear et al (1983b) provided evidence that a depression in action potential depolarization and an increase in effective axial resistance contribute to uniform conduction slowing in the infarcted myocardium. Conduction within the infarcted region suggests abnormal cellular coupling (decrease in space constant) associated with slow conduction, fractionated electrograms and a disruption of normal anisotropy (Spear et al, 1983b). High resolution mapping has identified localized areas of block possibly due to decreased connections between cells (Kienzle et al, 1987). Heptanol is an agent that affects junctional conductance; since there is more junctional resistance per unit of distance in the transverse than in the longitudinal direction, the effect of an uncoupling agent is expected to be greater on impulse propagation in the direction transverse to the fiber orientation. The preferential slowing of conduction in the transverse direction by heptanol in normal anisotropic epicardium is consistent with this idea (Balke et al, 1988). Spear et al (1990) found that heptanol concentrations which had small effects on conduction in normal myocardium selectively depressed conduction and induced local conduction block in infarcted tissues in areas showing the most severe degree of preexisting conduction abnormality. They suggested that the abnormality of conduction was due to an abnormality in gap junction distribution and/or function in the infarcted region (Spear et al, 1990). In a rabbit model of infarction with a uniform anisotropic epicardial border zone, slow conduction in the reentrant circuit was due to anisotropic properties of the epicardial muscle (Nassif et al, 1993); however, even though slow conduction may partly result from poor transverse coupling, heptanol did not terminate the arrhythmia. Rather, heptanol slowed conduction in both transverse and longitudinal directions of the reentrant circuits and sometimes produced an extended line of functional block (Nassif et al, 1993). Depression of the fast sodium channel and electrical coupling may both contribute to the initiation and maintenance of reentrant arrhythmias by having differential effects on propagation of the electrical impulse relative to fiber orientation (Brugada et al, 1991a).

Rudy and Quan studied the effects of discrete cellular structure on electrophysiological propagation in cardiac tissue with a mathematical

model, showing its potential importance in arrhythmogenesis (Rudy et al, 1987). Subsequent mathematical simulations showed the potential role of cellular uncoupling in the initiation and maintenance of reentry (Quan and Rudy, 1990).

2.2.4.2.4 Effects on refractoriness

The refractory period may be prolonged in the infarct zone, and local differences in refractoriness may contribute to arrhythmia induction (Gough et al, 1985; Hope et al, 1980). A graded increase in the effective refractory period occurs from the margin of the epicardial border zone towards the center of the infarct, and the sites located proximal to the arc of block possess shorter refractory periods compared with distal sites (Gough et al, 1985). Slow conduction around the zone of block is thought to be due to propagation in regions possessing prolonged refractoriness, and to conduction in tissue which have not regained full excitability. Tachycardias are often initiated by a premature beat which encounters unidirectional block in a region possessing a large dispersion in local refractoriness (Restivo et al, 1990).

2.2.4.3 Mechanisms of chronic arrhythmias

Various studies suggest reentry as the most likely mechanism for ventricular arrhythmias in the presence of chronic transmural infarcts. Earlier evidence was based on mapping techniques which analyzed the extracellular electrograms of the region from which the arrhythmia was thought to arise (El-Sherif et al, 1977a). Continuous activity was observed at the same time as the induction of ventricular arrhythmias by either ventricular pacing or premature stimulation, and electrical activity resumed upon termination of the arrhythmia (El-Sherif et al, 1977a and 1977b). High-density activation maps of both ventricles have shown that in arrhythmias induced by electrical stimulation, the surviving epicardial muscle overlying the infarct zone comprises an important portion of the reentrant circuit (Kramer et al, 1985; Wit et al, 1982).

From epicardial activation maps it is possible to calculate conduction velocity in the longitudinal and the transverse directions. During ventricular stimulation, conduction velocities in either direction are not significantly different from normal (Cardinal et al, 1988). During ventricular tachyarrhythmias, conduction velocity may be slowed and the region of conduction block may form a line referred to as an arc of conduction block (Cardinal et al, 1988; Dillon et al, 1985; El-Sherif et

al, 1983 and 1985; Mehra et al, 1983; Wit et al, 1982). Conduction velocity around the arc of block may be an important determinant of the successful initiation of reentry. The length of the arc of conduction block represents another important determinant for reentry. A longer arc of conduction block may facilicite the recovery of excitability by increasing the pathlength for reentry.

The block resulting in reentry may be due to the effects of anisotropy (Dillon et al, 1988) or to dispersion of refractoriness (Gough et al, 1985). Some authors have observed that during tachycardia, block occurred in the transverse direction (Dillon et al, 1988; Delgado et al, 1990). Computer simulations point to the involvement of myocardial anisotropy in the generation of reentry (Panfilov and Keener, 1993; Saypol and Roth, 1992). Gough et al (1985) reported a graded increase in the effective refractory period from the margin of the epicardial border zone towards the center and that the tissues located proximal to the arc of conduction block have shorter refractory periods compared with distal sites. A combination of the two mechanisms may, in fact, be operative in producing the arc of block and reentry.

3. ANTIARRHYTHMIC AGENTS USED TO TREAT CARDIAC ARRHYTHMIAS IN FAST CHANNEL TISSUE

Antiarrhythmic agents are used to prevent or terminate cardiac arrhythmia and their efficacy will in large part depend on their pharmacological actions, the mechanism(s) of a specific arrhythmia and the particular conditions or state of disease. Antiarrhythmic drugs may also provide tools to gain insight into mechanisms of cardiac arrhythmias. Class I drugs have been used to treat cardiac arrhythmias in fast channel tissue and they share sodium channel blocking actions. Before the results of the CAST study became known, the sodium channel was the most common target for antiarrhythmic drug development.

3.1 Classification of antiarrhythmic drugs

According to the modified Vaughan Williams classification (Vaughan Williams, 1984) antiarrhythmic agents are grouped in four classes. This classification divides drugs according to four major pharmacological actions: (I) sodium channel blockade, (II) ß-adrenergic receptor blockade, (III) prolongation of action potential duration and (IV) calcium channel blockade. Class I agents are thought to act primarily on fast channel tissue. A subsequent subclassification of class I drugs is based on the kinetics of sodium channel blockade (Campbell, 1983) and drug effects on action potential duration. This classification has been and is still being widely used. The strength of the current classification is due to the clinical importance of the pharmacological properties upon which it is based. The major limitations of the current classification reside in the existence of agents whose actions fall into multiple classes and the subsequent problem of subclassification of these drugs. Separating drugs according to clinically relevant pharmacological actions results in classes with distinct electrophysiological effects and actions on arrhythmias. Because of these limitations, the present classification of antiarrhythmic agents is being reevaluated. In his review on antiarrhythmic drug classification Nattel (1991) proposes to address the problems of current classification by defining classes of action rather than of drugs *per se*, and by considering the multiplicity of actions of drug individually instead of attributing drug actions to a group. Characterizing antiarrhythmic agents in terms of the classes of action they possess would allow to incorporate various pharmacological properties. Subclassification of drugs would be done by describing their properties based on the degree of class I channel blocking effects, the degree of voltage-dependent actions and the kinetics properties. The

advantages of the proposed approach is that pharmacological properties would be incorporated more realistically and that it would better represent the complexities of clinically relevant effects.

3.2 Electrophysiological effects of class I agents

Typical properties of subclasses have become part of standard pharmacologic teaching (Goodman and Gilman, 1985; Luchessi, 1990). Class IA drugs, with slow recovery kinetics, reduce phase 0 slope of the cardiac action potential in normal and diseased tissue (moderate conduction slowing and QRS prolongation on ECG), reduce ventricular automaticity and increase action potential duration. Class IB agents, with fast recovery kinetics, reduce phase 0 slope of the cardiac action potential in diseased tissue more than in normal tissue (little effect on conduction and QRS duration), reduce ventricular automaticity of the His-Purkinje system and tend to decrease action potential duration, more in His-Purkinje system than in ventricular tissue. Class IC drugs, with very slow kinetics, are potent sodium channel blockers and cause a larger reduction of phase 0 slope of the cardiac action potential (important conduction slowing and QRS prolongation) than class IA agents, both in normal and diseased tissue, decrease ventricular automaticity and tend to decrease action potential duration in His-Purkinje system, but produce either no change or an increase in ventricular action potential duration (Elharrar and Zipes, 1982; Ikeda et al, 1985; Varro et al, 1986).

3.3 Rate-dependence of drug action on sodium channels

Use-dependent sodium channel blockade by antiarrhythmic drugs manifests in greater reduction of V_{max} (phase 0 sodium current) at faster stimulation rates (Grant et al, 1984; Hondeghem and Katzung, 1977; Courtney, 1980; Campbell and Vaughan Williams, 1983). Class I drugs produce rate-dependent effects on cardiac sodium channels and quantitative studies have shown kinetics of action of sodium blockers on conduction *in vivo* in animal models (Nattel, 1985; Davies et al, 1987; Anderson et al, 1990) paralleling their kinetic effects on phase 0 of cardiac action potential and conduction *in vitro* (Nattel, 1985, Nattel, 1987a and 1987b). Campbell (1983) showed that various sodium channel blockers produced frequency-dependent depression of V_{max} and that rate-dependent block for each drug developed with characteristic kinetics which could be used for antiarrhythmic agents classification. Voltage-clamp techniques allow direct measurement of sodium conductance and confirm both frequency- and

voltage-dependence of sodium channel block by antiarrhythmic drugs.

The affinity of antiarrhythmic drugs for the sodium channel is modulated by the state of the channel. In general, antiarrhythmic agents have a low affinity for the rested state and preferentially bind to either the inactivated or open state of the channel (sometimes to both states), with corresponding association and dissociation rate constants (Hondeghem and Katzung, 1984). As a result, rate-dependence can be due to inactivated and/or open-state block(s) and the association and dissociation rate constants for a drug can be determined experimentally over a range of stimulation rates and voltages. Voltage-clamp techniques allow for the design of experimental protocols to determine tonic block, use-dependent block, inactivation block and recovery from block, and to discriminate between drug affinity for rested, activated and inactivated states of the sodium channels. For example, amiodarone (Mason et al, 1984) and imipramine (Habuchi et al, 1991) have a selective affinity for sodium channels in the inactivated state. Activated and inactivated channels have a different affinity for quinidine (Snyders and Hondeghem, 1990). Experiments using measurements of V_{max} have suggested that lidocaine binds to both activated and inactivated channels (Matsubara et al, 1987). Use-dependent block by lidocaine was studied in experiments using patch-clamp technique which suggest that sodium channel block is characterized by two components which may result from an interaction of lidocaine with sodium channels in the activated as well as inactivated states (Clarkson et al, 1988; Jia et al, 1993). Using similar techniques, other drugs such as flecainide (Varro et al, 1985a; Ideka et al, 1985; Anno and Hondeghem, 1990), quinidine (Snyders, 1990; Snyders and Hondeghem, 1991) and penticainide (Carmeliet, 1988) have shown to bind to the activated state.

The effects of antiarrhythmic agents have been described as being rate-, frequency- use- and voltage-dependent. Although the terms rate-, frequency- and use-dependent are sometimes utilized interchangeably when referring to antiarrhythmic drug actions, a distinction can be made. Rate- and frequency-dependency has been used to characterize the property of antiarrhythmic drugs to produce larger depression of V_{max} (index of sodium current) or conduction velocity at faster stimulation rates or frequencies. Use-dependent sodium channel blockade by antiarrhythmic drugs is used to describe block developing and accumulating with every action potential as the rest interval becomes insufficient for recovery from block, ie. block requires channel use - when channels are rested continuously, no block occurs. The terms use-, rate- and frequency-

describe a time-related drug effect without identifying the mechanism of drug interaction with the channel.

The affinity of the cardiac channel receptor for the drug is modulated by the membrane's voltage. Rested, activated and inactivated channels can have different interaction or kinetics with antiarrhythmic drugs. In addition, antiarrhythmic agents shifts the voltage-dependence of inactivation of drug-associated channels.

State-dependent effects have been incorporated in molecular models of antiarrhythmic drug action: the modulated receptor hypothesis (Hondeghem and Katzung, 1977 and 1984) and the guarded access hypothesis (Starmer et al, 1984 and 1985).

3.4 Models of antiarrhythmic drug action

3.4.1 Modulated receptor hypothesis

The modulated receptor hypothesis was developed by Hille (Hille, 1977) and Hondeghem and Katzung (Hondeghem and Katzung, 1977) to explain the use-dependent effects of local anesthetics and class I antiarrhythmic agents. This model is based on different affinities of drugs for the various sodium channel states. The model makes four assumptions: drugs bind to a receptor associated with the cardiac sodium channel; drug binding occurs with state-specific association and dissociation rate constants (Figure 5); drug-associated channels do not conduct, even when activated; and finally, the inactivation voltage-dependence of drug-associated channels is shifted to more negative potentials. Rates of interaction with each state of the channel are characteristic for each drug.

3.4.2 Guarded access model

This model is based on drug binding to a constant affinity receptor, with receptor access determined by channel gating. Channel gates restrict drug movement to and away from the receptor, thus controlling binding (Starmer and Grant, 1984 and 1985). Drug binding immobilizes the channel gates, resulting in sodium channel blockade. The frequency- and voltage-dependent behavior of channel gates accounts for the frequency- and voltage-dependent interaction of the drugs with the sodium channel (Grant et al, 1984).

Figure 5

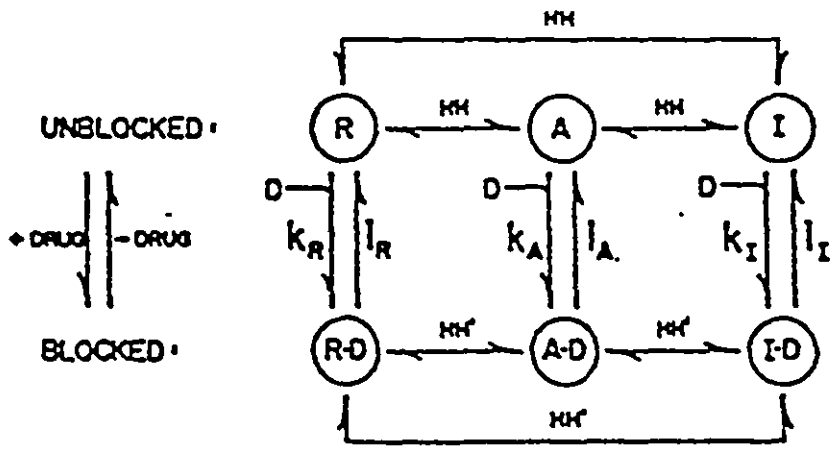


Figure 5: Schematic representation of Hodgkin-Huxley model of antiarrhythmic drug action and the modulated receptor hypothesis.

R = rested
A = activated or open
I = inactivated

R·D, A·D and I·D = respective drug-associated channels

k_R, k_A and k_I = association rate constants between drug-free and the corresponded blocked channels

l_R, l_A and l_I = dissociation rate constants between drug-free and the corresponded blocked channels

H-H = the reactions governed by the Hodgkin-Huxley parameters

H-H' = similar to H-H, except for the inactivaton (*h*) parameter

Adapted from:
Hondeghem LM, Katzung BG. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochimica et Biphysica Acta 1977; 472: 373-398.

3.5 Rate-dependence of conduction *in vivo*

Quantitative studies have shown kinetics of action of sodium channels blockers on conduction in *in vivo* animal models (Nattel, 1985; Davies et al, 1986; Bajaj et al, 1987; Anderson et al, 1990) paralleling their kinetic effects on phase 0 of the cardiac action potential and conduction *in vitro* (Nattel, 1985; Nattel, 1987a and 1987b). Rate-dependent conduction slowing may also be modified by pathological conditions. For example in conditions of acute ischemia, the distribution of high extracellular potassium concentration ($[K^+]_o$) and resting depolarization are inhomogeneous. In abnormal myocardium, an amplification of drug-induced rate-dependent conduction slowing has been demonstrated in depolarized tissue (Cascio et al, 1987; Kojima et al, 1986; Saito et al, 1978; Campbell et al, 1990 and 1991; Ye et al, 1993) and in conditions of ischemia (Donaldson et al, 1984).

Electrophysiologic studies using programmed stimulation have shown qualitative frequency-dependent effects on ventricular conduction of sodium channel blocking antiarrhythmic drugs in humans (Gang et al, 1985; Morady et al, 1985).

3.6 Molecular aspects of the sodium channel as a target for antiarrhythmic agents

3.6.1 Structure of the sodium channel

The protein components of the sodium channel have been identified, isolated, purified and reconstituted to provide function (Catterall, 1988). Presently, at least five members of this family have been cloned and expressed. The sodium channel purified and isolated from rat brain (Hartshorne and Catterall, 1984; Messner and Catterall, 1985) consists of three subunits: α (260 kD), β_1 (36 kD) and β_2 (33 kD). The ß2 subunit is attached to the α subunit by disulfide bonds; the ß1 subunit is noncovalently linked to the α subunit. The sodium channel from mammalian skeletal muscle (Barchi, 1983; Casadei et al, 1986) is composed of an α subunits (260 kD) and one ß subunit (38 kD) analogous to those of the brain sodium channel. The sodium channel purified from eel electroplax (Miller et al, 1983) and from chick cardiac muscle (Lombet and Lazdunski, 1984) consists of a single α subunit (260 kD), although some evidence suggest that there may be an additional subunit (Cribbs et al, 1990). It appears that the principal α subunits of sodium channels are expressed in

association with a variable number of smaller subunits in different excitable tissues. The large α subunit contains the binding site for tetrodotoxin and saxitoxin and functional expression of sodium channels in *Xenopus* oocytes from cloned cDNA suggest that this large α subunit most probably represents the main functional component of the sodium channel (Noda et al, 1986a; Cribbs et al, 1990).

The overall arrangement of α, β_1 and β_2 subunits in the membrane has been deduced from biochemical experiments. All three subunits are heavily glycosylated, indicating that they are all exposed to the extracellular surface (Barchi, 1983; Miller et al, 1983; Messner and Catterall, 1985). The α subunit is phosphorylated by cAMP-dependent protein kinase (Costa and Catterall, 1984a) and protein kinase C (Costa and Catterall, 1984b), indicating that it is exposed to the intracellular surface and implying that it is a transmembrane protein. The β_1 and β_2 subunits are also intrinsic membrane proteins and they have substantial hydrophobic domains (Reber and Catterall, 1987).

The properties of the purified sodium channels incorporated into macroscopic planar phospholipid bilayers have been studied after activation of the channels by batrachotoxin (a toxin that prevents inactivation). In experiments on purified sodium channels from brain, skeletal muscle and electroplax, channels retained the single channel conductance, ion selectivity and voltage-dependence of native sodium channels activated by batrachotoxin (Hartshorne et al, 1985; Furman et al, 1986).

Complementary DNA (cDNA) clones encoding the primary structure of the principal subunits of sodium channels has been isolated (Noda et al, 1984). The primary structure of rat brain subunit α of sodium channel comprises four homologous domains with multiple hydrophobic segments (Noda et al, 1986b) (Figure 6). The presence of these four homologous domains in the primary structure of the sodium channel α subunit suggests that the transmembrane pore of the sodium channel is formed in the center of a pseudosymmetric square array of these four homologous domains (Noda et al, 1986b). They also report that each of the four homologous domains of the sodium channel contains six regions of probable α-helical structure long enough to be membrane-spanning segments and designated them S_1 to S_6 (Figures 6 and 7). Many researchers have proposed that the S_4 segment is involved in the voltage-dependent gating of the sodium channel. The S_4 segment is thought to adopt an helical conformation (sliding helix model)

Figure 6

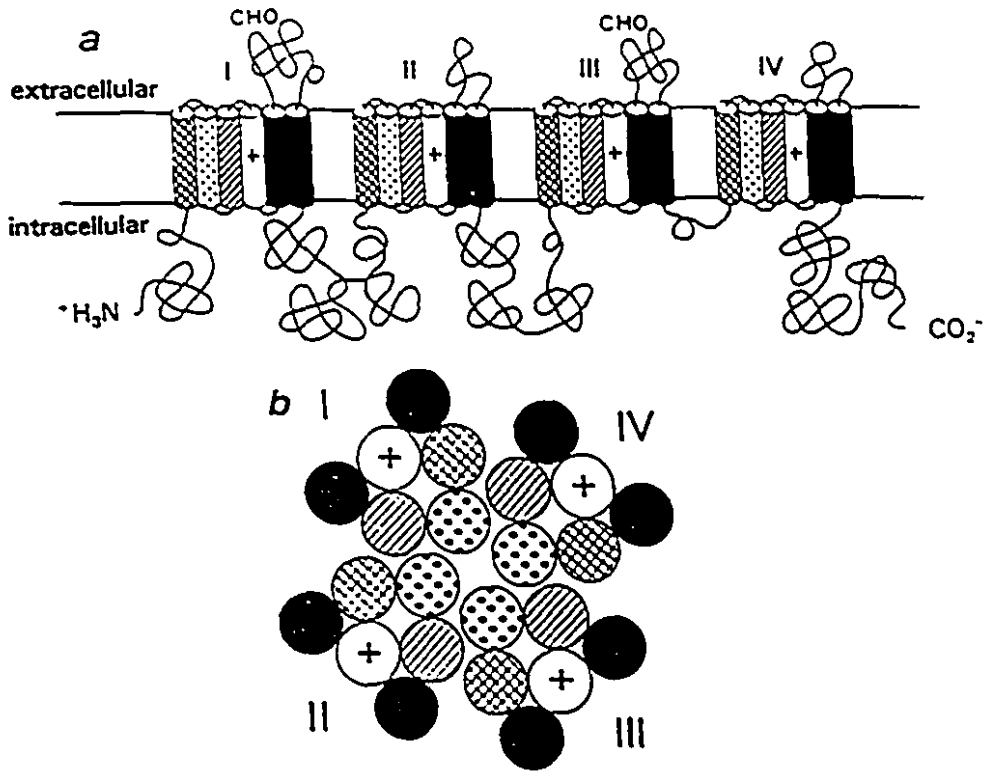


Figure 6: *a*: Transmembrane topology of the sodium channel.

The four units of homology spanning the membrane are displayed linearly. Segments S1-S6 in each repeat (I-IV) are indicated by cylinders as follows: S1, cross-hatched; S2, stippled; S3, hatched; S4, indicated by a plus sign; S5 and S6, solid. Putative site of N-glycosylation (CHO) are indicated.

b: Arrangement of the transmembrane segments (direction perpendicular to the membrane)

The ionic channel is represented as a general pore surrounded by the four units of homology. Segments S1-S6 in each repeat (I-IV) are represented by circles identified as in *a*.

Adapted from:

Noda M, Ideka T, Kayano T, Suzuki H, Takeshima H, Kurasaki M, Takahashi H, Numa S. Existence of distinct sodium channel messenger RNAs in rat brain. Nature 1986; 320: 188-192.

Figure 7

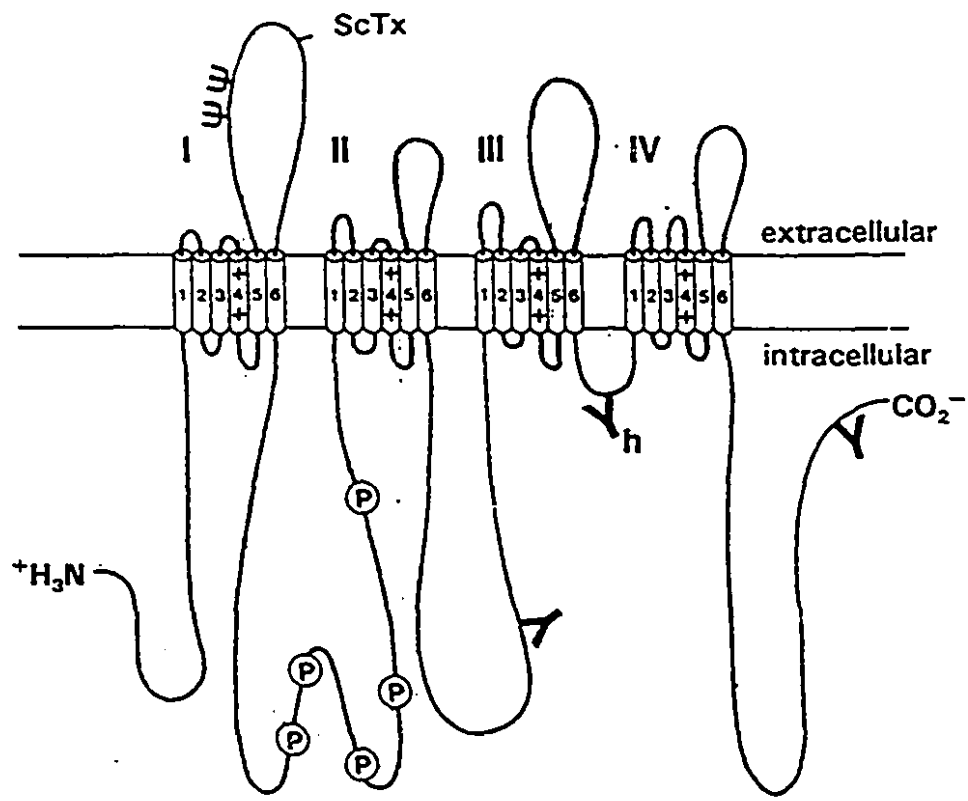


Figure 7: A functional map of the Na^+ channel subunit.

The transmembrane folding model of the α subunit is depicted with experimentally demonstrated sites of cAMP-dependent phosphorylation (P), interaction of site-directed antibodies that define transmembrane orientation (>), covalent attachment of α-scorpion toxins (ScTx), glycosylation ($\Psi\Psi$), and modulation of channel inactivation (h).

Adapted from:

Catterall WA. Structure and Function of Voltage-Sensitive Ion Channels. Science 1988; 242: 50-61.

(Catterall, 1986). This is consistent with the experiment demonstrating that a sodium-channel polypeptide, synthesized with a sequence identical to segment four, when incorporated into lipid bilayers, formed a cation-selective channel showing voltage-dependent characteristics (Tosteson et al, 1989). The short intracellular segment connecting domains III and IV has been proposed to comprise the site responsible for inactivation in sodium channels (Meiri et al, 1987; Vassilev et al, 1988) (Figure 7). The conformational changes proposed to occur in the inactivation process would take place along the channel between those two sites.

3.6.2 Receptor sites

Receptors sites differ in its ability to bind ligands (and toxins) and its location in distinct regions of the sodium channel structure (Catterall, 1986). Receptor site 1 binds the water-soluble heterocyclic guanidines toxins tetrodotoxin and saxitoxin (Catterall, 1980; Ritchie and Rogart, 1977). These toxins inhibit sodium-channel ion transport by binding to a common receptor site located near the extracellular opening of the ion-conducting pore of the sodium channel. Toxins that bind to site 2 are lipid-soluble toxins including grayanotoxin and alkaloids such as aconitine, veratridine and batrachotoxin, and cause persistant activation at the resting potential by blocking sodium channel inactivation (Conti et al, 1976). Neurotoxin receptor site 2 is likely to be located in a region of the sodium channel involved in voltage-dependent activation and inactivation. Receptor site 3 binds polypeptides purified from North African α scorpion toxins or sea anemone nematocysts. These toxins slow or prevent inactivation of the channel and they also act synergistically with site 2 toxins. The receptor site 3 is localized in neuronal channels at the extracellular loop between S_5 and S_6 of domain I of the α-subunit, on part of the sodium channel involved in inactivation (Thomsen and Catterall, 1989). Toxins that bind to site 4 include ß scorpion toxins, and site 5 toxins such as brevitoxin also cause persistant channel activation.

Class I antiarrhythmic agents are thought to bind to a receptor site closely associated with the channel (Sheldon et al, 1987), blocking sodium influx through the sodium channel and thereby causing electrophysiologic changes. Besides the study of structure and function of the sodium channel, the development of radiolabelled neurotoxins provides a biochemical approach to study local anesthetics and antiarrhythmic agents. The most common approach uses a tritiated derivative of batrachotoxin,

[^{3}H]batrachotoxinin A 20α-benzoate ([^{3}H]BTXB) (Postma and Catterall, 1983), and sea anemone toxin (ATX II), which bind to sodium channels on freshly isolated rat cardiac myocytes (Sheldon et al, 1986). ATX II promotes persistent activation by alkaloid toxins through an allosteric mechanism that also enhances alkaloid toxin binding. Class I antiarrhythmic drugs including procainamide, lidocaine, O-desmethylencainide (Sheldon et al, 1987), and amiodarone (Sheldon et al, 1989) inhibit [^{3}H]BTXB binding in a fashion consistent with binding to a specific receptor site on cardiac myocytes. The dissociation constant (K_d) for antiarrhythmic drug displacement of BTXB correlates with the EC_{50} for electrophysiologic actions.

4. PROARRHYTHMIC EFFECTS OF CLASS IC ANTIARRHYTHMIC AGENTS

4.1 Definition of proarrhythmia

Proarrhythmic events have been associated with a variety of antiarrhythmic drugs, causing the worsening of an arrhythmia already present in a patient (Velebit, 1982; Morganroth et al, 1987) or provoking new and qualitatively different arrhythmias. Classification of proarrhythmia have been based on changes in the frequency of ventricular premature complexes (VPC's) on the surface electrocardiogram (Morganroth and Horowitz, 1984; Morganroth et al, 1987; Velebit et al, 1982), in terms of clinical severity (Morganroth, 1987), in terms of the time of occurrence after initiation of drug therapy, ie early (Velebit et al, 1982; Minardo et al, 1988) versus late occurrence (CAST Investigators, 1989), and in terms of type of ventricular arrhythmias (Bigger and Sahar, 1987; Morganroth et al, 1986; Podrid, 1993).

The first proarrhythmic effects reported with a clinically used medication was the risk of ventricular fibrillation during anaesthesia with chloroform (Hill et al, 1932). An increasing number of clinically used substances possess proarrhythmic potential (Martyn et al, 1993), and proarrhythmia has been reported with a variety of antiarrhythmic agents (Zipes, 1987; Horowitz et al, 1987). In the case of potentially fatal proarrhythmic effects associated with antiarrhythmic drugs, the arrhythmia treated is often ventricular tachycardia and fibrillation, which may be similar to the potentially fatal proarrhythmic effect. Consequently, the identification and classification of proarrhythmic effects associated with antiarrhythmic drugs has been problematic.

4.2 Types of proarrhythmia associated with class I and III antiarrhythmic agents

4.2.1 Acquired long QT syndrome

Long QT syndromes termed "acquired" can be precipitated by certain drugs (including antiarrhythmic agents), electrolyte abnormalities (hypokalemia, hypomagnesemia), altered nutritional states, and severe bradyarrhythmias. "Idiopathic" or "congenital" long QT syndromes are generally familial and present from early in life. Torsades de pointes is a form of ventricular tachyarrhythmia characterized on the ECG by a changing and alternating QRS morphology and axis during each episode. Drug-induced torsades de pointes is frequently associated with an acquired long QT syndrome (Jackman et al,

1988).

This type of drug-induced proarrhythmia can occur early after initiation of therapy, can occur at low dosage and can be expressed as classical torsades de pointes or ventricular fibrillation (Minardo et al, 1988). This type of proarrhythmic response has been found to occur with quinidine (Roden et al, 1986; El-Sherif et al, 1989; Davidenko et al, 1989), and may occur with class IA and III antiarrhythmic drugs which prolong the APD of the ventricles. Many drugs alter the QRS duration or the QT intervals and this represents a pharmacologic effect of some antiarrhythmic agents. However, some of the early proarrhythmic responses are clinically characterized by a prolongation of the QT or QTU intervals, with drug-induced prolonged repolarization, early afterdepolarizations, and triggered activity as possible underlying mechanisms (El-Sherif et al, 1990). The mechanism of arrhythmogenesis of the class IA and III antiarrhythmic drugs appears to be the development of early afterdepolarizations (EADs) as a consequence of prolongation of action potential duration (Roden et al, 1986; Jackman et al, 1988).

4.2.2 Early afterdepolarizations and torsades de pointes

Experimental studies point towards the development of EADs as the most likely mechanism for proarrhythmic events associated with the long QT syndrome. Electrolyte abnormalities, particularly hypokalemia and perhaps low magnesium concentrations, may be factors responsible for arrhythmogenesis under certain circumstances and have been associated with torsades de pointes (Keren et al, 1981; Davidenko et al, 1989). Slow driving rates and bradycardia may result in prolonged repolarization and may be contributing factors in the development of early afterdepolarizations and torsades de pointes (Gadsby and Cranefield, 1977). A relationship between early afterdepolarization and torsades de pointes have been suggested, based on observations such as the long-coupled interval of the first beat of arrhythmia, its occurrence with bradycardia and hypokalemia, and its association with prolonged repolarization.

4.3 Ventricular proarrhythmic effects with class IC drugs

Proarrhythmic effects may be associated with excess sodium channel blockade, particularly due to class IC drugs. An increase of the duration of the QRS complex, incessant monomorphic ventricular tachycardia, or episodes of nonsustained polymorphic ventricular tachycardia have been

reported with this type of arrhythmia. Animal experiments and clinical studies suggest that strong drug-induced slowing of conduction and a minimal prolongation of the refractory period is often associated with proarrhythmia, consistent with a reentrant mechanism (Hellestrand et al, 1982; Coromilas et al, 1988; Brugada et al, 1991b; Rinkenberger et al, 1982). Sodium channel blockers which produce significant conduction delay may predispose to the development of ventricular arrhythmias or may increase the incidence of proarrhythmia particularly in the presence of ischemia (Elharrar et al, 1977; Nattel et al, 1981a).

4.3.1 Clinical manifestations

Proarrhythmic events have been reported on electrocardiographic (ECG) recordings (Winkle et al, 1981; Morganroth and Horowitz, 1984; Lui et al, 1982), on ambulatory Holter monitoring (Morganroth et al, 1987) and during exercise testing (Anastasiou-Nana et al, 1987; Cascio et al, 1988) in patients with antiarrhythmic drug therapy. Flecainide produces changes in the electrographic PR and QRS intervals and these increases are related to dose and plasma concentrations (Morganroth and Horowitz, 1984). The QRS duration on electrographic recordings may be used as an index of ventricular conduction slowing of drugs having use-dependent blocking properties and the degree of QRS prolongation at rest represents a clinical indicator of its pharmacologic action (Cascio et al, 1988).

In the Cardiac Arrhythmia Suppression Trial (CAST) three class I antiarrhythmic drugs, flecainide, encainide and moricizine, were used in patients with previous myocardial infarction or a history of ventricular arrhythmias (CAST investigators, 1989). This study was conducted to address the hypothesis that the suppression of premature ventricular complexes by class I drugs would reduce the incidence of sudden cardiac death. After a follow-up period of ten months, CAST Investigators reported that 4.5% of the encainide-flecainide patients had a nonfatal cardiac arrest or sudden death compared to 1.2% in the placebo group ($p<0.001$). The total mortality was 7.7% in patients treated with flecainide or encainide in contrast to 3.0% in patients receiving placebo ($p<0.001$). The increased mortality in the flecainide-encainide groups was attributed to proarrhythmic events but the mechanisms of action of lethal cardiac arrhythmias have not been thoroughly investigated.

Aggravation of existing arrhythmias have been reported in about 6 to 16% of patients taking any antiarrhythmic drug (Velebit et al, 1982). However with class IC drugs, the incidence of drug-induced serious ventricular

arrhythmias has been relatively higher than for other drugs (Torres et al, 1985; Rae et al, 1988; Levine et al, 1989, Podrid, 1993). Malignant ventricular tachycardias in association with propafenone was reported in five patients, of these, three patients had non-self-terminating ventricular tachycardia degenerating into ventricular fibrillation and sudden death occurred in two of them (Buss et al, 1985). In studies with flecainide- and encainide-treated patients, potentially lethal ventricular tachycardia developed in 16 and 11 percent respectively (Soyka, 1987). Patients with nonsustained ventricular tachycardia that developed incessant ventricular tachycardia were taking antiarrhythmic agents with class IC properties such as encainide (Winkle et al, 1981), flecainide (Morganroth and Horowitz, 1984; Morganroth et al, 1986) or propafenone (Stavens et al, 1985). The conversion of nonsustained ventricular tachycardia into sustained ventricular tachycardia requiring pharmacologic or electrical termination was reported to occur more frequently with class IC drugs (Rinkenberger et al, 1988).

Drug-induced proarrhythmia has also been reported during electrophysiologic studies (EPS) evaluating antiarrhythmic drug therapy in patients. Aggravation of ventricular tachyarrhythmia inducibility or some form of arrhythmogenic response has been observed with a wide variety of class I drugs, including disopyramide, procainamide, quinidine, mexiletine amiodarone, propafenone, encainide and flecainide (Velebit et al, 1982; Rinkenberger et al, 1982; Stavens et al, 1985; Torres et al, 1985; Poser et al, 1985; Sager et al, 1992). Electrophysiologic testing has been used to evaluate the proarrhythmic potential of antiarrhythmic drugs in patients with ventricular arrhythmias (Rae et al, 1988).

4.3.2 Experimental studies

Just as in the above clinical studies, EPS has been used to evaluate animal models of proarrhythmia. In a canine occlusion-reperfusion infarction model flecainide facilitated the induction of proarrhythmic events (sustained ventricular arrhythmias); this proarrhythmic effect was attributed to the selective effect of flecainide on myocardial conduction (Kidwell and Gonzalez, 1993). Proarrhythmic risk associated with flecainide has been investigated in Langendorff-perfused rabbit hearts (Brugada et al, 1991b). In this study ventricular tachycardia induced by programmed stimulation was enhanced in the presence of flecainide. All arrhythmias were due to reentry around an arc of functional block (Brugada et al, 1991b). Flecainide's electrophysiologic effects consisted of moderate slowing of conduction (27% longitudinal conduction slowing and

26% transverse conduction), with minor prolongation of the effective refractory period (8%, $p<0.003$). It is not obvious how these relatively small electrophysiologic changes caused proarrhythmia.

The most common model used to study flecainide-induced proarrhythmia is the post-infarction dog. In this model, class IC agents such as flecainide and encainide facilitated induction of new ventricular arrhythmias by programmed stimulation (Kou et al, 1987; Wallace et al, 1993). Preliminary findings suggested that flecainide facilitates the induction of sustained ventricular tachycardia by extending the arc of block and by slowing conduction in both the longitudinal and transverse direction (Coromilas et al, 1988). Proarrhythmic events were reported to occur at high concentrations of flecainide (Zimmerman et al, 1985). The proarrhythmic effects observed with class IC drugs such as propafenone and flecainide were seen in the presence of an increased dispersion of local refractoriness as well as a marked reduction of conduction velocity within the infarction zone (Miyazaki et al, 1988).

Other systems have also been used to study proarrhythmia. Flecainide was found to have proarrhythmic effects during coronary artery occlusion but possible antiarrhythmic actions in preventing ventricular fibrillation during reperfusion (Lederman et al, 1989). In a theoretical model, Starmer and coauthors defined proarrhythmic potential as "the duration of the vulnerable window" and concluded that drugs which possess use-dependent class I properties have a greater proarrhythmic potential (Starmer et al, 1991; Nesterenko et al, 1992).

4.3.3 Potential mechanisms of proarrhythmia

Drug-induced sodium channel blockade may depress myocardial conduction enough to result in unidirectional block, or may depress conduction in the abnormal myocardium enough to allow the normal myocardium to recover excitability and permit reentry. Class IA drugs, which slow conduction and prolong repolarization, may be less likely to cause such proarrhythmia because lengthening of refractoriness would tend to prevent reexcitation even in the presence of slowing of conduction. The zone of block necessary for a reentrant circuit may be due to nonuniform recovery of excitability in the presence of premature beats, anatomic factors in the infarcted myocardium, or differential depression of conduction by sodium channel blockade. The significant slowing of conduction with minor effects on refractoriness associated with class IC drugs represent a combination which would be expected to increase the likelihood of proarrhythmic events

due to reentry. Class IC drugs have been proposed to be inherently more proarrhythmic (Torres et al, 1985; Rae et al, 1988; Levine et al, 1989; Podrig, 1993) than the class IB and IA drugs (Velebit et al, 1982; Levine et al, 1989; Dhein et al, 1993).

4.4 Factors predisposing to proarrhythmia

4.4.1 Structural heart disease

Clinical reports suggest that diseased hearts are predisposed to drug-induced proarrhythmia (Levine et al, 1989). Patients with extensive heart disease, impaired left ventricular function, clinical congestive heart failure and a history of sustained ventricular tachycardia have a higher incidence of proarrhythmia (Slater et al, 1988) than patients with a structurally normal heart and only nonsustained ventricular tachycardia or supraventricular tachycardia. Flecainide-induced proarrhythmia occurs more often in presence of structural heart disease such as coronary artery disease, valvular disease, congenital heart disease, cardiomyopathy or previous cardiac surgery (Morganroth et al, 1986; Morganroth, 1987). The presence of structural disease increases the risk of drug-induced proarrhythmia by 2- to 3-fold in patients treated with flecainide or encainide (Morganroth, 1987). The presence of structural heart disease may act to provide a substrate that can support reentry. Patients with a history of sustained ventricular tachycardia may also have the appropriate substrate for persistent reentry that may be induced by alterations in conduction velocity and/or refractoriness.

Patients with reduced left ventricular function (<40%), previous myocardial infarction, left ventricular aneurysms and bundle branch block have an higher incidence of proarrhythmic events during electrophysiologic study (Sager et al, 1992). The risk of arrhythmia worsening in patients with left ventricular ejection < 35% is twice that of those with an ejection fraction > 35% (Slater et al, 1988). There is thus evidence that left ventricular pathology facilitates the occurrence of proarrhythmia.

4.4.2 Antiarrhythmic drug concentration

A low incidence of clinical proarrhythmic events (ventricular tachycardia) is reported with therapeutic concentrations of flecainide (Anderson et al, 1983; Platia et al, 1985) but the rapid escalation of class IC antiarrhythmic drug regimen has been identified as a factor increasing the likelihood of proarrhythmia in patients (Morganroth et al, 1986;

Morganroth, 1987). Therapeutic doses of class IC drugs (flecainide and encainide) prolong intraventricular conduction time as measured by QRS duration (Morganroth and Horowitz, 1984; Winkle et al, 1981). The degree of intraventricular conduction slowing increases with increasing flecainide doses (Morganroth and Horowitz, 1984) concomitantly with the incidence of clinical proarrhythmic events.

4.4.3 Ischemia and myocardial infarction

The CAST study has shown that patients with a recent myocardial infarction, no overt congestive heart failure, single ventricular VPC's and no history of sustained ventricular tachycardia, treated with a class IC agent (flecainide or encainide) have an increased incidence of mortality (CAST Investigators, 1989). Drug-induced proarrhythmia occurred despite the suppression of spontaneous ventricular arrhythmia by antiarrhythmic therapy. One explanation is myocardial remodelling during the healing period that may alter underlying electrophysiological properties. Another explanation is the potential presence of recurrent acute myocardial ischemia which produces an unstable and potentially arrhythmogenic substrate capable of generating a reentrant arrhythmia. Under these conditions, antiarrhythmic drugs may enhance the likelihood of arrhythmia. Transient ischemia may create a transient proarrhythmic risk.

Experimentally, the incidence of lethal ventricular arrhythmias during acute myocardial ischemia is much greater in the presence of potent Na^+ channel blockers (Elharrar et al, 1977; Nattel et al, 1981a; Lederman et al, 1989). In a model of acutely ischemic heart, lidocaine tended to increase the risk of profibrillatory effects and these were dose-dependent (Carson et al, 1986; Aupetit et al, 1995). Although flecainide-induced proarrhythmia has been reported in healthy hearts (Salerno et al, 1991) the proarrhythmic effects of various class I drugs (flecainide, tocainide, procainamide, propafenone and lidocaine) has been demonstrated most consistently in models of myocardial infarction (Zimmerman et al, 1985; Lynch et al, 1987; Miyazaki et al, 1988; Kidwell and Gonzalez, 1993; Wallace et al, 1993; Steinberg et al, 1992).

4.4.4 Heart rate

Some physiologic factors such as changes in heart rate (Hoffmann et al, 1986; Anastasiou-Nana et al, 1987) and in autonomic activity (Podrig et al, 1990) may predispose to proarrhythmia. Class I antiarrhythmic agents have frequency-dependent effects on cardiac sodium channels. This use-

dependent behavior causes increased sodium channel blockade at faster stimulation rates (Grant et al, 1984; Hondeghem and Katzung, 1977). The kinetics of drug association and dissociation from the Na^+ channel are believed to be responsible for the differences observed in the use-dependence of antiarrhythmic drug actions (Campbell, 1983). Quantitative studies showed the kinetics of action of sodium channel blockers on conduction in *in vivo* animal models (Davis et al, 1986; Nattel, 1985; Bajaj et al, 1987). *In vitro* studies, have shown use-dependent changes in V_{max} in the presence of class I drugs that parallels changes in conduction velocity (Nattel, 1985; Nattel, 1987a; Nattel, 1987b). The rate-dependent conduction slowing associated with the class IC drugs may be implicated in cardiotoxic effects occurring at usual dosages of antiarrhythmic agents. Drugs which possess slow unbinding time constants, like class IC drugs, are more likely to have heart rate-dependent effects even at therapeutic doses and physiologic heart rates.

Electrophysiologic studies with electrical stimulation have shown qualitative frequency-dependent sodium channel blockade for antiarrhythmic drugs in human (Gang et al, 1985; Morady et al, 1985). The sinus tachycardia during exercise testing may play an important role in exposing proarrhythmic effects during drug therapy. Exercise-induced ventricular tachycardia have been observed in patients during therapy with flecainide, suggesting a proarrhythmic effect associated with class I toxicity (Anastasiou-Nana et al, 1987; Hoffmann et al, 1985; Cascio et al, 1988). Quantitative work studying consequences of use-dependent sodium channel blockade for antiarrhythmic drugs in man was not done prior to our work. Use-dependent class I type of proarrhythmia has been observed in animal models, although the underlying mechanisms have not been defined (Nattel, 1985; Carson et al, 1986; Anderson et al, 1990).

In a theoretical model, Starmer and colleagues have shown that drugs with slow use-dependent kinetics are capable of increasing the duration of the "vulnerable window" during which premature stimuli may cause unidirectional block and reentrant activation (Starmer et al, 1991). They showed that this proarrhythmic response was enhanced at faster stimulation rates.

4.4.5 Autonomic activity

The autonomic nervous system modulates cardiac rhythms. Sympathetic and parasympathetic nervous systems, α- and ß-adrenergic, muscarinic and

purinergic receptors may influence cardiac electrophysiological properties and play a role in arrhythmogenesis associated with myocardial ischemia and infarction.

When transmural infarct extends to the epicardial surface it may produce heterogeneous denervation, not only in the infarcted region, but also in some normal regions distal to the infarct (Barber et al, 1983). The heterogeneity of sympathetic denervation due to infarction may influence refractoriness and experimental evidences has shown that it could have an arrhythmogenic effect (Gaide et al, 1983; Kozlovskis et al, 1986). Clinical and experimental evidences suggested that a sudden augmentation of sympathetic activity with a concomitant reduction of vagal tone, may alter cardiac electrical properties and precipitate the occurrence of ventricular arrhythmias, particularly in a setting of acute myocardial ischemia (Malliani et al, 1980; Lombardi et al, 1983; Podrid et al, 1990). Increased vagal activity has been shown to reduce the incidence of fibrillation during acute myocardial ischemia (Vanoli et al, 1991) an effect that could be partially mediated by a decrease in heart rate. However vagal stimulation may enhance ventricular arrhythmias, possibly by unmasking ventricular automaticity in one-day post-myocardial infarction (Scherlag et al, 1974). In the ischemic heart, vagal stimulation may improve the marked disparities in repolarization between the ischemic region and normal region, a phenomenon linked to initiation of malignant ventricular arrhythmias in the ischemic heart (Janse et al, 1980). Clinically, there is indirect evidence (heart rate variability) that a decrease in vagal activity could be associated with increased mortality in post-myocardial patients (Kleiger et al, 1987).

Thus for drugs which interact with the sympathetic or parasympathetic nervous system, changes in autonomic nervous system function could affect their proarrhythmic potential. Regional sympathetic denervation and supersensitivity could modulate drug actions and cause the drugs to affect the myocardium heterogeneously, resulting in proarrhythmia (Stanton and Zipes, 1989). Myerburg et al have suggested that class IC type of proarrhythmia in patients may be reversed by treatment with propanolol (Myerburg et al, 1989). They suggest a possible interaction of antiarrhythmic drugs with autonomic function in the occurrence of proarrhythmia. ß-adrenergic stimulation shortens ventricular refractoriness (Nattel et al, 1981b) and ß-blockade increases the ventricular refractory period, which in theory could prevent the induction of VT (Brodsky et al, 1989).

4.5 Cardiotoxicity by antiarrhythmic drugs and its reversal

4.5.1 Cardiotoxic manifestations

Arrhythmia aggravation may occur at antiarrhythmic drug blood levels in the therapeutic range, but there is evidence that proarrhythmia incidence is dose-related and rises with increasing plasma concentration of class IC drugs (eg. flecainide) (Morganroth, 1987). Blood levels in the toxic range are associated with the occurrence of proarrhythmic effects such as a new tachyarrhythmia or bradyarrhythmia and this suggests that drug toxicity may play a role in class I antiarrhythmic agent proarrhythmia.

At high plasma concentrations of class I drugs such as quinidine and procainamide, cardiac toxicity may result in prolongation of the QRS duration and QT interval on the electrocardiogram (Heissenbuttel and Bigger, 1970; Wasserman et al, 1958). The cardiotoxic effects include conduction slowing in all parts of the heart leading to polymorphic tachycardia, ventricular premature depolarization, ventricular tachycardia and ventricular fibrillation (Nathan et al, 1984; Winkelmann et al, 1987). The Purkinje fibers may become depolarized and develop abnormal automaticity. SA block or arrest, AV block, ventricular tachyarrhythmias and asystole may occur (Biggs et al, 1977). Class IC drugs are potent sodium channel blockers and produce depression of conduction even at therapeutic plasma concentrations. Agents such as flecainide and encainide produce slowing of conduction of all specialized cardiac conduction system and ventricular myocardium as seen by a marked increase in the QRS duration, the AH, HV, PR and QTc intervals on the electrocardiogram (Platia et al, 1985; Roden and Woosley, 1986; Woosley et al, 1988; Anderson et al, 1990; Nathan et al, 1984; Estes et al, 1984; Hellestrand et al, 1982). Life-threatening ventricular arrhythmias represent a manifestation of the cardiotoxicity associated with class IC antiarrhythmic agents (Chouty et al, 1987; Winkle et al, 1981; Buss et al, 1985). The marked QRS prolongation seen with these arrhythmias may result from the enhancement of sodium channel blocking properties of class I drugs. Cardiac toxicity is also a frequent complication of overdose with tricyclic antidepressants that have sodium channel blocking properties (Biggs et al, 1977). In experimental studies high plasma concentrations of class IC drugs have been shown to produce severe conduction slowing and to be arrhythmogenic (Dawson et al, 1984; Zimmerman et al, 1985).

4.5.2 Use of sodium salts to treat drug-induced cardiotoxicity

Case reports suggest that intoxication with class I antiarrhythmic drugs associated with symptoms such as widening of QRS duration on the electrocardiogram, bradycardia, low blood pressure and arrhythmias may be treated or partially reversed with the administration of sodium lactate (Wasserman et al, 1959; Pentel et al, 1986; Winkleman and Leinberger, 1987; Chouty et al, 1987; Camous et al, 1987). Experimental studies report that sodium lactate, sodium chloride and sodium bicarbonate (Bellet et al, 1959a and 1959b; Cox and West, 1961; Nattel et al, 1984; Nattel and Mittleman, 1984; Sasyniuk and Jhamandas, 1984; Pentel and Benowitz, 1984; Bajaj et al, 1989; Keyler and Pentel, 1989; Gardner et al, 1990; Beckman et al, 1991; Turgeon et al, 1992) can partially reverse cardiac toxicity associated with drugs possessing sodium channel blocking properties such as quinidine, procainamide, amitriptyline, desipramine, flecainide, propafenone, encainide and cocaine.

The potential mechanisms by which sodium salts may reverse the drug-induced cardiotoxic effects are not well understood and no consensus has been reached concerning the exact mechanisms of action of sodium salts in reversing drug-induced cardiotoxic effects. Beneficial effects have been attributed to an increase of extracellular sodium concentration, a decrease in potassium concentration, changes in pH, a metabolic effect of lactate, a decrease in plasma drug concentration, intravascular volume expansion and improvement of longitudinal propagation. Alkalinization by sodium bicarbonate and hyperventilation had similar effects in reversing drug-induced arrhythmias by amitriptyline intoxication (Nattel et al, 1984, Nattel an Mittleman, 1984). However Pentel and Benowitz reported that the major factor in reversing slowing of conduction (QRS prolongation) due to desipramine in rats was increasing the sodium concentration rather than correction of acidosis or increasing blood pH. In addition they attributed smaller beneficial effects to the improvement of intravascular volume expansion (Pentel and Benowitz, 1984). The increase in extracellular concentration could also enhance the driving force for sodium entry during phase 0 (increase in sodium current) thereby improving ventricular conduction. Bajaj et al (1989) reported that partial reversal of slowing of conduction induced by C-desmethyl encainide was mostly due to sodium loading itself, rather than to concomitant changes in plasma drug concentration, pH, serum potassium concentration, or osmolarity. The effects of sodium salts may also depend on factors such as the individual properties of the different sodium channel blockers: their pKa (degree of ionization), lipid solubility or molecular weight.

Kohlhardt (1982) suggested that sodium ions play an important role in the interaction between antiarrhythmic drugs and sodium channels. He reported that tonic block is more sensitive to changes of extracellular sodium concentration than phasic block. Drugs like propafenone shift the steady state inactivation of sodium current to more negative potentials, and this shift is altered by changing extracellular sodium concentration (Kohlhardt, 1982; Sasyniuk and Jhamandas, 1984). The modulation of drug-induced sodium channel blockade by sodium ions could be due to the alterations of sodium channel protein charges (Brown and Noble, 1978) or by sodium ions directly regulating access of the drug to its receptor (Calahen and Almers, 1979). The precise mechanism by which sodium ions antagonize drug-induced sodium channel blockade has not been defined.

Experiments using whole-cell voltage clamp showed that lidocaine, a drug which has a fast unbinding constant, may reverse the cardiotoxic effects of a second drug (propoxyphene) which has slow unbinding kinetics. These results suggest that the two drugs compete for the same receptor site (Whitcomb et al, 1989). Using a similar technique, another study has investigated the potential mechanisms by which extracellular sodium concentration may modulate the blocking effect of lidocaine, a drug which binds to inactivated sodium channels, and disopyramide, a drug which binds to open channels. They concluded that increase in extracellular sodium concentration can reverse the sodium blocking actions of drugs by either an increase in single-channel amplitude or by a decrease in drug association rate with the sodium channel, depending on the mode of block of the specific drug (Barber et al, 1992).

5. STATEMENT OF THE PROBLEM

The information presented in the Introduction indicates the clinical importance of the cardiotoxicity of class I drugs, especially class IC agents. Cardiotoxic effects of antiarrhythmic agents may be rate-related or due to intoxication, and may lead to potentially lethal ventricular arrhythmias. The mechanisms by which class I drugs cause toxicity are unclear, as are the mechanisms by which sodium salts reverse their cardiotoxic effects.

The goals of my research were (1) to study the mechanism by which class IC antiarrhythmic drugs cause ventricular arrhythmias and (2) to explain the mechanism of action of sodium salts in the reversal of the class IC cardiotoxicity.

Specifically, we aimed to determine (1) whether the reported relationship between exercise and ventricular proarrhythmia could be due to rate-dependent increases in drug-induced conduction slowing, (2) whether rate-dependent conduction slowing is related to use-dependent sodium channel blocking actions, (3) whether the mechanisms of proarrhythmia can be studied in detail in an animal model and, if so, what factors determine the occurrence and manifestations of proarrhythmia and finally, (4) what mechanisms underly the reversal of class IC cardiotoxicity by hypertonic sodium salts.

Rate-dependent conduction slowing by class I antiarrhythmic agents have clinical implications which are important regarding the mechanisms of action of antiarrhythmic agents *in vivo*. In Publication 1, we used flecainide as a prototype class I agent because it is a potent sodium channel blocking agent and it would be likely to show *in vivo* drug-induced ventricular conduction slowing. Previously it was demonstrated that flecainide-induced reduction of V_{max} is augmented by increases in stimulation frequencies throughout the physiologic range of heart rates (Varro et al, 1985; Campbell and Williams, 1983). Although there had been reports of proarrhythmia occurrence during exercise (Anastasiou-Nana et al, 1987), the mechanisms of the interaction between exercise and antiarrhythmic drug action had not been defined and the role of heart rate *per se* in the rate-dependent effects of class IC drugs has not been evaluated. The goals of the clinical studies (Publications 1 and 2) were to determine the mechanisms by which class IC antiarrhythmic drugs may induce ventricular conduction slowing during the sinus tachycardia of

exercise (Publication 1), to evaluate the kinetics of conduction slowing caused by a variety of sodium channel blockers and to compare these to the kinetics of depression of sodium current indexes *in vitro* (Publications 1 and 2). If drug-induced ventricular conduction slowing by class I antiarrhythmic agents is due to sodium channel blockade, the kinetics of conduction slowing *in vivo* should be related to those of sodium channel blockade.

Proarrhythmic events have been reasonably well described, classified and identified in patients subjected to commonly used antiarrhythmic drugs such as quinidine, lidocaine, mexiletine, amiodarone, propafenone, flecainide and encainide (Morganroth et al, 1986; Morganroth, 1987; Velebit et al, 1982; Podrig, 1993). A variety of mechanisms (similar to the basic mechanisms of cardiac arrhythmias described in section 2) may be responsible for the different types of drug-induced proarrhythmic events clinically observed. Antiarrhythmic drugs could potentiate an arrhythmia mechanism already present or create the electrophysiologic substrate for an arrhythmia based on a new mechanism.

In Publication 1, during the course of the exercise testing, a patient receiving flecainide therapy experienced a sustained ventricular tachycardia at peak exercise. This patient, who had the greatest increase in QRS duration during exercise, had a previous myocardial infarction and was diagnosed as having coronary artery disease. This observation prompted questions about the mechanism of proarrhythmia associated with marked slowing of conduction caused by class IC drugs. Many factors predisposing to proarrhythmia have been identified: myocardial ischemia or previous myocardial infarction, a history of sustained ventricular tachyarrhythmia, chronic coronary artery disease, structural heart disease (poor left ventricular function) and high dose of class IC agents (Morganroth and Horowitz, 1984; Morganroth et al, 1986; Morganroth, 1987; Slater et al, 1988; Podrig, 1993). Some experimental studies have used normal animals to assess drug-induced proarrhythmia or cardiotoxic effects (Hodess et al, 1979; Salerno et al, 1991), but a more pertinent animal model is to reproduce post-infarction conditions where there may be a substrate that can support reentry (El-Sherif et al, 1977; Karagueuzian et al, 1979; Wit et al, 1982; Garan et al, 1985). In Publication 3, we used both normal and infarcted canine models (anesthetized, whole dog experiments) to perform a systematic evaluation of the occurrence, concentration-dependence and mechanisms of flecainide-induced proarrhythmia. Our experimental model took into consideration some important factors suggested (clinically and/or experimentally) to promote proarrhythmia such as previous

myocardial infarction, drug concentration, ectopic activity (premature stimulation for induction).

The exact mechanism of drug-induced proarrhythmia had not been determined precisely. Proarrhythmia associated with class IC agents was thought to be due to excess sodium channel blockade, and clinical and experimental studies proposed that drug-induced conduction slowing and changes in the refractory period are involved in arrhythmias due to reentry (Coromilas et al, 1988; Brugada et al, 1991b; Rinkenberger et al, 1982). Functionally, the initiation and maintenance of reentry depends on a critical balance between conduction velocity and refractoriness (Mines, 1913; Allessie et al, 1973), and conditions for reentry include unidirectional conduction block and conduction slowing. If drug-induced conduction slowing potentiates reentry and represents the mechanism underlying proarrhythmia with class IC drugs, then drugs possessing strong channel blocking properties and slight effects on refractoriness, such as flecainide, should be associated with proarrhythmic actions due to the promotion of ventricular reentry. In Publication 3, we examined the electrophysiological properties of the region supplied by the left anterior descending coronary artery, using mapping techniques, and we determined the mechanisms leading to flecainide-induced arrhythmias.

Some of the cardiotoxic effects of class IC drugs due to intoxication, in particular prolongation of the QRS duration and conduction slowing, resembles those observed with rate-related cardiotoxic effects. Experimental studies have shown that high class IC drug plasma concentration produce severe conduction slowing and can cause arrhythmias (Dawson et al, 1984; Zimmerman et al, 1985). Clinically, hypertonic sodium salts have been found to reverse cardiotoxic effects of class I drugs (Chouty et al, 1987; Camous et al, 1987; Pentel et al, 1986; Winkelmann and Leinberger, 1987) but their mechanisms of action were not clear. Beneficial effects have been attributed to an increase in extracellular sodium concentration, changes in plasma drug concentration, a decrease in potassium concentration, alkalanization, a change in osmolarity. In Publication 4 both electrophysiological and biochemical approaches were used to study the mechanisms by which sodium salts reverse flecainide's cardiotoxicity.

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CHAPTER II

PUBLICATIONS

As described in section 4 of the Introduction, proarrhythmic events have been reported with a variety of class I antiarrhythmic agents but it is class IC drugs that have a higher propensity to proarrhythmia. The clinical manifestations of proarrhythmia associated with potent sodium channel blockers include severe conduction slowing and potentially lethal ventricular arrhythmias.

To investigate clinically the mechanism by which sodium channel blockers induce ventricular conduction slowing, we used flecainide as a prototype of its class and QRS duration on the ECG recorded during exercise testing as an index of ventricular conduction slowing. If drug-induced ventricular conduction slowing during the sinus tachycardia of exercise is due to sodium channel blockade then the kinetics of QRS prolongation should be similar to those of sodium channel blockade, and QRS prolongation during tachycardia caused by exercise should be similar to that caused by ventricular pacing.

Amplification of Flecainide-Induced Ventricular Conduction Slowing by Exercise

A Potentially Significant Clinical Consequence of Use-Dependent Sodium Channel Blockade

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Proarrhythmic effects of flecainide acetate have been reported during exercise, but the mechanism for the arrhythmogenic interaction between flecainide and exercise is unknown. We hypothesized that the sinus tachycardia of exercise may enhance flecainide-induced conduction slowing by increasing use-dependent sodium channel blockade, thereby facilitating the occurrence of ventricular reentry. To evaluate the modulation of flecainide's effects by exercise, we studied 19 patients who were receiving therapeutic doses of flecainide for the treatment of cardiac arrhythmias. Sixteen patients underwent treadmill exercise testing by a modified Bruce protocol. During exercise, QRS duration increased progressively from 94±22 msec (mean±SD) at rest to 116±25 msec ($p<0.001$) at a mean heart rate increase of 84±32 beats/min. The patient with the greatest QRS increase developed a monomorphic ventricular tachycardia at peak exercise. At rest, the QRS duration after treatment with flecainide increased 12.1±10.0% compared with the pretreatment value, and with exercise, the QRS duration increased by a further 28.1±17.0% compared with the predrug value. We found that the best predictor of further exercise-induced QRS slowing was the change in QRS duration produced by flecainide at rest ($r=0.76$, $p=0.001$). In an age- and disease-matched control group, the QRS duration did not change during exercise that caused a similar heart rate increase. Abrupt increases in heart rate by ventricular pacing during electrophysiologic study in seven patients prolonged their QRS duration as an exponential function of beat number, with an onset rate constant (0.033±0.006/beat) that is comparable to flecainide's rate constant for use-dependent changes in $\dot{V}_{max}$ in vitro. The QRS increases were similar when compared for corresponding heart rate changes produced by ventricular pacing (13.9±3.1%) and by exercise (12.6±6.7%) in four patients undergoing both. We conclude that exercise causes a rate-dependent augmentation of flecainide's effects on ventricular conduction by enhancing state-dependent sodium channel blockade, potentially causing ventricular arrhythmogenesis in predisposed patients. (*Circulation* 1989;79:1000–1006)

Class I antiarrhythmic drugs have frequency-dependent effects on cardiac sodium channels, leading to greater reductions in $\dot{V}_{max}$ of ventricular tissue at faster stimulation rates. These effects have been incorporated into recent molecular models of antiarrhythmic drug action.[1–4] The first model[1,2] is based on the interaction between drugs and sodium channels on different affinities of various drugs for different sodium channel states. The second model[3,4] is based on drug binding to a constant affinity channel receptor, with receptor access determined by channel gating. These models have important potential clinical implications[2] related to the mechanisms of antiarrhythmic drug action in vivo. Electrophysiologic studies with programmed electrical stimulation have shown qualitative frequency-dependent sodium channel blockade for antiarrhythmic drugs in humans.[5,6] In addition, quantitative studies have shown kinetics of action of sodium channel blockers in in vivo animal models[7–9] parallelling their kinetic effects in vitro.[7,10,11] There

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TABLE 1. Characteristics of the Patient Population

Characteristic	Patients receiving flecainide		Controls
	Exercise study	Electrophysiologic study	
n	16*	7*	6
Age (yr)	54.4±15.2	58.6±10.8	51.3±7.4
Sex			
Male	14	7	5
Female	2	0	1
Cardiac diagnosis			
Coronary artery disease	10	6	5
Mitral valve prolapse	1	0	0
Other†	5	1	1
Flecainide			
Plasma concentration (μmol/l)	1.5±0.6	1.2±0.3	—
Dosage (mg/day)	250±52	271±49	—
Medication			
Digitalis (*n*/total)	4/16	4/7	0/7
β-Blockers (*n*/total)	3/16	1/7	3/7

*Four patients underwent both the exercise and the electrophysiologic studies.

†Consists in the exercise group of five patients with cardiac arrhythmias (paroxysmal atrial fibrillation, 2; nonsustained ventricular tachycardia, 1; isolated premature ventricular contractions, 1; and paroxysmal atrial tachycardia, 1) and no known heart disease. In the electrophysiologic study group, one patient had a cardiomyopathy due to alcoholism. In the control group, one patient had isolated premature ventricular contractions and no structural heart disease.

is, therefore, good evidence to suggest that the predictions of basic molecular models of antiarrhythmic drug action[1-4] apply to the effects of these agents on conduction in vivo.

Flecainide acetate is a class IC antiarrhythmic agent according to the modified Vaughan-Williams classification[12] and is effective in treating a variety of cardiac arrhythmias.[13] Flecainide has caused occasional proarrhythmic reactions, including the de novo occurrence of sustained ventricular tachyarrhythmias or an increase in the severity of preexisting ventricular arrhythmias.[14,15] A recent study suggests that flecainide-induced ventricular tachyarrhythmias have a propensity to occur during exercise.[16] The mechanisms underlying exercise-induced arrhythmogenesis in the presence of flecainide have not been previously examined.

The conduction slowing typically produced by flecainide is probably due to sodium channel blockade, as reflected by the depression of $\dot{V}_{max}$ that it produces in vitro.[17,18] Like other class I drugs, flecainide reduces $\dot{V}_{max}$ slightly in the absence of stimulation, but with stimulation, a substantial decline in $\dot{V}_{max}$ to a new steady state occurs.[17] A similar rate-dependent change in block occurs with an abrupt change in stimulation frequency.[17,18] Flecainide-induced reductions in $\dot{V}_{max}$ are substantially augmented by increases in stimulation frequency throughout the physiologic range of heart rates.[17,18]

We reasoned that the sinus tachycardia of exercise may amplify the sodium channel blockade produced by flecainide acetate through rate-dependent mechanisms. This amplification would result in an increase in drug-induced conduction slowing and possibly lead to ventricular arrhythmogenesis in predisposed patients. The purpose of this study was to determine whether flecainide-induced ventricular conduction slowing is increased by exercise and to determine the mechanism responsible for this phenomenon.

Methods

Patient Population

The study group consisted of 19 patients receiving flecainide acetate for the treatment of cardiac arrhythmias. Six patients not treated with flecainide served as the control group. The characteristics of both groups are summarized in Table 1. Groups did not differ significantly in age, sex, or cardiac diagnosis.

Exercise Testing

Treadmill exercise testing was conducted according to a modified Bruce protocol.[19] A 12-lead electrocardiogram (ECG) was obtained before exercise. ECG leads CC5, CM5, and CL were used for monitoring during exercise. Recordings were obtained at a paper speed of 100 mm/sec before the exercise test and at frequent intervals during the test.

Electrophysiologic Study

Electrophysiologic testing was performed in seven patients in the fasting, nonsedated state as part of the clinical evaluation of drug efficacy in treating ventricular tachycardia. Four of these patients also underwent exercise testing while receiving oral flecainide. A quadripolar electrode catheter was positioned in the right ventricular apex by way of

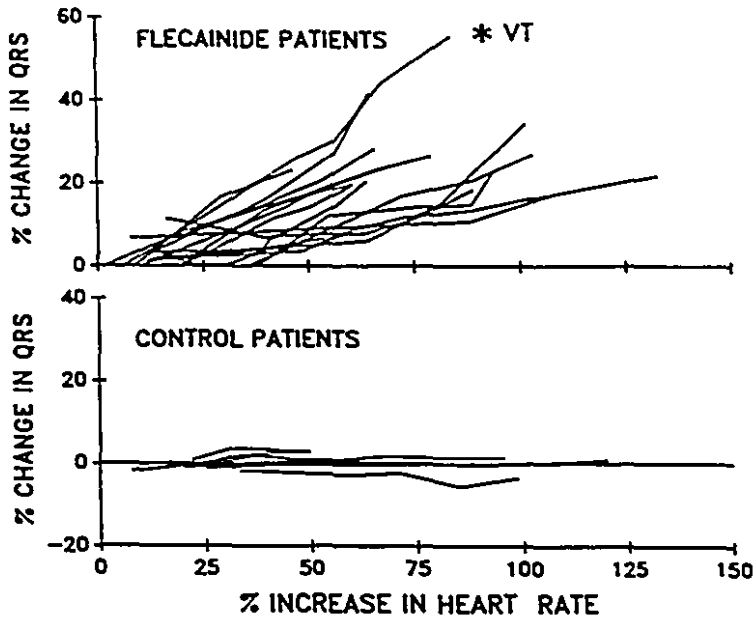

FIGURE 1. *Plots of changes in QRS duration relative to increases in heart rate in patients treated with flecainide* (top panel) *and in the control group* (bottom panel). *All changes are expressed as a percent increase from the values measured at rest immediately before exercise.* Top Panel: *In all patients receiving flecainide, QRS duration increased progressively during exercise. QRS increases at peak heart rate varied from 6.7 to 55.9% (mean, 24±12.7%) among patients. The patient who had the greatest exercise-induced change in QRS duration (•) had a ventricular tachycardia (Figure 2) at peak heart rate during exercise.* Bottom Panel: *In control patients, the percent change in QRS duration did not deviate significantly from the zero line; values vary from −5.6 to 3.8%.*

the right femoral vein. Stimulation was performed with 1.5-msec square wave pulses with twice late diastolic threshold current controlled by a programmable stimulator (Bloom Associates, Flying Hills, Pennsylvania). Electrocardiographic leads I, aV_F, and V_1 and a right ventricular electrogram were recorded simultaneously at 100 mm/sec with a paper recorder (Mingograf T16, Siemens-Elema, Stockholm, Sweden). The kinetics of flecainide-induced conduction slowing were assessed by 30–60-second trains of ventricular stimulation at rates of 100, 120, and 150 beats/min. No stimulation was performed for a minimum of 60 seconds between trains of ventricular stimuli. All runs of test stimuli were begun abruptly during sinus rhythm, and stimulation was continued in all cases until changes in QRS duration had stabilized. The study protocol lasted approximately 10 minutes and was performed before the routine clinical study in each case.

Drug Dosage and Assay

All patients had been receiving flecainide at the same dose for at least 3 days at the time of either exercise or electrophysiologic study. The attending physician selected the flecainide dose based on clinical criteria. Blood samples for subsequent flecainide assay were drawn at the time of the exercise test or electrophysiologic study. Plasma flecainide concentrations were measured with a commercially available fluorescence polarization immunoassay technique (Abbott Laboratories, Mississauga, Ontario, Canada).

Data Analysis

Exercise-induced QRS changes. Only ECG tracings showing normal sinus rhythm were used for analysis. QRS duration was measured independently by two observers who were unaware of the identity and treatment status of each patient. The electrocardiographic lead that best allowed identification of the onset and offset of QRS complexes was used for all QRS measurements in a given patient. Each observer measured the average duration of three consecutive QRS complexes at each heart rate. The average result of the two observers was used for analysis of the relation between heart rate and QRS duration.

Changes in QRS duration resulting from ventricular pacing. QRS duration was measured as a function of beat number after the onset of ventricular pacing. A steady state was always achieved within 90 complexes after the onset of stimulation. The mean QRS duration of three complexes at steady state was considered representative of the QRS duration at that frequency. Only QRS complexes of consistent configuration were used for analysis. Capture or fusion complexes occasionally occurred and were excluded from consideration. Rate-related changes were evaluated by comparing steady-state QRS duration at a more rapid ventricular pacing rate with the steady-state duration of QRS complexes of the same configuration at a slower rate. The kinetics of the onset of rate-dependent block were analyzed by previously described methods.[11,12,20]

Statistical Analysis. Group data are presented as the mean±SD. Comparisons between two groups of experimental data were made with unpaired Student's *t* tests. We used multilinear regression analysis to relate drug-induced QRS prolongation during exercise to possible determining factors (flecainide dose, serum flecainide concentration, percent change in QRS duration at rest resulting from flecainide, and percent change in heart rate

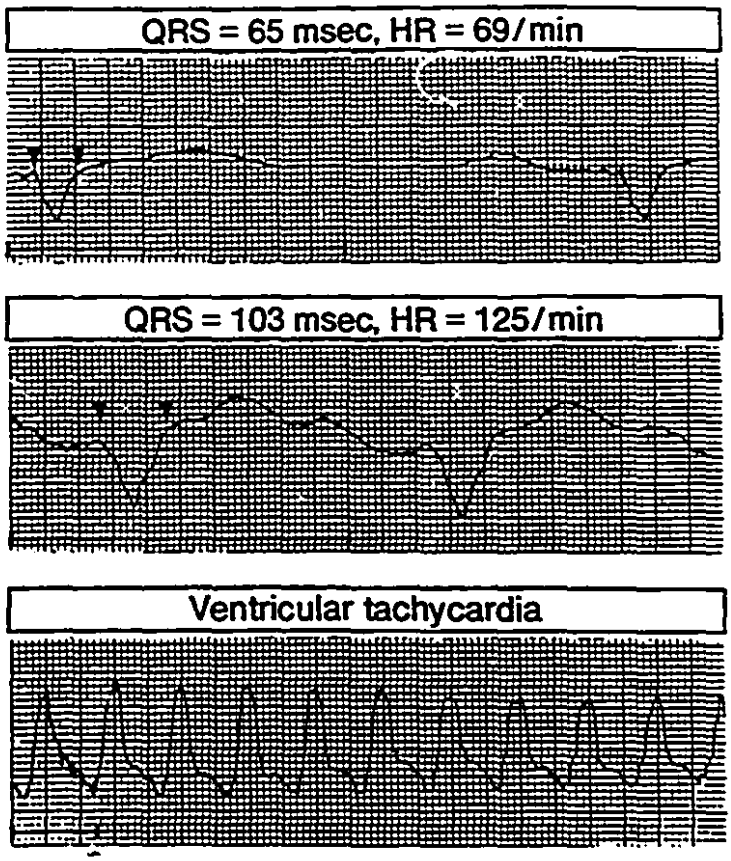

FIGURE 2. *Electrocardiographic tracings of exercise-induced ventricular tachycardia in a patient receiving flecainide. ECG lead CC5 was recorded at 100 mm/sec paper speed (top and middle panels) or 25 mm/sec (bottom panel).* Top Panel: *Recording at rest, showing a QRS duration of 65 msec at a heart rate of 69 beats/min. Arrows indicate the onset and the termination chosen for QRS measurement.* Middle Panel: *Recording at peak exercise. Heart rate increased with exercise to 125 beats/min (81% compared with the rate at rest), and the QRS duration increased to 103 msec, without any change in QRS configuration.* Bottom Panel: *Recording obtained just after the electrocardiogram shown in the middle panel, showing a monomorphic ventricular tachycardia at 155 beats/min. The ventricular tachycardia began and ended spontaneously (onset and termination not recorded), and lasted for a total of 30 seconds. This patient had had an exercise test before flecainide therapy and achieved a similar workload without QRS duration changes or ventricular arrhythmias. Scale markers, 0.5 seconds.*

during exercise).[21] The significance of regression was determined by an analysis of covariance. Two-tailed tests were used for all statistical comparisons, and $p<0.05$ was considered to be significant.

Results

Exercise

In all patients receiving flecainide, QRS duration increased progressively during exercise from 94±22 to 116±25 msec ($p<0.001$) at a mean heart rate increase of 84±32 beats/min (Figure 1, top). In the control group, the QRS duration was 79±10 msec before and 80±11 msec (NS) after exercise, which caused a heart rate increase of 84±29 beats/min (Figure 1, bottom). The patient receiving flecainide who had the greatest QRS increase during exercise developed a ventricular tachycardia at peak heart rate. His ventricular tachycardia (Figure 2, bottom) was monomorphic and was sustained for 30 seconds, after which it terminated spontaneously. This patient had had a previous exercise test while not receiving flecainide and had shown neither arrhythmia nor QRS prolongation at a similar work load.

The QRS durations of patients before treatment were compared with those of patients at rest and at peak exercise during treatment with flecainide. The QRS duration of patients at rest after treatment with flecainide increased by 12.1±10.0% compared with the pretreatment value. During exercise, the QRS increased a further 28.1±17.0% compared with the predrug value. Stepwise multilinear regression analysis showed that the only variable that significantly correlated with the extent of exercise-induced QRS increase was the percent increase in QRS duration on the resting ECG produced by flecainide compared with pretreatment values ($r=0.76$, $p=0.001$). The only other variable that improved the multilinear correlation coefficient was the percent heart rate increase resulting from exercise, which improved the r value to 0.84 ($p<0.001$) when included in the analysis. Neither flecainide dose nor serum flecainide concentration were independent predictors of the degree of exercise-induced QRS prolongation.

Electrophysiologic Study

Seven patients who received flecainide were studied during electrophysiologic testing. In all seven, abrupt changes in heart rate by ventricular pacing increased QRS duration. Changes in QRS duration after the onset of ventricular pacing followed an exponential relation with beat number (Figure 3). The mean rate constant for QRS prolongation in the seven patients studied was 0.033±0.006/beat. Four patients underwent both electrophysiologic study and exercise testing. In these four patients, the QRS increase resulting from exercise (12.6±6.7%) was very similar to the QRS

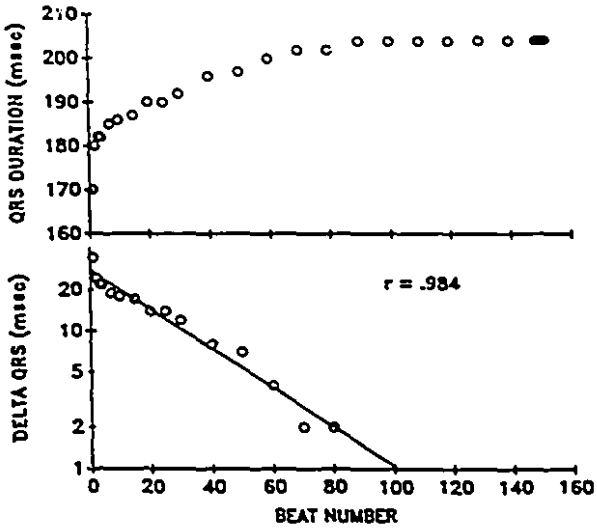

FIGURE 3. *Plots of time-dependent increase in QRS duration during ventricular pacing in a representative patient treated with flecainide.* Top Panel: *An abrupt change in heart rate produced by ventricular stimulation at a basic cycle length of 400 msec produced a gradual prolongation in QRS duration.* Bottom Panel: *Changes in QRS duration (DELTA QRS) are calculated as the difference between the value attained at steady state and the value for a given beat. Plotting of DELTA QRS on a logarithmic scale as a function of beat number shows a linear relation with an onset rate constant of 0.033/beat.*

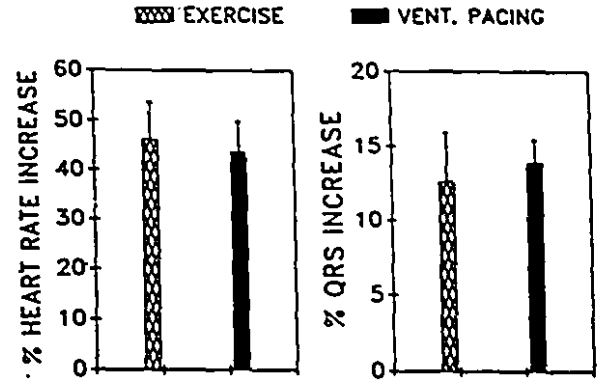

FIGURE 4. *Bar graphs of rate-dependent changes in QRS duration resulting from exercise (hatched bars) and ventricular pacing (solid bars). When a similar degree of heart rate augmentation was produced by either technique* (left graph), *the corresponding QRS prolongation was very similar* (right graph). *Results are mean±SD from four patients undergoing both the exercise and pacing protocols.*

increase produced by ventricular pacing (13.9±3.1%, p=NS) when values were compared for a corresponding increase in heart rate (Figure 4).

Discussion

We have shown that flecainide-induced QRS prolongation is increased by exercise. The degree of additional change in QRS duration produced by exercise was similar to that produced by ventricular pacing throughout a corresponding range of heart rates. A comparable degree of exercise did not alter QRS duration in a set of disease-matched control patients. These observations suggest that the tachycardia associated with exercise is the major factor responsible for the additional conduction slowing resulting from exercise in patients treated with flecainide. Although other factors such as autonomic changes and myocardial ischemia may have had a modulating role, the occurrence of exercise-induced QRS prolongation in patients treated with flecainide while on β-blocker therapy, as well as in several patients without coronary artery disease, suggests that these other factors were not of primary importance. Furthermore, the changes in QRS duration produced by ventricular pacing displayed an exponential onset that had a rate constant of 0.033±0.006/beat. This onset rate constant is very similar to that determined for flecainide effects on $\dot{V}_{max}$ (an index of inward sodium current) in vitro, 0.029±0.006/beat.[17] Therefore, our results provide strong evidence that exercise enhances flecainide's effects on ventricular conduction by increasing the drug's rate-dependent interaction with cardiac sodium channels.

Our results provide further insights into the underlying mechanisms of the interaction between exercise and flecainide. In a recent study, Cascio et al[22] showed that exercise accentuated the QRS prolongation produced by amiodarone, which also has use-dependent class I properties.[23,24] Our study differs from theirs in that we have both evaluated the specific role of heart rate by comparing QRS changes during exercise with those resulting from tachycardia produced by ventricular pacing and studied the onset time course of conduction changes during pacing at a constant frequency. We found that, in patients receiving flecainide, the increase in QRS duration at rest was the most important determinant of the degree of further QRS prolongation resulting from exercise. Similarly, Cascio et al[22] found that the degree of amiodarone-induced QRS prolongation at rest is an important predictor of further QRS prolongation produced by exercise. These results are not surprising because the degree of QRS prolongation on the resting ECG is a direct indicator of the drug's pharmacodynamic action of interest.

Antiarrhythmic drugs could have arrhythmogenic actions by a variety of mechanisms, including abnormal impulse formation (afterdepolarizations and abnormal automaticity) and abnormal impulse propagation resulting in reentry.[25,26] Class IC drugs do not result in cellular calcium overload or action potential prolongation.[12] Therefore, they would be unlikely to produce delayed or early afterdepolarizations.[27-29] The most characteristic property of class IC agents is their potent sodium channel

blocking and conduction-slowing action,[12] which is most readily related to their arrhythmogenic potential in the context of a reentrant arrhythmia mechanism. The ability to sustain reentry depends on a critical balance between conduction velocity and refractoriness in the reentrant circuit.[26,30] A potential reentrant circuit could exist, particularly in the presence of heart disease, but if refractoriness exceeded circuit time, no manifest reentry would occur. If a drug slowed conduction in this circuit to the point at which conduction time exceeded refractory period, sustained reentry would then become possible.

The occurrence of this type of arrhythmogenic mechanism should depend on the presence of a preexisting substrate that can support reentry and on the magnitude of drug-induced conduction slowing. Consistent with this mechanism is the observation that flecainide is much more likely to produce proarrhythmic effects in patients with a history of structural heart disease or ventricular tachyarrhythmias or both than in patients without such a history.[31] Similarly, flecainide, even at toxic doses, does not induce ventricular arrhythmias in normal dogs,[32] but it does cause dose-related arrhythmogenicity in dogs with prior myocardial infarctions.[33] In patients predisposed to develop reentrant ventricular arrhythmias, the enhancement of flecainide-induced conduction slowing by exercise may be sufficient to allow manifest reentry to occur. This may account for the occurrence of ventricular tachycardia in our patient with the greatest exercise-induced conduction slowing and for the occurrence of proarrhythmia described by Anastasiou-Nana et al.[16]

Routine exercise testing has been advocated as a means to detect potential proarrhythmic responses to flecainide.[16] Our results indicate that the degree of flecainide-induced QRS prolongation on the resting ECG is a good predictor of further conduction slowing during exercise, and they suggest that changes in the resting ECG could be used to select patients at increased risk of proarrhythmia during exercise. The value of exercise testing for the prediction of proarrhythmia due to flecainide, either routinely or in selected subgroups, needs to be tested prospectively.

We used QRS duration as an indicator of flecainide's effects on ventricular conduction. Recent work with epicardial mapping shows that QRS duration changes accurately reflect antiarrhythmic drug effects on ventricular conduction, provided that the QRS configuration remains constant.[34] The similarity between the onset kinetics that we observed for flecainide's effects on conduction in humans and the values reported for changes in $\dot{V}_{max}$ in vitro[17] indicates the relevance of basic models of antiarrhythmic drug action[1-4] to achieve an understanding of the clinical properties of these compounds. Moreover, we have shown that the use-dependent actions described by these models account for exercise-induced ventricular conduction slowing in patients treated with flecainide. The latter phenomenon may have an important role in producing ventricular proarrhythmic actions in predisposed patients, indicating the potential clinical importance of the rate-dependent actions of antiarrhythmic drugs.

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KEY WORDS • ventricular arrhythmias • antiarrhythmic drugs • toxicity • heart rate • conduction • pharmacodynamics • cardiac

Publication 2

In Publication 1 we demonstrated that in patients on flecainide therapy, QRS duration progressively increased as the heart rate was augmented by exercise. We also showed that there was no significant difference between QRS increases resulting from exercise or ventricular pacing. In order to isolate the role of heart rate *per se*, we evaluated the time constant for the onset of QRS prolongation upon the abrupt initiation of ventricular pacing in control patients and in patients treated with flecainide. We found that the time constant that we calculated for flecainide was very similar to that of depression of sodium current indexes *in vitro* reported in the literature. These results provided evidence that ventricular conduction slowing in humans may be due to use-dependent sodium channel blockade.

In our next study, we wanted to confirm that the results obtained with flecainide were valid for a variety of antiarrhythmic drugs with sodium channel blocking properties and different kinetics of blockade. This would exclude some nonspecific effect related to sodium channel inhibition.

Kinetics of Use-Dependent Ventricular Conduction Slowing by Antiarrhythmic Drugs in Humans

Suzanne Ranger, BSc; Mario Talajic, MD; Robert Lemery, MD; Denis Roy, MD; Christine Villemaire, BSc; and Stanley Nattel, MD

Background. Rate-dependent conduction slowing by class I antiarrhythmic agents has clinically important consequences. Class I drugs are known to produce use-dependent sodium channel blockade. If rate-dependent conduction slowing by class I agents is due to sodium channel blocking actions, the kinetics of conduction slowing should be similar to those of depression of sodium current indexes in vitro. The purpose of the present investigation was to study the onset time course of ventricular conduction slowing caused by a variety of class I agents in humans.

Methods and Results. Twenty-seven patients undergoing electrophysiological evaluation for antiarrhythmic therapy were studied. Changes in QRS duration at initiation of ventricular pacing at cycle lengths of 400 and 500 msec were used to evaluate the kinetics of drug action. Mean time constants for each drug were similar to values for $\dot{V}_{max}$ depression reported in vitro studies: flecainide, 24.9±11.6 beats in eight patients (versus 34.5 beats reported for $\dot{V}_{max}$ block); propafenone, 17.8±6.9 beats in five patients (versus 8.4–20.8 beats); quinidine, 7.0±2.4 beats in six patients (versus 5.6–6.2 beats); and amiodarone, 3.6±2.0 beats for eight patients (versus 3.0 beats). Time constants were significantly different among the various drugs tested ($p=0.0002$ at a cycle length of 400 msec; $p=0.002$ at 500 msec), and there was a strong correlation ($r=0.89$, $p<0.0001$) between values obtained at a cycle length of 400 msec and those at a cycle length of 500 msec. No rate-dependent changes in QRS duration were seen at onset of ventricular pacing among eight age- and disease-matched control patients not taking class I antiarrhythmic drugs, including three patients subsequently showing such changes during type I antiarrhythmic drug therapy.

Conclusions. We conclude that class I agents produce use-dependent QRS prolongation in humans with characteristic kinetics for each agent that are similar to the kinetics of $\dot{V}_{max}$ depression in vitro. These results suggest that rate-dependent ventricular conduction slowing by antiarrhythmic drugs in humans is due to use-dependent sodium channel blockade. (*Circulation* 1991;83:1987–1994)

Sodium channel blocking drugs are commonly used for the treatment of cardiac arrhythmias. Conduction slowing by these agents in humans has been found to depend on heart rate.[1,2] These observations are consistent with fundamental models of antiarrhythmic drug interaction with cardiac sodium channels.[3,4] According to these models, depolarization, which results in channel opening and then inactivation, tends to facilitate drug binding to the sodium channel, whereas repolarization, which returns sodium channels to their resting state, tends to facilitate drug dissociation. Increases in cardiac rate enhance drug action by increasing the amount of time in the activated and inactivated states at the expense of time in the resting state.

Models of use-dependent channel blockade have important implications for mechanisms of clinical drug action.[5,6] Rate-dependent blockade may contribute to the beneficial antiarrhythmic actions of both calcium[7,8] and sodium[9] channel blockers. In

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TABLE 1. Characteristics of Patient Population

	Flecainide	Propafenone	Quinidine	Amiodarone	Control
n	8	5	6	8	8
Age (yr)	59±10	63±14	56±12	57±9	64±10
Sex (male/female)	8/0	3/2	5/1	8/0	6/2
Cardiac diagnosis					
Coronary artery disease	8	3	6	8	6
Valvular		1			
No known cardiac disease		1			2
Dosage (mg/day)	225±104	555±146	790±253	925±512	NA
Plasma concentration (μmol/l)	1.2±0.4	0.8±0.3*	8.0±4.4	2.0±0.6*	NA
Plasma concentration (mg/l)	0.5±0.2	0.3±0.1	2.6±1.4	1.25±0.4	NA
Ejection fraction (%)	35±9	37±9	33±8	25±9	41±14
Medication					
Digitalis	3/8	3/5	1/6	3/8	2/8
β-Blocker	1/8	0/5	1/6	1/8	2/8
Drug efficacy	2/8	3/5	1/6	1/8	NA

Values are mean±SD where applicable.

Ejection fractions were available for all patients except for one taking propafenone and one control patient. Drug efficacy was defined as suppression of ability to induce sustained ventricular tachycardia.

NA, not applicable.

*The concentrations shown are for the parent drugs. Concentrations of the active metabolite averaged 1.2±0.5 μmol/l for desethylamiodarone and 0.2±0.1 μmol/l for 5-hydroxypropafenone.

other instances, rate-dependent blockade may result in proarrhythmic properties.[10–12]

A characteristic feature of use-dependent channel blockade is that it develops and dissipates with a typical time course for each antiarrhythmic agent.[3–5] Studies in experimental animals have shown that the kinetics of conduction slowing due to both sodium[10,13–18] and calcium[19] channel blockers parallel their actions on $\dot{V}_{max}$ or inward current in vitro. We have shown that the onset of additional QRS prolongation upon an abrupt increase in ventricular rate in patients taking flecainide parallels the time dependence of flecainide's effects on $\dot{V}_{max}$ in vitro.[12] To our knowledge, this is the only quantitative study of the kinetics of use-dependent conduction slowing by an antiarrhythmic drug in humans. The possibility remains that the response we observed in the presence of flecainide was due to a nonspecific rate-dependent phenomenon such as myocardial ischemia, ion accumulation or depletion, and so on, and that the similarity to flecainide's in vitro blocking kinetics is purely coincidental.

The latter possibility could be critically assessed by evaluating the time course of conduction slowing upon an abrupt rate change in the presence of a variety of antiarrhythmic drugs with different kinetics of sodium channel blockade in vitro. If a specific use-dependent blocking action is responsible for conduction slowing, a characteristic time course should be observed for each agent. If, instead, a nonspecific mechanism is responsible for conduction slowing, the time course of the latter should be constant irrespective of the drug studied. The present study was designed to determine whether abrupt increases in heart rate result in ventricular conduction slowing in the presence of a variety of class I antiarrhythmic drugs and to establish the time course of any changes seen. Preliminary results have been presented in abstract form.[20]

Methods

Patient Population

The study group consisted of 27 patients receiving class I antiarrhythmic drug therapy for the treatment of cardiac arrhythmias. Eight patients not treated with class I antiarrhythmic drugs served as a control group. All patients were undergoing clinically indicated electrophysiological studies for the assessment of possible tachyarrhythmias (control group) or of the efficacy of antiarrhythmic drug therapy for ventricular tachyarrhythmias. Antiarrhythmic drugs studied were flecainide, propafenone, quinidine, and amiodarone. The clinical characteristics of both patient groups are summarized in Table 1. The results presented for left ventricular ejection fraction were obtained from radionuclide angiography performed as part of the clinical management of each patient.

Electrophysiological Study

The electrophysiological protocol was performed at the beginning of the routine clinical study. All patients gave written consent to the invasive electrophysiological study. A quadripolar electrode was positioned in the right ventricular apex by way of the right or left femoral vein. Stimulation was performed with 1.5-msec square-wave pulses with twice diastolic threshold current controlled by a programmable stimulator (Bloom Associates, Flying Hills, Pa.). Recordings of electrocardiographic leads I, aVF, and V_1 and a right ventricular electrogram were obtained at

100 mm/sec paper speed (Mingograf T16, Siemens-Elema, Stockholm, Sweden). Electrocardiographic signals were obtained with electrocardiographic amplifiers (Electronics for Medicine, Pleasantville, N.Y.) with a band width of 0.1 to 100 Hz and a standardized signal amplitude of 1 mV/cm of recording paper. The kinetics of drug-induced blockade were assessed after an abrupt rate change induced by ventricular pacing. Ventricular pacing was initiated during sinus rhythm and was maintained for 1 minute at cycle lengths of 400, 500, and 600 msec. Because the onset of the pacing train was not coupled to the last sinus beat, analysis began with the second ventricular-paced complex, that is, the first ventricular complex at the selected RR interval. Patients were allowed to recover for at least 2 minutes after a series of stimuli at a given rate, which was a time found to be sufficient for the dissipation of all rate-dependent electrocardiographic changes.

Drug Dosage and Assay

Drug doses had been selected by the treating physician based on standard clinical criteria. All patients had received continuous oral therapy for at least 3 days before study. The rather large mean dose of amiodarone (Table 1) reflects the fact that several patients were in the loading phase of amiodarone therapy. At the end of the pacing protocol, a blood sample was drawn for drug concentration measurement. Plasma concentrations of quinidine, procainamide, amiodarone, and propafenone were measured by high-performance liquid chromatography as previously described.[18,21-23] Flecainide was assayed with a fluorescence polarization immunoassay (Abbott Laboratories, Mississauga, Ontario).

Data Analysis

QRS duration was used as an index of ventricular conduction time. Only QRS complexes of consistent morphology were analyzed. Changes in QRS duration resulting from ventricular pacing were measured as a function of beat number by two observers. Beats 1–10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, and 150 were separated (beat by beat), coded, and read by two observers who were unaware of both beat number and treatment received. The observers independently measured QRS duration, using leads they selected as giving the most reliable onset and offset of the QRS complex.

We performed nonlinear regression with a Marquardt procedure (Statgraf software) to fit data to an equation of the form:

$$QRS_n = QRS_0 + (QRS_\infty - QRS_0) \exp(-n/\tau)$$

Where QRS_n, QRS_0, and QRS_∞ are the QRS duration of the n^{th} beat, first paced beat, and at steady state, respectively, and τ is a time constant expressed in terms of a number of beats. The magnitude of rate-dependent conduction slowing was calculated as QRS_∞ minus QRS_0 in each study. Analysis was performed separately on measurements obtained by each observer, and the values obtained for τ and the magnitude of rate-dependent slowing by the two observers were averaged to obtain representative values for each study.

Group data are presented as mean±SD. The significance of regression was determined by analysis of variance (ANOVA).[24] The statistical significance of differences among time constants and magnitude of rate-dependent slowing for different drugs was assessed by one-way ANOVA with a Scheffé test.[24] The overall significance of each drug as a determinant of the time constant and magnitude of rate-dependent slowing was determined by ANOVA. The statistical significance of differences between values determined at a cycle length of 400 msec and those at 500 msec was determined by paired t tests, by use of only studies during which measurable changes were noted at both cycle lengths. A probability of less than 5% was taken to indicate statistical significance.

Results

In the eight control patients, abrupt changes in heart rate by ventricular pacing did not produce any rate-dependent changes in QRS duration (Figure 1). However, in the presence of class I antiarrhythmic drugs, QRS duration increased progressively as an exponential function of beat number (Figure 1). Figure 2 shows analog data to illustrate the types of changes in the QRS that occurred after the onset of ventricular pacing at a cycle length of 400 msec, both in the absence and presence of class I antiarrhythmic drugs.

There was excellent agreement between the measurements made by either observer. Figure 3 shows the regression of QRS measurements made by observer 1 on QRS measurements made by observer 2. The correlation coefficient was 0.96 ($p<0.0001$), and the regression line was close to the line of identity with a slope of 0.94 and an intercept of 9.63 msec. There was also a highly significant correlation ($r=0.85$, $p<0.0001$) between the values of time constants obtained by use of the measurements of either observer. Figure 4 shows the results for an individual patient before and after quinidine therapy as measured by both observers. In this patient, as in the other two patients studied both before and during drug therapy, there were no changes in QRS duration after the onset of ventricular pacing in the control study, but clear use-dependent QRS prolongation occurred during ventricular pacing in the presence of drug therapy. The results obtained by either observer, unaware of beat number and treatment and measured independently, are quite similar.

The magnitude of rate-dependent conduction slowing depended on pacing cycle length. The time constants of rate-dependent QRS prolongation were estimated separately for data at basic cycle lengths of 400 and 500 msec. Although similar phenomena were observed at a basic cycle length of 600 msec, the magnitude of the changes seen was too small for

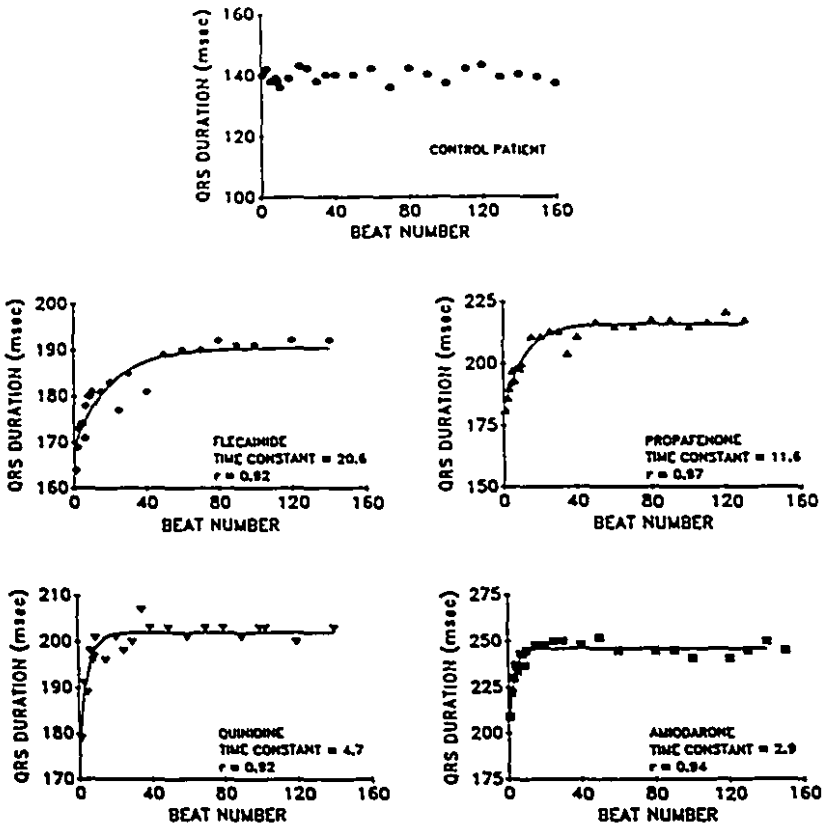

FIGURE 1. *Plots of kinetics of QRS prolongation upon the initiation of ventricular pacing in a control patient* (top) *and for four representative patients taking antiarrhythmic drugs. Onset time constants for the best-fit nonlinear regression lines (shown) are in units of beats. Results shown are at a pacing cycle length of 400 msec.*

precise calculation. The changes observed were largest at a cycle length of 400 msec, and the curve fits were consequently better than those at 500 msec. Mean time constants for the onset of block are shown in Table 2, with corresponding values for previously reported in vitro studies. In three patients, the changes at a cycle length of 500 msec were too small for the onset time constant to be calculated (one with propafenone and two with flecainide). In two patients taking amiodarone, time constants were only obtained at a cycle length of 500 msec. The time constants for flecainide and propafenone were significantly longer than those for quinidine and amiodarone, and the drug taken was a highly significant determinant of the time constant of rate-dependent QRS prolongation.

Figure 5 shows the relation between time constants measured at a cycle length of 400 msec and those at a cycle length of 500 msec. There was a highly significant correlation between the two sets of results (r=0.89, p<0.0001). Although the magnitude of time-dependent conduction slowing was significantly greater at a cycle length of 400 msec than that at 500 msec (p<0.0001), there was no significant difference between the mean time constants obtained at either cycle length.

Discussion

Class I antiarrhythmic agents are all able to slow ventricular conduction, an action presumably due to the sodium channel blockade that they demonstrate in vitro. Their conduction slowing action has been shown to be qualitatively rate dependent in humans[1,2] and has potentially important clinical consequences.[9–12] In the present study, we present data that show for the first time that a variety of class I antiarrhythmic agents produce use-dependent conduction slowing in humans with kinetics similar to those of $\dot{V}_{max}$ blockade in vitro.

Determinants of Rate-Dependent Conduction Slowing

There was a close correlation between time constants measured at a cycle length of 400 msec and those measured at 500 msec (Figure 5), and the mean time constants at either rate were not significantly different. While the onset rate constant is expected to decrease at shorter cycle lengths,[26] the magnitude of the change resulting from a decrease in cycle length from 500 to 400 msec in likely to be too small to be detected by the techniques we used. Changes in QRS duration were larger at faster pacing rates, as expected for a rate-dependent phenomenon. This resulted in generally poorer curve fits at slower rates and presumably a lesser precision of the estimated time constants. We, therefore, used the values obtained at a cycle length of 400 msec for comparison with previously reported in vitro data.

As calculated at a cycle length of 400 msec (Table 2), the mean onset time constants ranged from 3.6±2.0 (for amiodarone) to 24.9±11.6 beats (for flecainide). The value we obtained for flecainide is in

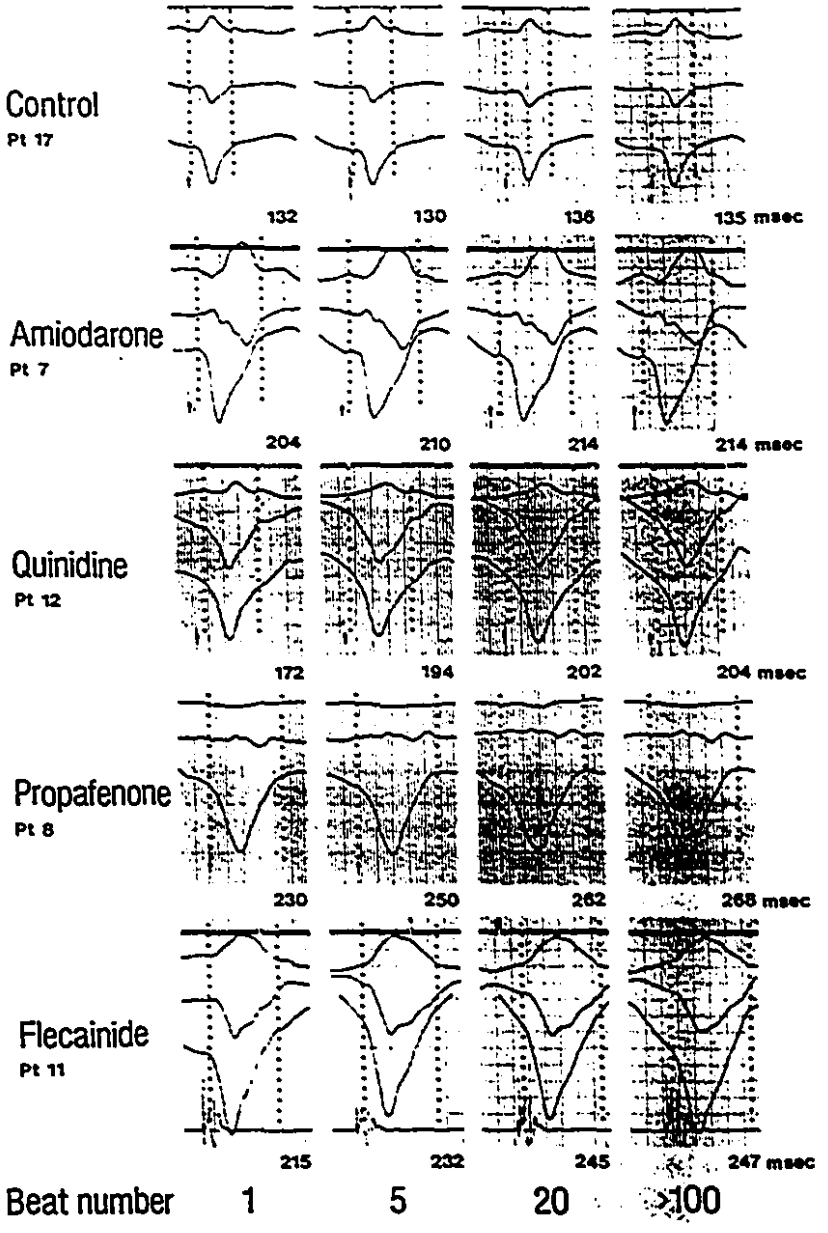

FIGURE 2. *Tracings showing QRS prolongation during ventricular pacing at a cycle length of 400 msec in individual patients taking amiodarone, quinidine, propafenone, and flecainide, as a function of the beat number (in the pacing train) of the complex shown. Corresponding data from a control patient are shown* (top). *Vertical dotted lines indicate the onset and offset of the QRS complex. We chose points that could be measured accurately and reproducibly rather than try to measure total QRS duration in each case. Small vertical arrows indicate the time of the stimulus artifact. Measured QRS durations are shown at the lower right of each complex, and the patient's number for each case is given under the drug taken.*

the same range as the time constant (34.5 beats) for $\dot{V}_{max}$ depression at a cycle length of 300 msec reported in guinea pig papillary muscles by Campbell.[27] Our result for propafenone (17.8±6.9 beats) is similar to the range of values (8–21 beats) for $\dot{V}_{max}$ depression in ventricular tissues at cycle lengths of 300–400 msec.[28,29] For quinidine, Valois and Sasyniuk[30] noted an onset time constant of 5.6±0.5 pulses at a cycle length of 600 msec in canine Purkinje fibers, and Grant et al[25] reported a value of 6.2±1.5 pulses at a cycle length of 500 msec. These are quite close to the time constant of 7.0±2.4 beats that we measured at a cycle length of 400 msec. We were unable to find onset time constants for amiodarone to compare with the value (3.6±2.0 beats) that we obtained in humans. However, fitting data of Mason et al[31] (their Figure 1) to a single exponential, we

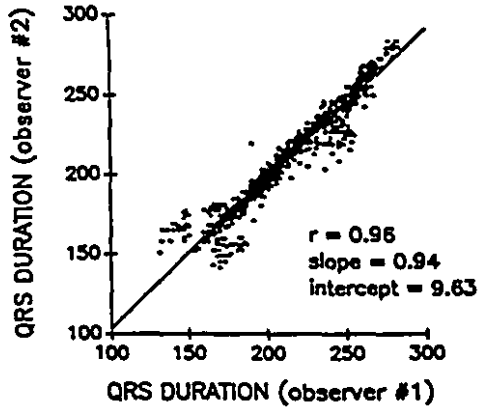

FIGURE 3. *Regression plot of QRS duration as measured by both observers. There was a highly significant correlation, with the regression line (shown) being close to the line of identity.*

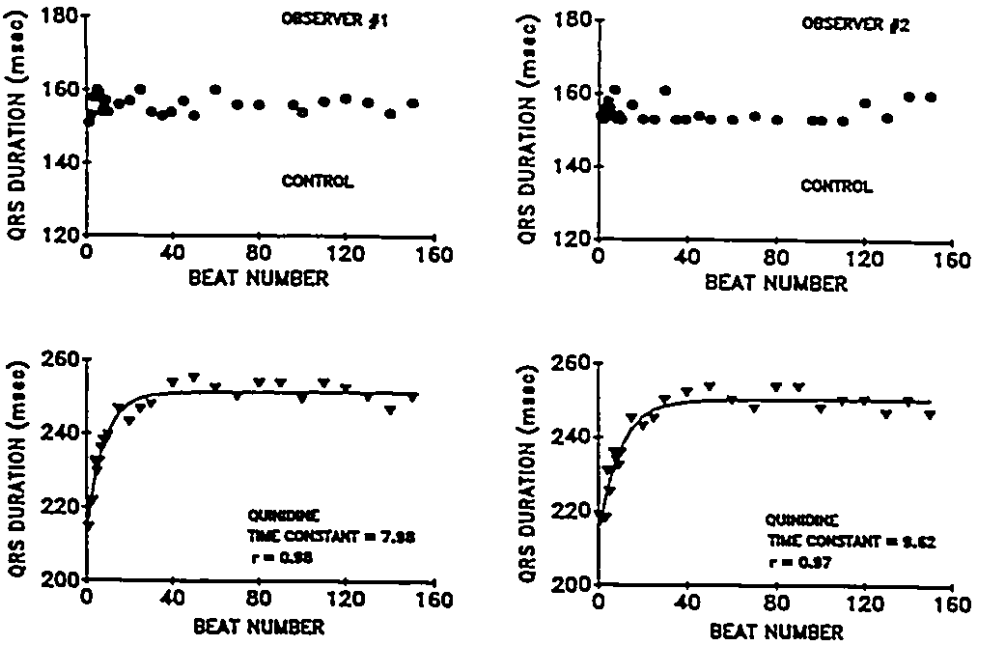

FIGURE 4. *Plots of QRS changes resulting from the onset of ventricular pacing in a single patient before* (top panels) *and after* (bottom panels) *treatment with quinidine. Measurements of observer 1* (left panels) *and observer 2* (right panels) *based on the same tracings are shown, with the best-fit exponential curve for data obtained during drug therapy.*

obtained a time constant for amiodarone of 3 pulses, in the same range as our results. Overall, the values that we measured in humans were in the same range as those reported in vitro, with the same rank order (flecainide>propafenone>quinidine>amiodarone). Comparison is limited by the different experimental animal species tested and by the fact that values are not always available at the same cycle length(s) in vitro as those we studied in humans.

Potential Limitations

We used QRS duration as an in vivo index of antiarrhythmic drug-induced conduction slowing. Drug-induced changes in QRS duration are linearly related to directly measured changes in epicardial activation time,[18,32] provided overall morphology is unchanged (indicating a constant activation pattern). Even though ventricular conduction velocity is proportional to the square root of phase 0 inward current, interval-dependent changes in conduction time approximate a first-order function over the range of values obtained in this study.[18,33]

We studied patients on therapeutic doses of antiarrhythmic drugs as selected by their treating physicians. Their plasma concentrations (Table 1) were in

TABLE 2. Time Constants for the Onset of Drug-Induced Changes in QRS Duration

Drug	Flecainide	Propafenone	Quinidine	Amiodarone
Basic cycle length 500 msec				
n	6	4	6	8
r	0.73±0.08	0.77±0.13	0.69±0.18	0.54±0.18
Magnitude (msec)	17.4±5.3	21.3±5.9	14.3±6.7	14.4±6.7
τ (beats)	26.6±17.3	9.7±2.7	8.4±3.0*	4.4±2.3†
Basic cycle length 400 msec				
n	8	5	6	6
r	0.89±0.07	0.94±0.04	0.86±0.06	0.85±0.06
Magnitude (msec)	25.8±5.6	30.0±10.8	26.0±8.9	26.7±13.0
τ (beats)	24.9±11.6	17.8±6.9	7.0±2.4†	3.6±2.0‡
Values reported from in vitro studies				
τ (beats)	34.5[27]	8.4[28], 20.8[29]	5.6[30], 6.2[25]	3.0[31]

Values are mean±SD where applicable.

n, number of patients with analyzable kinetic data for drug and cycle length indicated; *r*, nonlinear correlation coefficient; τ, time constant.

*$p<0.05$, †$p<0.01$, ‡$p<0.001$ compared with τ for flecainide. In addition, τ at 400 msec was significantly different between amiodarone and propafenone ($p<0.01$). Overall, drug was a highly significant determinant of τ, both at cycle length 400 msec ($p=0.0002$) and 500 msec ($p=0.002$).

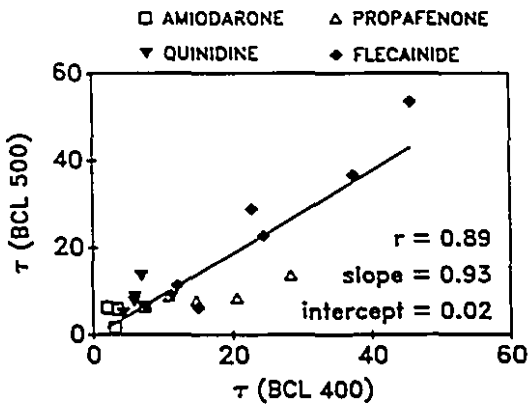

FIGURE 5. *Regression plot between time constants measured at a cycle length of 500 msec and those at a cycle length of 400 msec. Best-fit regression line is shown.*

the therapeutic range,[34] which varies among the drugs selected. This is relevant because the magnitude of drug action, and to some extent the onset time constant, depend on drug concentration. On the other hand, the magnitude of rate-dependent QRS prolongation was not significantly different among the various drugs studied, suggesting that the concentrations achieved were roughly equivalent in biological action. Furthermore, with corrections for protein binding, the concentrations were roughly in the same range as those in the in vitro studies used for comparison.[27-31]

We cannot exclude the possibility that myocardial ischemia may have contributed to some of the conduction slowing seen. Evidence against a primary role for ischemia is provided by the absence of rate-related slowing in control patients with coronary artery disease, its presence in patients on class I drugs without coronary disease, and the similarity of time constants for conduction slowing by a given drug in vivo and its depression of $\dot{V}_{max}$ in vitro.

Clinical Relevance

The similarity between the time constants we observed for rate-dependent QRS prolongation in vivo and the values previously noted for $\dot{V}_{max}$ depression by the same drugs in vitro support the concept that their sodium channel blocking action is responsible for rate-dependent conduction slowing in humans. Such sodium channel blockade is probably responsible for the important QRS prolongation during exercise in patients taking flecainide[12] and propafenone.[35] Rate-dependent conduction slowing by the sinus tachycardia of exercise may be responsible for some cases of proarrhythmia caused by class IC drugs.[12] Evidence for rate-dependent arrhythmogenic mechanisms has been advanced in experimental animal models.[10,11,17] Rate-dependent conduction block may play a role in other proarrhythmic actions of class IC agents, such as the facilitation of lethal ventricular tachyarrhythmias during canine acute myocardial ischemia[36,37] and the potentially detrimental effects of IC drugs in postinfarction patients.[38]

Although amiodarone was not initially classified as a class I antiarrhythmic drug, unlike the other compounds we studied, it clearly has class I properties in vitro[31,39] and in vivo.[2,22,40] Patients taking amiodarone may have QRS prolongation with exercise,[41] which our results suggest are due to use-dependent sodium channel blockade. Amiodarone's use-dependent effects during exercise may be greater in patients with evidence of acute ischemia.[42] This observation may be due to a sensitizing effect of acute ischemia to the drug's sodium channel blocking action. On the other hand, the degree of QRS prolongation by amiodarone at rest in the latter study was greater in the ischemic group, indicating a greater baseline drug effect and itself predicting greater QRS increases with exercise.[12,41]

Use-dependent conduction slowing by class I drugs may be beneficial in reducing the rate of a ventricular tachycardia. Marchlinski et al[9] showed that ventricular tachycardia slowing by procainamide can be predicted by rate-dependent QRS prolongation. Kidwell et al[43] provided preliminary evidence for use-dependent ventricular tachycardia slowing by flecainide in humans. They estimated a time constant of 15 seconds for the onset of ventricular tachycardia slowing by flecainide, which is in the same range as the time constant we obtained for QRS prolongation (10 seconds at a cycle length of 400 msec).

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KEY WORDS • arrhythmias • sodium channel blockers • cardiac conduction • antiarrhythmic drugs • pharmacodynamics

We and others have reported the occurrence of proarrhythmia in patients on flecainide therapy (Publication 1: Ranger et al, 1989; Chouty et al, 1987; Anastasiou-Nana et al, 1987; Morganroth and Horowitz, 1984). At the time of publication of our clinical work, the Cardiac Arrhythmia Suppression Trial (The CAST Investigators, 1989; Echt et al, 1991) reported an higher incidence of mortality in patients with previous myocardial infarction and treated with class IC drugs (encainide and flecainide). We choose to investigate in an animal model the mechanism by which flecainide causes ventricular arrhythmias and to assess the role of myocardial infarct in flecainide-induced proarrhythmia.

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Determinants and Mechanisms of Flecainide-Induced Promotion of Ventricular Tachycardia in Anesthetized Dogs

Suzanne Ranger, BSc; Stanley Nattel, MD

Background Class IC antiarrhythmic agents such as flecainide are known to have potentially significant ventricular proarrhythmic actions, but the underlying mechanisms are incompletely understood. While some studies have reported proarrhythmia in both healthy dogs and dogs that previously have had a myocardial infarction (MI), there are no published, controlled studies comparing proarrhythmia in healthy dogs vs in dogs with MI. In addition, the concentration dependence of proarrhythmia is unknown and the electrophysiological changes associated with proarrhythmia are not well established.

Methods We administered successive loading and maintenance infusions of flecainide until ventricular tachyarrhythmia or death occurred in 13 healthy dogs and 19 dogs with 72-hour-old MIs (MI dogs). Ventricular proarrhythmia, defined as reproducible ventricular tachycardia absent under control conditions and occurring in the presence of flecainide, was observed in 4 of 13 healthy dogs (31%) and 15 of 19 MI dogs (79%, $P=.02$), and drug-induced spontaneous ventricular tachycardia occurred in 8 of 19 MI dogs but in no healthy dogs ($P=.007$). Activation data at the time of proarrhythmia were available for 11 MI dogs and provided evidence for reentry in 9, with a complete epicardial reentry circuit identified in 4 dogs and a partial circuit in 5. While flecainide slowed ventricular conduction in both the longitudinal and transverse directions, there were no significant differences between overall drug-induced conduction changes in MI dogs compared with healthy dogs. However, in 7 MI dogs for whom activation data were available during ventricular pacing at concentrations comparable to those causing proarrhythmia, flecainide induced a new arc of block in 6 of 7, whereas an arc of block was never observed in the absence of proarrhythmia. Conduction block was induced transverse to fiber orientation in a rate-dependent fashion and was caused by a regionally-specific effect of the drug. No differences were noted between refractory periods proximal and distal to the site of block.

Conclusions Prior MI strongly predisposes dogs to flecainide proarrhythmia, which occurs in the majority of such dogs in a concentration-related way. In most cases, activation data suggest that anisotropic reentry around a localized arc of rate-dependent transverse conduction block underlies proarrhythmia. These results provide insights into the conditions and mechanisms underlying the ability of flecainide to promote the occurrence of ventricular tachycardia. (*Circulation*. 1995;92:1300-1311.)

Key Words • myocardial infarction • antiarrhythmia agents • sodium • arrhythmia • death, sudden

Ventricular proarrhythmia, consisting of the de novo induction or aggravation of preexisting ventricular tachyarrhythmias (VTs) by an antiarrhythmic agent, is probably the most important factor limiting the application of antiarrhythmic drug therapy.[1,2] Proarrhythmic responses are believed to underlie the propensity of class I drugs, particularly the IC agents flecainide and encainide, to increase the mortality rate in patients with a recent myocardial infarction (MI).[3,4] Indirect evidence suggests that similar phenomena may be operative in patients resuscitated from sudden cardiac death[5] and those with atrial fibrillation.[6,7] Improved understanding of the mechanisms of drug-induced proarrhythmia is needed to develop strategies to optimize the risk to benefit ratio of antiarrhythmic drug therapy.

Several studies have suggested that class IC agents can result in the induction of VT by programmed electrical stimulation in dogs with a previous MI even when VT was not inducible before drug administration.[8-14] Results have also been presented that suggest that flecainide causes proarrhythmia (including a 50% prevalence of sustained VT) in a large percentage of normal dogs.[15] These findings raise questions about the need for a pathological substrate to promote proarrhythmia. Limitations of previous studies have included a lack of consideration of the concentration dependence of drug action, the variable definition of proarrhythmia that often did not require reproducibility of VT induction, study protocols that did not include observations in the presence of stable drug concentrations, and the lack of a comparison between healthy dogs and those with prior infarction. In addition, activation mapping studies of mechanisms have been presented in a very limited form (48-channel recordings from two dogs) in only one published study,[12] and preliminary data from more detailed studies have been presented but published only in abstract form.[10] A recent study showed that flecainide permitted the induction of sustained VT in rabbit hearts through a thin layer of surviving epicardial tissue after extensive ventricular cryoablation.[16] Proarrhythmia in this model was associated with a small decrease in the wavelength for reentry and the induction of arcs of conduction block of variable location.

The present experiments were designed to address several questions regarding the ability of flecainide to

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TABLE 1. Flecainide Doses and Plasma Concentrations

Dose Level	Loading Dose, mg/kg†	Maintenance Dose, mg·kg^{-1}·min^{-1}	Plasma Concentration, mg/L				Systolic/Diastolic Blood Pressure, mm Hg	
			Healthy Dogs		MI Dogs			
			Beginning	End	Beginning	End	Healthy Dogs	MI Dogs
Control	...	...	...	...	...	...	146±14/95±12	132±14/90±12
1	1.875	0.056	1.7±0.9	1.8±0.8	2.2±0.7	2.4±0.6	135±14/87±12	129±16/89±13
2	3.750	0.113	4.8±1.6	5.3±2.2	6.2±1.2	6.2±1.4	122±15*/88±16*	121±15/84±13
3	5.625	0.168	8.6±1.6	8.7±1.1	8.9±1.6	9.5±1.5	102±23*/67±23*	89±26*/45±17*

MI dogs indicates dogs that sustained a myocardial infarction 72 hours before the experiment. Loading dose was given over 15 minutes, and the maintenance dose was begun immediately after the loading dose. Flecainide was 10 mg/mL in 5% dextrose. Experimental procedures were begun 15 minutes after onset of the maintenance dose. Plasma concentrations were measured at the beginning and end of each experimental protocol.
*P<.05 vs control.

induce proarrhythmic responses in anesthetized, open-chest dogs: (1) To what extent does a previous myocardial infarction predispose to such proarrhythmic responses, and at what drug concentrations do they occur? (2) What electrophysiological changes permit the development of drug-induced proarrhythmia? (3) How frequently can epicardial mapping reveal a functional mechanism of proarrhythmia, and what mechanisms are involved? Preliminary findings have been presented in abstract form.[17,18]

Methods

Infarct Model

Mongrel dogs (17 to 27 kg) were anesthetized with sodium pentobarbital 30 mg/kg IV and ventilated through an endotracheal tube. A left thoracotomy was performed under sterile conditions, and a small opening was created in the pericardium. The left anterior descending coronary artery (LAD) was dissected proximal to the first diagonal branch and then occluded in two stages.[19] Nadolol 0.5 mg/kg IV was given before occlusion and then daily (20 mg PO) for 2 days. Benzathine penicillin G 150 000 U and procaine penicillin G 150 000 U SC were also given daily. Levorphanol 0.056 mg/kg SC BID was used to control postoperative pain.

Study Procedures

The proarrhythmic effects of flecainide were studied in 13 healthy dogs and 31 dogs subjected to LAD occlusion 72 hours before study (MI dogs). On the day of study, dogs were anesthetized with morphine 2 mg/kg SC and α-chloralose 120 mg/kg IV, intubated, and mechanically ventilated. Catheters were inserted into one femoral artery and two femoral veins. Arterial blood gases were measured at 1-hour intervals and maintained in the physiological range (sAO_2 >90%, pH 7.35 to 7.45) by adjusting the ventilator or using supplementary oxygen. A left thoracotomy was performed at the fifth intercostal space, and a pericardial cradle was created. The chest cavity was covered with plastic wrap to prevent cooling or dehydration, and body temperature was maintained at 37°C to 38°C with a heating blanket.

An array of 56 or 112 bipolar electrodes in a 4×6-cm plastic sheet was sewn to the surface of the left ventricle over the LAD territory. A bipolar platinum electrode was fixed to the left atrial appendage to record the left atrial electrogram. ECG leads II, III, aVL, and aVR were filtered at 0.05 to 40 Hz, and electrograms were filtered at 30 to 400 Hz (amplifiers, Bloom Associates Ltd) recorded, along with stimulus artifacts and arterial pressure, with a paper recorder (MT-95000 Host Control, Astromed Inc).

Activation Mapping

Previously-described techniques were used to create activation maps.[20,21] The interpolar distance was 2 mm, and electrodes were arranged in a parallel fashion with an interelectrode distance of 4 mm for the 112-electrode array and 6 mm for the 56-electrode array. The 56-electrode array was used in the first 18 dogs, and the 112-electrode array was used in the remaining 26 dogs. A bipolar electrode at the center of the array was used for programmed ventricular stimulation with the use of 2-ms current pulses of twice diastolic threshold (model EP-2 Stimulator, Digital Cardiovascular Instruments). Electrograms were filtered at 40 to 300 Hz, digitized with 12-bit resolution and a 1-kHz sampling rate, and transmitted by means of duplex fiber-optic cables into a microcomputer. The activation time at each electrode site was defined as the time of maximal rate of voltage change as calculated by the computer. Each electrogram was reviewed manually to exclude low-amplitude recordings resulting from poor contact, electrical artifacts, and interference by electrical noise. The presence of block was defined by a conduction velocity <0.1 m/s between adjacent electrode sites, generally with an abrupt alteration in the direction of wave front propagation across the line of block. Isochrones were constructed with a computer-based interpolation algorithm.

Experimental Procedures

Successively increasing doses of flecainide (Table 1) were administered until ventricular proarrhythmia or death occurred in 19 MI dogs and 13 healthy dogs ("controlled series"). Under control conditions and at each drug concentration, ventricular activation, ventricular refractory period, and arrhythmia occurrence were evaluated over a range of cycle lengths from 180 to 500 ms. Two minutes of stimulation were allowed at each basic cycle length before the introduction of extrastimuli to determine the refractory period. Single extrastimuli were then applied after every 15 basic stimuli at the central electrode site to determine effective refractory period (ERP, the longest S_1S_2 interval failing to capture the ventricle) and to induce VT.

Proarrhythmia was defined as the reproducible occurrence of spontaneous or inducible VT (≥5 successive ventricular complexes) in the presence of flecainide but absent under control conditions. Sustained VT was defined by the occurrence of >30 successive ventricular complexes. Plasma flecainide concentrations were measured by high-performance liquid chromatography.[22] Infarct size was determined with triphenyl tetrazolium chloride.[23]

An additional 12 MI dogs ("additional series") were studied to relate the occurrence of proarrhythmia to infarct histopathology and to differences in ERP on either side of lines of block. The same drug infusion protocol described above was used, but instead of acquiring detailed mapping data at each dose, we identified the dose causing proarrhythmia and then determined the ERP at multiple sites on either side of the line of block. Portions of myocardium were cut into 0.3×1×2.4-cm sections, which were stained with hematoxylin-phloxine-saffron or Gomori's one-step trichrome[24] and subjected to microscopic examination.

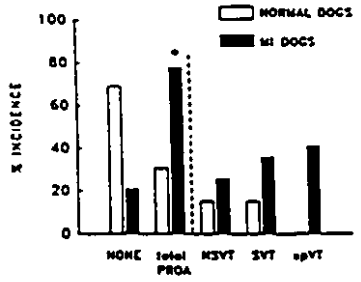

FIG 1. Bar graph showing the incidence of various forms of proarrhythmia (PROA) in healthy (normal) dogs and dogs that had prior myocardial infarction (MI dogs). The percentage of dogs with any form of proarrhythmia (total PROA) was significantly greater (*P=.02) for MI dogs. NSVT indicates nonsustained ventricular tachycardia (VT); SVT, sustained VT; and spVT, spontaneous VT. Some dogs had more than one form of proarrhythmia.

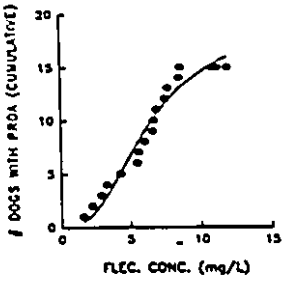

FIG 2. Line graph showing cumulative concentration-response curve for flecainide proarrhythmia (PROA) in dogs that had prior myocardial infarction. Flec. Conc. indicates flecainide concentration; dots, data points; solid line, nonlinear best-fit curve to the equation $N=N_{max}(1/[1+\{FC/EC_{50}\}^{K}])$, where N is the cumulative number of dogs with proarrhythmia (PROA); N_{max}, maximum predicted number of dogs with PROA; FC, flecainide concentration; EC_{50}, concentration for 50% of maximum incidence of PROA; and K, a constant.

Data Analysis

Conduction velocity was analyzed by linear regression of interelectrode distance on activation time. Group values are presented as mean±SD. Statistical comparisons were performed by ANOVA with a range test (Student's t test with the Bonferroni correction)[25] or for contingency data by Fisher's exact test or χ^2 test. All comparisons were two-tailed, and a value of $P<.05$ was taken to indicate statistical significance.

Results

Occurrence and Type of Proarrhythmia in Controlled Series

Flecainide caused proarrhythmic responses in 15 of 19 controlled series MI dogs, and 4 of 13 healthy dogs ($P=.02$). MI dogs also presented more severe forms of proarrhythmia, such as sustained and spontaneous VT (Fig 1). Spontaneous VT did not occur in healthy dogs, but was observed in 8 of 19 MI dogs ($P=.007$). Drug concentrations at the time of VT averaged 5.3±1.9 mg/L in healthy dogs and 5.6±2.3 mg/L in MI dogs (P=NS). In dogs without proarrhythmia, death occurred because of progressive hypotension at plasma concentrations averaging 9.8±4.8 mg/L in healthy dogs (n=9) and 10.4±1.9 mg/L in MI dogs (n=4, P=NS versus healthy dogs). Flecainide significantly reduced blood pressure in MI dogs only at the highest doses, with blood pressure remaining unchanged before proarrhythmia in most cases.

General Determinants of Proarrhythmia

In the four MI dogs without proarrhythmia, infarct size averaged 3±2% of the left ventricle, compared with 32±5% in dogs with proarrhythmia ($P<.05$). The cumulative concentration-response curve for proarrhythmic responses in infarct dogs is shown in Fig 2. The EC_{50} for proarrhythmia was ~6 mg/L, and the maximum proarrhythmic incidence of 79% occurred at a plasma concentration of 9 mg/L. Mean plasma concentrations at the time of proarrhythmia were higher for spontaneous VT (6.8±1.4 mg/L, n=7) than for sustained (4.0±2.5 mg/L, n=7) and nonsustained (4.4±2.2 mg/L, n=5) VT. When multiple forms of VT occurred within a given dog, more severe forms occurred later in the protocol at higher drug concentrations. Because of the smaller number of normal dogs experiencing proarrhythmia, a concentration-response curve for proarrhythmia could not be constructed, but the mean drug concentration at the time of proarrhythmia was similar to that in MI dogs.

Drug-Induced Changes in Conduction and Refractoriness

Drug-induced conduction slowing that exceeds changes in refractoriness is believed to play an important role in the arrhythmogenic properties of class IC drugs.[1,16,26-28] Fig 3, left, shows an analysis of the effects of flecainide on conduction velocity. Drug-induced conduction slowing was not significantly different for longitudinal versus transverse propagation and was not significantly different in infarcted versus healthy hearts. Flecainide did not significantly alter ventricular ERP in either infarcted or healthy hearts (Fig 3, right). The very similar changes produced by the drug in healthy versus infarcted hearts do not clarify the mechanism of the increased susceptibility to proarrhythmia of the latter

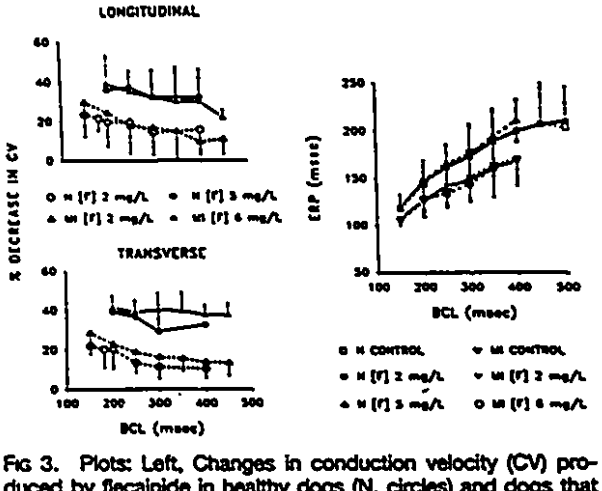

FIG 3. Plots: Left, Changes in conduction velocity (CV) produced by flecainide in healthy dogs (N, circles) and dogs that had prior myocardial infarction (MI, triangles), when conduction was assessed in the longitudinal (top) or transverse (bottom) direction. For a similar mean concentration ([F]), drug effects were not significantly dependent on direction of propagation or the presence or absence of MI. BCL indicates basic cycle length. Right, Ventricular effective refractory period (ERP) as a function of BCL in the absence and presence of flecainide. No significant changes were seen.

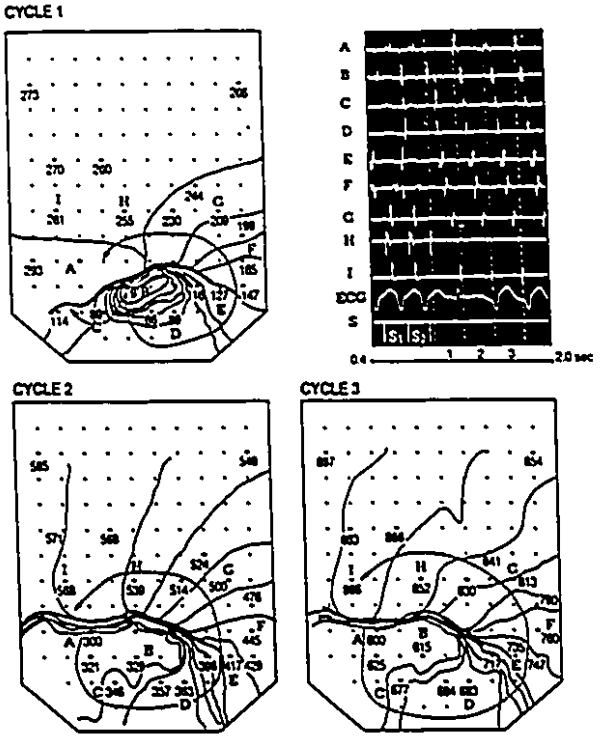

FIG 4. Top left, bottom left, and bottom right, Maps of cycles 1, 2, and 3, respectively, showing a complete reentry circuit responsible for proarrhythmic ventricular tachycardia (VT) in dog No. 34. The activation patterns during these cycles are delimited by the vertical dashed lines in the analogue recordings (top right). The electrode array is shown schematically for each activation map, with dots representing the sites of bipolar recording electrodes. The long axis of the array is oriented parallel to the left anterior descending coronary artery, which runs along the left side of the array; the numbers on each map are activation times at selected sites; and 20-ms isochrons are shown. All activation times are relative to the time of the extrastimulus (S_2) that initiated VT. The heavy lines represent arcs of block, and the dashed arrows indicate the sequence of activation. Top right, Analogue recordings from nine electrode sites (A through I), with locations indicated on each map, from the surface ECG and from a stimulus artifact channel (S). (For detailed discussion, see text).

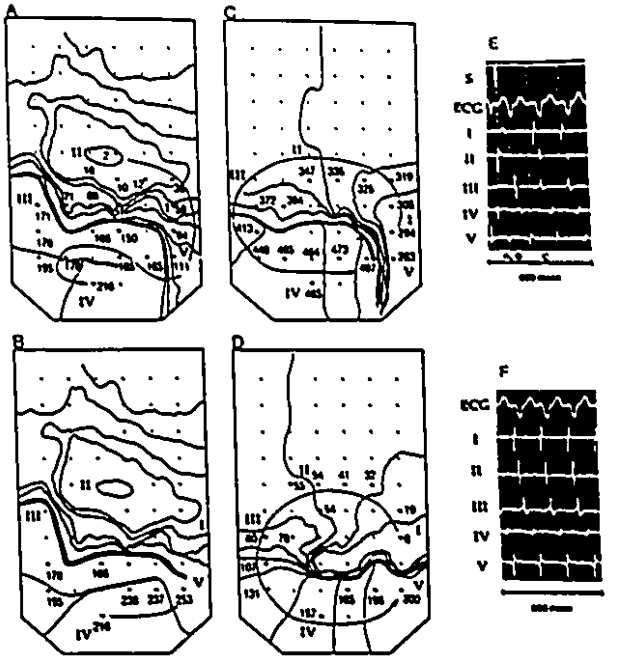

FIG 5. A through D, Activation maps during sustained ventricular tachycardia (VT) initiated by an extrastimulus at a coupling interval of 145 ms in dog 26. Format for activation maps as in Fig 4. A and B, Activation during the beat initiated by the extrastimulus; C, activation during the first reentrant beat of VT; and D, activation during a cycle of VT several seconds after VT onset. E, Analogue recordings of stimulus artifact (S), surface ECG, and electrograms from five locations in A through C. The beginning and end of each cycle is delimited by the vertical dashed lines, and activation times are given relative to the peak of the stimulus artifact. F, Analogue recordings of surface ECG and electrograms at selected sites (leads I through V) during VT. The cycle corresponding to D is delimited by the vertical dashed lines in F, and D shows the locations corresponding to electrogram leads I through V. (For detailed discussion, see text.)

group. Conduction slowing alone appears to be insufficient to cause proarrhythmic responses in a large percentage of hearts.

Activation During Ventricular Tachycardia

Fig 4 shows activation data at the onset of VT in one dog (dog No. 34): recordings of selected electrograms and a surface ECG lead (top right) and maps of the first three activations of VT (cycles 1 through 3, corresponding to the cycles delimited by the vertical dashed lines on the analogue panel). All activation maps are oriented in the same fashion, with the LAD running parallel to the long axis of the array along its left border. Electrode locations indicated by letters on each map correspond to the electrograms shown in the analogue recordings. After the last basic stimulus (S_1) at a cycle length of 250 ms, a single extrastimulus is applied (S_1S_2, 210 ms), initiating a run of sustained VT (200 per minute). Selected activation times relative to S_2 are shown on the activation maps, along with 20-ms isochrons. The activation initiated by S_2 (cycle 1) is shown at the upper left. The tissue underlying the top two thirds of the array (toward the base of the heart) was not captured by the extrastimulus, and a line of horizontal unidirectional block (solid line) resulted. Propagation occurred slowly in the inferior direction, and the counterclockwise limb propagated around the lateral border of the arc of block near site E. The impulse then continued to propagate counterclockwise around a transverse arc of functional block to produce cycles 2 and 3, and all subsequent beats of VT displayed a similar activation pattern.

Fig 5 shows the initiation of ventricular tachycardia in another dog (dog No. 26). An extrastimulus (S_1S_2, 145 ms; basic cycle length, 200 ms) initiates activity at the center of the array (activation times in Fig 5A through 5C are referenced to S_2), which propagates rapidly in the longitudinal direction (perpendicular to the LAD and horizontally in Fig 5A) and more slowly in the transverse direction. A broad arc of conduction block is encountered (solid line), forcing the impulse to travel around the lateral margin of the arc of block. The impulse travels toward the LAD, past a shorter arc of transverse conduction block, and then appears to propagate around the inferior border of the array in a counterclockwise direction (Fig 5B). The excitation of tissue at the

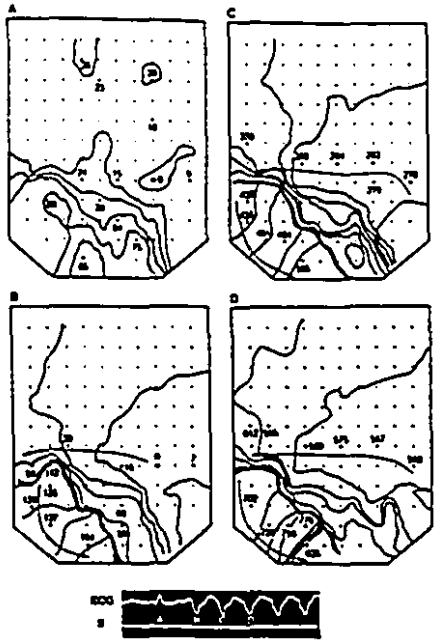

Fig 6. Activation during ventricular tachycardia (VT) of spontaneous onset. A through D, Activation maps corresponding to complexes A through D in the ECG tracing (bottom). Format as in Fig 4. *Site (S) of earliest activation (time 0) for A through D. A, Activation during last sinus beat B, Activation of ventricular ectopic beat that initiated VT. C and D, Activation during second and third beat, respectively, of VT. Note that site of earliest activation has moved to lateral margin of array. Activation times for B through D are referenced to earliest activation during cycle B. (For detailed discussion, see text.)

inferolateral portion of the array then initiates a full counterclockwise reentry circuit around the arc of transverse block, as shown in Fig 5C, producing the first unstimulated beat of VT. A stable reentry pattern results, with the activation shown in Fig 5D recorded several seconds later during VT (vertical dashed lines in Fig 5F delimit cycle mapped in Fig 5D). The ECG and selected electrogram tracings corresponding to Fig 5A through 5C are shown in Fig 5E, and those corresponding to Fig 5D are shown in Fig 5F.

Fig 6 shows activation during a spontaneously occurring episode of VT in dog No. 42. An ECG and stimulus channel (showing lack of stimulation) are provided on the bottom, and activation maps of beats A through D on the ECG recording are shown in the corresponding sections of the figure (Fig 6A through 6D). Epicardial breakthrough of the last sinus beat (Fig 6A) occurs at the site marked by an asterisk, and a large region of the LAD territory is activated within 30 ms. Activation is delayed towards the apex, as expected in the presence of an almost transmural infarction that interferes with normal endocardial to epicardial impulse propagation.[29] A spontaneous ventricular ectopic beat, with initial activation indicated by the asterisk in Fig 6B, initiates 14 beats of VT at a cycle length of 280 ms. The propagating impulse encounters an arc of conduction block (solid line in Fig 6B) and appears to turn around the arc of block at the medial border of the electrode array. Activation proceeds in a counterclockwise direction beyond the arc of block. Initial activation of the next cycle (C) occurs at the lateral border of the array, 106 ms after the last recorded activation during the preceding cycle. The impulse conducts medially, encountering an arc of block similar to that shown in B, and returns in a counterclockwise direction beyond the arc of block. A similar activation pattern was recorded for the next beat (D) and for subsequent cycles of VT. Unlike the data shown in Figs 4 and 5, which show complete circuses of impulse propagation during VT, the activation data in Fig 6 account for only part of a potential reentry cycle. In addition, VT occurs spontaneously in Fig 6, whereas it results from premature stimulation in the other figures. In both cases of spontaneous VT recorded with the mapping system, the ventricular beat initiating tachycardia had a pattern of activation different from that recorded during VT, consistent with the possibility that the mechanism of the premature beat initiating reentry is different from the mechanism of VT. Enhanced automaticity is known to cause ventricular ectopy in this model[30-32] and could explain the ventricular premature beat that initiated reentry. Alternatively, the beat initiating reentry could be a result of reentry with a different epicardial breakthrough point compared with sustained VT.

Of 15 dogs with myocardial infarction and proarrhythmic VT in the controlled series, activation data during VT were available in 11. In 4 dogs, a complete reentry circuit was resolved (Table 2), as in Figs 4 and 5. In 5 other dogs, the activation pattern was consistent with reentry, but recorded activation times did not account for all the reentry cycle. Among all dogs with a complete or partial reentry circuit visualized, an arc of transverse conduction block was noted between the central stimulation point and the apex of the left ventricle.

Activation During Ventricular Paced Rhythm and Relation to Proarrhythmia

Transverse conduction block appeared to play a central role in proarrhythmic responses. We therefore evaluated the hypothesis that while overall conduction changes caused by the drug were similar in infarcted and normal hearts, infarction may predispose localized regions to drug-induced conduction slowing. Figs 7 through 9 show activation in the LAD territory before and after flecainide in a representative healthy dog, an MI dog without proarrhythmia, and an MI dog with proarrhythmia, respectively. In the healthy dog (Fig 7), flecainide slowed conduction by 16% at a cycle length of 400 ms, without altering the pattern of activation (Fig 7C). When the pacing cycle length was decreased to 200 ms (Fig 7D), the drug slowed conduction by 34% without changing the activation pattern. In MI dogs without proarrhythmia (Fig 8), similar drug concentrations produced conduction changes very similar to those in the healthy dogs. Fig 9 shows corresponding results in a dog with proarrhythmia. Under control conditions, activation in the LAD territory was not perceptibly different from that in the healthy dog in the absence of drug at either cycle length. Flecainide, at a concentration similar to that in the healthy dog (Fig 7), slowed conduction by 18% at a cycle length of 400 ms (Fig 7C), without changing activation pattern. When the pacing cycle length was shortened to 200 ms (Fig 7D), conduction in the longitudinal and superior (basal) direction was uniformly slowed, as in the healthy dog in Fig 7D. In the apical direction, however, an arc of conduction block appeared (solid line).

TABLE 2. Occurrence of Arrhythmia and Block AFter Flecainide Infusion in Controlled Series of Dogs

	Control Results		Results in the Presence of Flecainide				
Dog	Conduction Block	Arrhythmia (CL)	[F] V Pacing, mg/L	Conduction Block	[F] A/D, mg/L	Arrhythmia (CL)	Activation maps
Healthy Dogs							
11	–	–	10.7	–	19.2	–	–
12	–	–	12.4	–	14.9	–	–
13	–	–	2.7	–*	2.7	SVT (220)	N/A
14	–	–	2.6	–	10.5	–	–
15	–	–	2.9	–	5.6	–	–
17	–	–	6.4	–	8.8	–	–
23	–	–	3.2	–	3.5	–	–
24	–	–	2.8	–	7.1	–	–
36	–	–	5.8	–*	6.4	NSVT (250)	NM
38	–	–	9.5	–	9.5	–	–
39	–	–	4.4	–	9.5	–	–
41	–	–	6.7	+ (L/S)*	7.1	NSVT (200)	N/A
45	–	–	4.2	–*	5.5	SVT (240)	NM
MI Dogs							
18	–	–	2.7	–	10.8	–	–
19	–	–	2.9	–*	3.3	NSVT (180)	C
20	–	–	4.2	–	8.6	spont VT (200)	P
21	–	–	3.2	–	N/A	spont VT (N/A)	N/A
22	–	–	2.6	+ (S)*	2.9	NSVT (200)	C
26	–	NSVT (190)	1.2	+ (S)*	1.6	SVT (245)	C
27	–	–	5.9	–	11.2	–	–
28	–	–	1.6	–	2.2	SVT (180)	N/A
31	–	–	7.7	+ (S)*	8.5	SVT (320)	N/A
32	–	–	N/A	N/A	4.3	spont VT (220)	P
33	–	–	1.8	–	7.7	spont VT (270)	NM
34	–	–	4.8	+ (S)*	5.5	SVT (270)	C
37	–	–	7.5	+ (S)*	7.5	spont VT (320)	P
42	–	–	2.5	+ (S)	6.6	spont VT (280)	P
43	–	–	5.6	–	11.9	–	–
44	–	–	5.7	+ (S)*	6.7	spont VT (300)	P
46	–	–	2.1	–	6.0	spont VT (250)	N/A
47	–	–	2.0	–	6.9	NSVT (240)	NM
48	–	–	4.4	–	7.7	–	–

Conduction block indicates presence or absence of conduction block during ventricular pacing at long (L) or short (S) basic cycle length (CL) of ventricular arrhythmia; Arrhythmia, most severe form of reproducible arrhythmia observed; [F] V Pacing, flecainide concentration at time of ventricular pacing before arrhythmia or death; [F] A/D, flecainide concentration at time of proarrhythmia or death; Activation maps, activation pattern during arrhythmia; –, no block; NSVT, non-sustained ventricular tachyarrhythmia (VT); +, transverse conduction block present; SVT, sustained VT; spont VT, spontaneous VT; NM, no mechanism revealed by activation map; C, complete reentry circuit; and P, partial reentry circuit.

*Dogs with proarrhythmia for which conduction data during ventricular pacing were available at plasma concentrations ≥75% of concentration associated with proarrhythmia.

In 7 MI dogs with proarrhythmia, activation data during ventricular pacing were obtained as part of the controlled series study protocol at a plasma concentration ≥75% (Table 2) of the concentration at which proarrhythmia occurred. In 6 of these dogs, an arc of conduction block was observed during rapid pacing. In all 6, conduction block was absent at slower rates and the location of block during rapid ventricular pacing remained the same as during VT. Arcs of block were never observed in dogs without VT. In the additional 12 dogs studied to evaluate regional refractoriness, all 6 with proarrhythmia had arcs of block during rapid ventricular pacing and no block was seen in the 6 without proarrhythmia.

Studies of Conduction and Refractoriness at Sites of Block

The above data indicate a close association between the development of regional block and the occurrence of proarrhythmia, consistent with a potentially important role for regional block in the mechanism of VT induced by flecainide. Fig 10 presents an analysis of results from the controlled series of dogs that was designed to establish whether the susceptibility of dogs to proarrhythmia was due to generally enhanced drug effects on conduction or to regional factors at the site of block. For equivalent drug concentrations, overall drug-induced conduction slowing during both longitudinal and transverse propagation was similar for dogs with (Fig 10, bottom) and without (Fig 10, top) block. During rapid pacing (bottom right), conduction slowing was much greater at the site of transverse conduction block (filled bar) than elsewhere over the infarct (bars with horizontal lines). In contrast also in Fig 10, during slower pacing (bottom left) transverse conduction slowing was the same at the site of block (filled bar) as in other regions (horizontal lines). Thus, block was due to an interaction between rate and the underlying substrate and not to a generalized susceptibility to drug action.

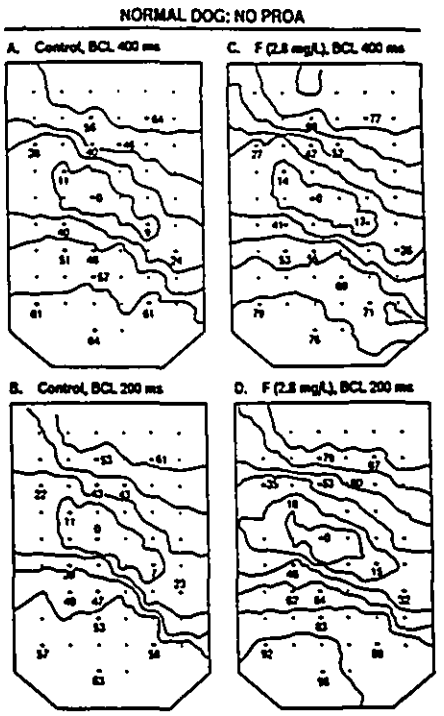

FIG 7. Activation maps from a healthy (normal in the figure) dog with no proarrhythmia (PROA). A and B, Control results as obtained at basic cycle lengths (BCLs) of 400 and 200 ms, respectively. C and D, Results in the presence of 2.8 mg/L flecainide at the same cycle lengths. Format for maps as in Fig 4.

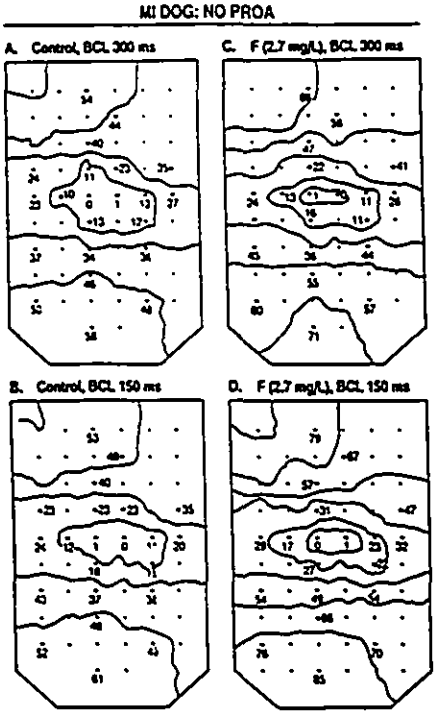

FIG 8. Activation maps from a dog that had a myocardial infarction (MI) but no proarrhythmia (PROA). A and B, Control results at basic cycle lengths (BCLs) of 300 and 150 ms, respectively. C and D, Results in the presence of flecainide (2.7 mg/L) at the same BCL values. Format for maps as in Fig 4.

We then determined whether the interaction is due to a progressive reduction in conduction velocity at the site of block with increased rate in the presence of flecainide. Fig 11 shows the cycle length dependence of transverse conduction velocity at the site of block under control conditions and in the presence of flecainide in the 7 MI dogs with block. While conduction tended to slow with decreased cycle length in the presence of the drug, in all but 1 dog (dog No. 26) there was an abrupt decrease in conduction velocity (averaging 58±17%) associated with block at the shortest cycle length.

The final possibility that we evaluated was that block results from spatially determined drug effects on refractoriness, with ERP prolonged to a greater extent distal (versus proximal) to the site of block. In an additional series of 12 dogs studied to evaluate this possibility, block occurred in 6. For each of these dogs, ERP was determined by local stimulation on either side of the arc of block at a cycle length similar to the VT cycle length in that dog. As shown in Fig 12, no systematic differences in ERP were observed between sites proximal and distal to the site of block. Overall, ERP averaged 246±16 ms at 13 sites proximal to the arc of block and 246±21 ms at 13 sites distal to the arc of block.

Histological Analysis

Examination of histological sections confirmed that epicardial muscle fibers were oriented in the direction of rapid impulse propagation, ie, perpendicular to the LAD. Infarctions became progressively denser and more transmural toward the apex, and at the zones of block there were areas in which the infarction extended to the epicardial surface (ie, zones in which there were no surviving epicardial cells). However, similar zones were present in dogs without an arc of block, so that no qualitative histological differences could be identified with the occurrence of an arc of block.

Discussion

We have shown that the presence of a prior MI predisposes dogs to the ventricular proarrhythmic actions of flecainide. The occurrence of proarrhythmia in our dogs was related to plasma drug concentration, and the mechanism of proarrhythmia appeared to be reentry involving an arc of rate-related transverse conduction block in the presence of the drug.

Comparison With Previous Studies of Flecainide-Induced Proarrhythmia in Dogs

Several studies have shown that flecainide can facilitate the occurrence of VT in dogs with prior MI.[8-13] In addition, one group reported a high incidence of flecainide proarrhythmia at high concentrations in dogs with healthy hearts.[15] We found that under controlled conditions, dogs with healthy hearts are relatively resistant to flecainide proarrhythmia, whereas MI dogs that do not experience proarrhythmia have relatively small infarctions. While clinical experience suggests that diseased hearts are predisposed to flecainide proarrhythmia,[27] the present study comprises, to our knowledge, the first direct experimental demonstration of this phenomenon.

Little information is available about changes in activation that underlie flecainide proarrhythmia. Only one published study[12] presented activation data that were

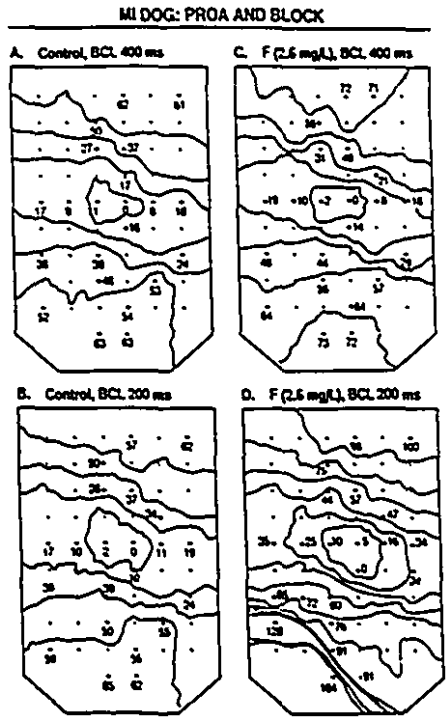

FIG 9. Activation data from a dog that had a myocardial infarction (MI) with proarrhythmia (PROA) (dog No. 22). A and B, Activation maps at basic cycle lengths (BCLs) of 400 and 200 ms, respectively, under control conditions. C and D, Activation maps at BCLs of 400 and 200 ms, respectively, in the presence of 2.6 mg/L flecainide. Format for maps as in Fig 4.

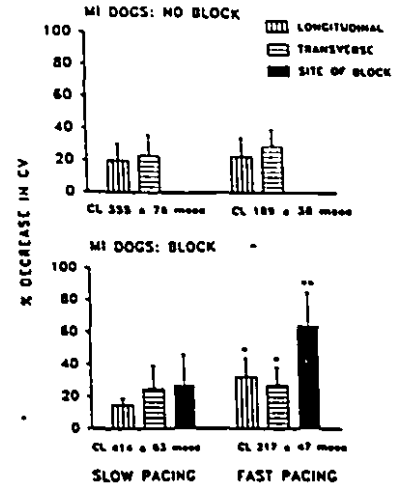

FIG 10. Bar graphs showing percentage decrease in conduction velocity (mean±SD) caused by flecainide in a controlled series of dogs that had a myocardial infarction (MI dogs) without block (top, 3.3±1.5 mg/L) and MI dogs with an arc of conduction block (bottom, 4.6±2.5 mg/L), during fast and slow pacing. Conduction velocity (CV) in the longitudinal and transverse directions were calculated by linear regression of distance on activation times, excluding zones of block. Drug-induced conduction velocity changes at the site of block were calculated from the interelectrode distance and difference in activation times across the site of block. CL indicates cycle length. *$P<.05$ for difference in drug-induced change in conduction velocity across the arc of transverse conduction block vs overall longitudinal or transverse conduction, **$P<.01$ for difference in conduction velocity during fast vs slow pacing.

obtained in two dogs with a 48-electrode array. The limited data presented in that paper are consistent with the arrhythmia mechanism that we observed, reentry around a drug-induced arc of conduction block. Another study, published only in abstract form,[10] showed that flecainide induced VT in four dogs by extending the line of block transverse to fiber orientation while slowing conduction equally in the longitudinal and transverse directions.

Mechanism of Proarrhythmia

In 9 of 11 MI dogs in the controlled series for which activation maps during proarrhythmic VT were available, activation compatible with a reentry circuit could be mapped around an arc of conduction block, which always occurred in the direction transverse to fiber orientation. The anisotropic properties of myocardium thus played an important role in proarrhythmia. According to the original descriptions of VT inducibility by programmed electrical stimulation in dogs with prior MI,[33-36] epicardial mapping studies showed that VT in this model is due to reentry around an arc of conduction block within the infarct,[37,38] with block occurring in the direction of impulse propagation transverse to myocardial fiber bundles.[39,40] The intrinsically anisotropic properties of myocardial conduction[41] appear to play an important role in producing this type of VT,[39,40] which has been called "anisotropic reentry."[40] Similar mechanisms underlay proarrhythmic VT in our dogs. While in normal tissues, the safety factor for impulse propagation is greater in the transverse direction,[41,42] transverse conduction block is predicted to occur more readily than longitudinal when active membrane properties are impaired.[43] A lower safety factor for transverse impulse propagation has been observed in the presence of hyperkalemia,[43] and the intrinsically anisotropic properties of myocardial cell coupling[44] are believed to play a central role in the genesis of reentrant clinical arrhythmias related to MI.[45-47] We obtained evidence that suggested that flecainide caused proarrhythmia by inducing reentry around arcs of transverse conduction block, as also noted in infarcted hearts in the absence of drugs.[39,40] Block was rate related and appeared abruptly at critical cycle lengths (see Figs 10 and 11). These findings are compatible with computer simulations of the behavior of anisotropic tissues in the presence of impaired active properties and suggest that flecainide causes proarrhythmic VT by impairing active membrane properties and causing conduction to fail in the direction of weaker cell-to-cell coupling, ie, transverse-to-fiber orientation.

New Findings and Potential Significance

The present study is the first to study experimentally the dose-response relation for flecainide proarrhythmia in an organized fashion, to evaluate associated electrophysiological changes in the presence of stable plasma concentrations, and to compare directly proarrhythmia and electrophysiological changes produced by flecainide in infarcted hearts with those in control dogs studied in parallel. While limited mapping data previously have been reported during flecainide-induced VT,[10,12] the present study provides a systematic analysis of activation during VT and relates this to ventricular activation at a

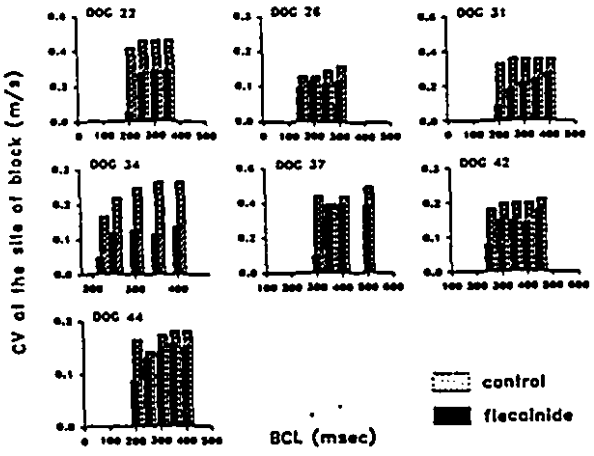

FIG 11. Bar graphs showing apparent conduction velocity (CV) between electrodes at the site of rate-dependent block, calculated from the interelectrode distance divided by difference in activation times. Conduction block was defined by a velocity <0.1 m/s. Results are shown under control conditions (hatched bars) and in the presence of flecainide (solid bars) during the dose that caused proarrhythmia. BCL indicates basic cycle length.

series of basic cycle lengths under control and drug conditions in healthy and infarcted hearts.

The specific proarrhythmic risks associated with class IC antiarrhythmic drugs were first noted in the early 1980s[48-51] and led to a distinction between proarrhythmic responses to drugs that block sodium channels compared with those that prolong action potential duration.[27,28] The presence of structural heart disease, a substrate that can support VT, and rapid escalation of flecainide dose have been identified as factors that increase the likelihood of proarrhythmia.[52,53] In addition, exercise has been identified to be especially likely to precipitate proarrhythmic reactions, particularly during flecainide therapy,[54,55] possibly by causing a sinus tachycardia.

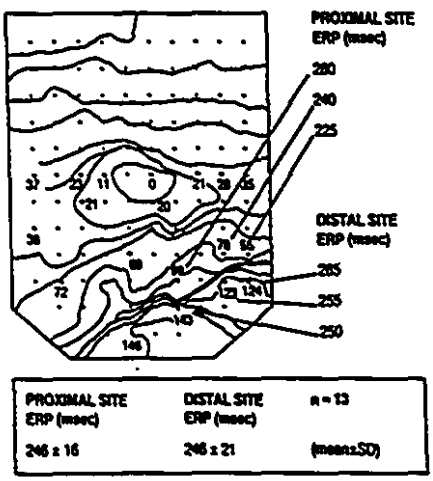

FIG 12. Activation map of a representative dog (during block) showing refractory periods (ERP) measured selectively by stimulating at sites proximal and distal to an arc of transverse conduction block. ERP values were measured at six adjacent sites across the arc of block in six dogs. Table. Mean results from 13 pairs of sites in the six dogs studied.

The present study sheds light on potential mechanisms underlying these clinical findings. Anisotropic reentry, strongly facilitated by the presence of previous infarction, was found to be the likely mechanism underlying VT in 9 of our 11 MI dogs for which activation data during VT were available. Abrupt conduction block at rapid rates appeared to underlie the rate dependence of drug-induced proarrhythmia. While use-dependent sodium channel blockade causes progressive conduction slowing in the presence of sodium channel blockers,[26,56] sudden decreases in conduction velocity are not seen in healthy tissues.[56] Sudden rate-dependent failure of transverse conduction occurs in infarcted hearts not exposed to antiarrhythmic drugs[37,40] and is predicted to occur during transverse propagation when sodium current is depressed.[43] Thus, in addition to enhancing drug-induced sodium channel block, tachycardia favors the occurrence of transverse block by exposing critical discrepancies in the source-sink relation. Our findings suggest that proarrhythmia is due to interaction among cycle length, the underlying substrate, and drug effects on conduction.

Model Limitations and Results

We found that, while proarrhythmia could be induced by flecainide in dogs with prior MI, relatively high drug concentrations were necessary in most dogs. These observations are consistent with clinical findings that therapeutic flecainide concentrations cause sustained VT to be inducible in a relatively small fraction (7% to 29%) of patients.[57-59] It is difficult to know to what extent the mechanisms of flecainide-induced VT in our dogs apply to drug-induced VT in humans. Because of important interspecies differences in drug sensitivity, it is important to evaluate indexes of drug pharmacodynamics to relate the effects of drugs seen in animal models to those noted in man. At therapeutic doses, flecainide slows intraventricular conduction (as indicated by the QRS duration) by ~25% in humans.[51] This degree of conduction slowing is in the range of the mean changes that we observed in our dogs (Fig 3). Furthermore, the risk of clinical proarrhythmia increases with increasing flecainide dose,[52] as does the degree of intraventricular conduction slowing.[51] In a well-documented series of patients with VT that was caused by a pharmacologically similar class IC agent, encainide, drug-induced QRS prolongation averaged 46±10%,[48] suggesting a degree of conduction slowing in the range that we observed in dogs exposed to 5 to 6 mg/L plasma flecainide concentrations (Fig 3). Thus, while VT in some dogs required concentrations that are equivalent to toxic levels in man, the similarity in conduction slowing to values in patients experiencing proarrhythmia at therapeutic doses suggests pharmacodynamic equivalence and possible relevance to clinical phenomena. Proarrhythmia in our dogs was always associated with significant conduction slowing. Clinical drug-induced VT can occur in the absence of substantial ventricular conduction slowing,[50] in which case it may be due to idiosyncratic predisposition to other drug-induced arrhythmia mechanisms. Potent sodium channel blockers such as flecainide strongly increase the risk of ventricular fibrillation during acute ischemia[60-62] at concentrations much lower than those required for proarrhythmia in the present model.[63] This

mechanism may well play an important role in class I drug effects on mortality.

We were able to account for a complete reentry circuit in just under half the dogs (4 of 9) in which reentrant activity could be mapped. In the other 5 dogs, activation accounting for part of a potential reentry circuit was obtained. As for most previous studies in the literature in this arrhythmia model, mapping was limited to epicardial sites over the infarct zone. Intramural and endocardial activation were not recorded. Our inability to account fully for reentry in some dogs may have been due to the involvement of intramural and subendocardial tissues in the reentry circuit, as previously demonstrated in both dog models[64-66] and human hearts with inducible VT.[67] Alternatively, some of the reentry circuit may have been epicardial but outside the field covered by our electrode array. This condition may also account for the fact that the circuits we mapped had only one limb of reentry, whereas reentry in the postinfarction VT model is frequently associated with a figure eight pattern.[39,40,66] Another possibility, particularly when activation data do not reveal evidence for reentry or account for only part of a reentry circuit, would be the participation of other arrhythmia mechanisms, such as abnormal and triggered automaticity, that can occur in infarcted preparations.[33,68]

In any case, it is important to be aware that the results presented in this manuscript, while pointing strongly to reentry mechanisms (particularly when a complete circuit is mapped), do not provide absolute proof for reentry. We were able to satisfy two of the Mines criteria for demonstrating reentry,[69] the identification of unidirectional block and the delineation of a repetitive recirculating wave front during tachycardia. We did not attempt to satisfy the final criterion, the termination of tachycardia by anatomic disruption of the reentry circuit, because the demanding technical requirements of such a demonstration (requiring a stable and hemodynamically tolerated tachycardia, rapid on-line delineation of the circuit, and precise localized interruption of the circuit without disruption of function in a beating heart) place it beyond the scope of the present study.

The model we used simulates many conditions of clinical ventricular tachyarrhythmia occurring after recovery from acute MI.[33-40,67,68] Nevertheless, the model cannot be considered to mimic directly any specific clinical condition, and many aspects of the procedures involved (general anesthesia, open-chest preparation, intravenous drug administration, and programmed electrical stimulation) create potentially important differences from the clinical setting in which proarrhythmia typically occurs. Caution is therefore warranted in extrapolating the results of the present studies to man.

Several studies have suggested that sodium channel blockers may have more profound effects on longitudinal versus transverse conduction.[70-74] However, the relative magnitude of directional differences was highly variable, and at some concentrations no differences were seen.[71] The apparent discrepancy may be due to differences in action between flecainide and previously studied drugs (procainamide,[70] mexiletine,[71] quinidine,[71] amiodarone,[72] lidocaine,[73] and 0-desmethyl encainide[74]), to the drug concentrations studied, or to some other technical factor.

Conclusions

We have shown that flecainide promotes the occurrence of ventricular tachyarrhythmias in a concentration-dependent fashion in a large proportion of dogs with prior MI. The presence and extent of infarction are important in determining the likelihood of VT, which appears to be caused by reentry around an arc of functional transverse conduction block. The occurrence of block is rate dependent and appears to be caused by an interaction between sodium channel blockade and the underlying substrate. These experiments support the importance of the anisotropy of ventricular conduction in the genesis of cardiac arrhythmias, particularly when intercellular coupling and active membrane properties are disturbed by MI and potent sodium channel blocking drugs.

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Publication 4

The cardiotoxic effects associated with class I antiarrhythmic agents and its reversal by hypertonic salts have been described in sections 4.5 of the Introduction. In a study (Bajaj et al, 1989) where different possible contributing factors were well-controlled, the beneficial effects of hypertonic salts in reversing cardiotoxic effects of class I antiarrhythmic were due to the sodium moiety. In publication 4 we studied the mechanisms of modulation of flecainide's effects by extracellular sodium concentration using both electrophysiological and biochemical approaches.

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Modulation of Flecainide's Cardiac Sodium Channel Blocking Actions by Extracellular Sodium: A Possible Cellular Mechanism for the Action of Sodium Salts in Flecainide Cardiotoxicity[1]

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ABSTRACT

Sodium salts reverse the clinical cardiotoxicity of class 1c antiarrhythmic agents, but the underlying mechanisms are unknown. We studied the modulation of flecainide's action by changes in extracellular sodium concentration ($[Na^+]_e$) produced by isotonic substitution of choline for sodium. Increasing $[Na^+]_e$ by 25 mM attenuated the depressant effects of 3.2 μM flecainide on $\dot{V}_{max}$ in canine cardiac Purkinje fibers, whereas decreasing $[Na^+]_e$ enhanced drug action. The voltage dependence of $\dot{V}_{max}$ was shifted by flecainide (activation potential for 50% decrease in $\dot{V}_{max}$, V_{50}: −77.4 ± 3.5 mV at 3.2 μM flecainide) compared to control (V_{50}: −73.7 ± 2.8 mV, mean ± S.D., $P < .05$). Increasing $[Na^+]_e$ in the presence of flecainide returned V_{50} toward control (−75.8 ± 3.1 mV, $P < .05$ vs. flecainide at normal $[Na^+]_e$). Increased $[Na^+]_e$ shifted the flecainide concentration-response curve to the right (EC_{50} 19.0 μM) compared to normal (EC_{50} 14.6 μM) and low (EC_{50} 10.8 μM) $[Na^+]_e$. $[Na^+]_e$ modulated the concentration-dependent displacement by flecainide of [^{3}H]batrachotoxin-A-benzoate, with increased $[Na^+]_e$ shifting the binding curve to the right and decreased $[Na^+]_e$ shifting it to the left compared to normal $[Na^+]_e$. There was a strong linear correlation ($r = 0.99$) between flecainide's EC_{50} for $\dot{V}_{max}$ depression and its IC_{50} for [^{3}H]batrachotoxin-A-benzoate displacement at various $[Na^+]_e$. We conclude that $[Na^+]_e$ modulates flecainide's interaction with the sodium channel. Sodium's ability to displace blocking drug from the sodium channel may underlie the efficacy of sodium salts in treating flecainide toxicity, and could play a similar role in antagonizing cardiotoxicity of other class 1 compounds.

The electrophysiologic actions of class 1c antiarrhythmic drugs are typified by substantial conduction slowing with relatively minor effects on refractory period (Roden and Woosley, 1986; Vaughan Williams, 1975), conditions which are expected to increase the risk of re-entry. In fact, class 1c drugs produce proarrhythmic responses associated with strong ventricular conduction slowing (Nathan *et al.*, 1984), particularly when there are pre-existing substrates capable of supporting ventricular re-entry (Slater *et al.*, 1988, Morganroth, 1987). These features have led to the identification of a particular form of proarrhythmic reaction caused by 1c agents (Levine *et al.*, 1989).

Recently, hypertonic sodium salts such as molar sodium lactate (Chouty *et al.*, 1987; Camous *et al.*, 1987) and sodium bicarbonate (Pentel *et al.*, 1986; Winkelmann and Leinberger, 1987) have been found to be effective in the treatment of class 1c drug toxicity. The mechanisms by which hypertonic sodium salts reverse 1c drug toxicity are unknown. Bajaj and coworkers showed that the beneficial actions of sodium bicarbonate on toxicity caused by an encainide metabolite *in vivo* were mimicked by equimolar sodium chloride (Bajaj *et al.*, 1989), but not by hyperventilation (to increase pH) or by mannitol (to increase osmolarity). They therefore concluded that the sodium moiety was responsible for the actions of sodium bicarbonate.

The purpose of the present experiments was to study the mechanisms by which $[Na^+]_e$ alters the actions of a class 1c agent, flecainide. Both electrophysiologic and biochemical methods were used, and isosmotic substitution of sodium by choline was applied to isolate the role of changes in $[Na^+]_e$ *per se*.

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ABBREVIATIONS: $[Na^+]_e$, extracellular sodium concentration; LNa, low sodium; NNa, normal sodium; HNa, high sodium; MAP, membrane activation potential; APA, action potential amplitude; APD, action potential duration; I_{Na}, sodium current; [^{3}H]BTXB, tritiated batrachotoxin A benzoate; TTX, tetrodotoxin; ATX II, toxin from *Anemonia sulcata*.

Methods

Microelectrode Experiments

General methods. Mongrel dogs were anesthetized with i.v. pentobarbital (30 mg/kg). Their hearts were excised *via* right thoracotomy and placed into oxygenated Tyrode's solution. False tendons were removed and pinned to the floor of a 30-ml Lucite tissue chamber. The tissue was initially superfused at 16 ml/min with "standard" Tyrode's solution containing (mM): Na^+, 141; HCO_3^-, 22; dextrose, 10; K^+, 4; $H_2PO_4^-$, 0.9; Mg^{++}, 0.5; Ca^{++}, 1 and Cl^-, 125; and was aerated with 95% O_2/5% CO_2 (bath pH 7.35). A heating element and proportional power supply were used to maintain a temperature of 37°C in the bath. Glass microelectrodes filled with 3 M KCl and with tip resistances of 8 to 20 megohms were coupled *via* an Ag-AgCl junction to a high-impedance amplifier (WPI KS-700). A bipolar platinum electrode positioned on ventricular muscle adjacent to the false tendon was used to apply square-wave 2-msec pulses (twice threshold current) under the control of a programmable stimulator (Bloom Associates, Flying Hills, PA). If significant changes in latency (>10%) occurred, the experiment was rejected. Previously described methods were used to digitize the amplified waveform and analyze standard action potential characteristics (Nattel and Zeng, 1984). All preparations were equilibrated for at least 1 hr before experimental protocols began. Continuous impalement of the same cell throughout each experiment was required.

Superfusion solutions. To modify $[Na^+]_o$ while maintaining constant extracellular chloride concentration and osmolarity, solutions were prepared with ionic contents as shown in table 1. These solutions will be referred to as "modified Tyrode's solutions." Other constituents were identical to those in the initial standard Tyrode's superfusion solution described above.

Experimental Protocols

Modulation of flecainide's cellular electrophysiologic actions by $[Na^+]_o$. Action potential characteristics were determined after ≥2 min of pacing at basic cycle lengths of 300, 500, 800, 1000 and 2000 msec. MAP was defined as the transmembrane potential at the time of stimulation. APD was measured as the time for 50% (APD_{50}) and 95% (APD_{95}) of repolarization. In each experiment, action potential characteristics were studied in the presence of NNa solution and at least one of LNa or HNa solution in the absence of flecainide, and with the same solutions in the presence of flecainide. In each experiment, the preparation was studied before and after 20 min of flecainide infusion at a given sodium concentration, and then after 30 min of washout. The mean $\dot{V}_{max}$ after flecainide washout averaged 95.1 ± 4.0% of the predrug value. Flecainide was studied at a concentration of 3.2 μM (60% above the therapeutic range of 0.8–2 μM in humans). The order of solutions was varied to eliminate bias from time-related electrophysiologic changes. A total of five preparations were used to compare drug effects in the presence of LNa and NNa, and seven preparations to compare NNa and HNa. In some preparations, we were able to study all three sodium concentrations under both control and drug conditions. The effects of flecainide were based on comparisons between values in the presence of the drug and a given $[Na^+]_o$ and values in its absence at the same $[Na^+]_o$.

Modulation of the flecainide concentration-response relation by extracellular sodium. The preparation was perfused with LNa, NNa or HNa solution during stimulation at a cycle length of 1000 msec. A perfusion pump (model 575, New England Medical Instruments Inc., Medway, MA) was used to infuse flecainide, achieving concentrations of 0.1, 0.5, 2.9, 10.7 and 21.4 μM in the bath. The maximum infusion rate never exceeded 0.27 ml/min, or 1.7% of the superfusate flow rate. The actions of flecainide were studied after 20 min of superfusion at sequentially higher drug concentrations, with all concentrations evaluated in each experiment. After completion of the dose-response protocol, action potential characteristics were monitored until they returned to ≥95% of control values. The superfusate was then changed to one with a different sodium concentration, and action potential characteristics were measured 30 min later. The flecainide concentration-response curve was then repeated. In all experiments, concentration-response data were obtained in both NNa and at least one of LNa ($N = 7$) or HNa ($N = 10$) solutions. In two experiments, complete concentration-response data were obtained at all three $[Na^+]_o$.

Effects of $[Na^+]_o$ on the $\dot{V}_{max}$ inactivation curve. Membrane activation potential tended to be more negative as $[Na^+]_o$ was increased (table 2). To exclude effects due to changes in MAP, V_{max} inactivation curves were constructed by increasing the superfusate K^+ concentration to 10 mM and then returning to a normal K^+ concentration. The inactivation curve was obtained at normal and high $[Na^+]_o$ for each cell, and superimposable inactivation curves during wash-in and washout of 10 mM K^+ were required. Because of the difficulty in maintaining continuous stable impalement for all conditions (normal and high $[Na^+]_o$, control and flecainide, K^+ wash-in and washout), we could not compare all conditions in many cells. We obtained data at both $[Na^+]_o$ in the same cell under drug-free conditions in seven preparations, and in the presence of 3.2 μM flecainide in nine preparations. In two preparations, we obtained complete data at both $[Na^+]_o$ and in the presence and absence of flecainide.

Electrophysiologic studies of choline. To assess possible direct effects, action potential characteristics were first measured in standard Tyrode's solution, and then 20 min after the addition of 25 or 50 mM choline chloride. Atropine was then added (0.1 μM) and the measurements were repeated. To control for possible osmotic effects, similar experiments were performed in which 25 or 50 mM sucrose was added to standard Tyrode's solution.

Whole-Cell Voltage-Clamp Experiments

To determine whether $[Na^+]_o$ alters the I_{Na}-blocking action of flecainide, whole-cell voltage-clamp techniques were used to evaluate the changes in I_{Na} caused by 3.2 μM flecainide in the presence of 25 and 50 mM $[Na^+]_o$. Male guinea pigs (350–400 g) were sacrificed by cervical dislocation, and their hearts were removed and rinsed in a modified Tyrode's solution (100% O_2, 37°C) containing (mM): NaCl 126, KCl 5.4, $MgCl_2$ 1.0, NaH_2PO_4 0.33, glucose 10 and HEPES 5; pH adjusted to 7.4 with NaOH. The heart was perfused briefly in the Langendorff mode with the same solution containing $CaCl_2$ (2.0 mM). This was followed by perfusion with Ca-free solution, and then by the same solution containing 0.2% collagenase (CLS II, Worthington Biochemical, Freehold, NJ) and 1.0% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) for 15 min. The heart was then washed for 2 min with Ca-free solution, and the left atrium was removed and placed in a solution containing (mM): KCl 20, KH_2PO_4 10, glucose 10, glutamic acid 70, β-hydroxybutyric acid 10, taurine 10 and EGTA 10, bovine serum albumin 1.0%; pH adjusted to 7.4 with KOH. Cells were dissociated by mechanical agitation with a Pasteur pipette. The storage solution was progressively replaced over 60 min with normal bath solution containing (mM): NaCl 126, KCl 5.4, $MgCl_2$ 0.8, $CaCl_2$ 1.0, $CoCl_2$ 2.0, NaH_2PO_4 0.33, HEPES 10 and glucose 5.5; pH adjusted to 7.4 with NaOH. After adhesion to the bottom of a 1.0-ml chamber on the stage of an inverted microscope, cells were superfused with the normal bath solution at 3 ml/min. The $[Na^+]_o$ was then reduced to 25 or 50 mM by equimolar substitution of CaCl for NaCl. Adequate voltage control was assured by the absence of "abominable notches" and a gradual increase in I_{Na} during the increasing phase (negative limb) of the current-voltage curve. All experiments were performed at room temperature.

Current recordings were obtained in the whole-cell, voltage-clamp

TABLE 1
Ionic content of superfusion solutions

Solution	$[Na^+]$	[Choline]	$[Cl^-]$
		mM	
LNa (low Na)	116	50	150
NNa (normal Na)	141	25	150
HNa (high Na)	166	0	150

TABLE 2

Effects of flecainide (Flec) on action potential characteristics at varying sodium concentrations and cycle lengths

Results are mean ± S.D.

	Experiments Studying LNa (N = 5)				Experiments Studying HNa (N = 7)			
	LNa	LNa/Flec	NNa	NNa/Flec	NNa	NNa/Flec	HNa	HNa/Flec
BCL 300								
MAP	−91 ± 3	−88 ± 7	−91 ± 3	−89 ± 3	−93 ± 6	−91 ± 1	−93 ± 3	−92 ± 3
APA	118 ± 2	103 ± 5*	119 ± 5	109 ± 7**	124 ± 3	113 ± 7**	129 ± 7	121 ± 3**
APD_{95}	234 ± 13	217 ± 17*	237 ± 21	223 ± 23	234 ± 5	232 ± 14	247 ± 3	238 ± 6*
$\dot{V}_{max}$	465 ± 87	270 ± 73**	530 ± 98	342 ± 65*	623 ± 145	386 ± 110**	607 ± 80	438 ± 86**
BCL 500								
MAP	−90 ± 3	−88 ± 5	−91 ± 3	−89 ± 1*	−92 ± 2	−90 ± 2*	−95 ± 4	−92 ± 3*
APA	119 ± 4	107 ± 5*	122 ± 5	111 ± 5**	126 ± 5	116 ± 6**	131 ± 5	123 ± 4**
APD_{95}	328 ± 22	295 ± 38*	338 ± 6	290 ± 29**	324 ± 35	293 ± 27**	355 ± 26	310 ± 25**
$\dot{V}_{max}$	481 ± 64	305 ± 69**	548 ± 104	383 ± 71**	617 ± 147	394 ± 99**	622 ± 120	450 ± 76**
BCL 800								
MAP	−90 ± 4	−88 ± 3	−91 ± 3	−88 ± 1	−91 ± 2	−89 ± 2	−94 ± 4	−91 ± 3
APA	119 ± 4	107 ± 7**	122 ± 5	111 ± 5**	125 ± 4	117 ± 6**	131 ± 5	124 ± 5**
APD_{95}	418 ± 42	368 ± 50**	427 ± 48	360 ± 33**	412 ± 58	357 ± 47**	452 ± 56	389 ± 47**
$\dot{V}_{max}$	497 ± 72	343 ± 74**	551 ± 110	404 ± 60**	619 ± 138	435 ± 100**	630 ± 126	482 ± 77**
BCL 1000								
MAP	−89 ± 3	−88 ± 6	−90 ±3	−87 ± 1	−91 ± 3	−88 ± 1	−93 ± 4	−91 ± 3
APA	120 ± 4	107 ± 6**	121 ± 5	111 ± 5**	124 ± 4	118 ± 4**	129 ± 4	124 ± 5**
APD_{95}	452 ± 48	403 ± 56**	465 ± 55	403 ± 31**	442 ± 63	387 ± 56**	494 ± 52	428 ± 59**
$\dot{V}_{max}$	501 ± 71	359 ± 70**	547 ± 105	412 ± 64**	619 ± 134	467 ± 94**	634 ± 129	506 ± 85**
BCL 2000								
MAP	−86 ± 4	−86 ± 5	−87 ± 2	−86 ± 1	−89 ± 2	−89 ± 2	−91 ± 2	−91 ± 2
APA	116 ± 4	107 ± 7*	118 ± 4	111 ± 4*	120 ±2	118 ± 4*	126 ± 3	124 ± 2
APD_{95}	518 ± 65	457 ± 95*	525 ± 55	468 ± 48**	489 ± 76	445 ± 62**	552 ± 41	515 ± 71**
$\dot{V}_{max}$	497 ± 76	364 ± 81**	535 ± 96	430 ± 62**	612 ± 137	509 ± 119**	652 ± 111	558 ± 83*

* $P < .05$ compared to corresponding control at the same cycle length and $[Na^+]_o$.
** $P < .01$ compared to corresponding control at the same cycle length and $[Na^+]_o$.

configuration of the patch-clamp technique with the use of an Axopatch 1-D amplifier (Axon Instruments, Burlingame, CA). Electrodes were made of 1.0 mm outside diameter borosilicate glass and had tip resistances of 3 to 10 megOhm when filled with (mM): CsCl 120, $MgCl_2$ 1.0, HEPES 5.0, tetraethylammonium 20, Mg_3ATP 5.0, Na_2-creatine phosphate 5.0 and EGTA 10; pH adjusted to 7.4 with CsOH. Command pulses were generated by pClamp software interfaced with a D/A convertor (Axon Instruments). Whole-cell currents were filtered at 1 kHz and series resistance was compensated. I_{Na} was measured as the difference between the peak and steady-state current at the end of a 20-msec depolarizing pulse from a holding potential of −100 mV (frequency 2 Hz). Control, flecainide (3.2 μM) and washout results were obtained at the same $[Na^+]_o$ in each experiment. In two cells, stable results were obtained long enough to allow for control, drug infusion and washout at two sodium concentrations in each cell.

Extracellular sodium and flecainide's interaction with [^{3}H] BTXB binding

To study further flecainide's interaction with the sodium channel, we examined changes in [^{3}H]BTXB binding to isolated cardiac myocytes. Cardiac myocytes were isolated from adult male Sprague-Dawley rats (250–300 g) using previously described methods (Sheldon *et al.*, 1986, 1987). The heart was first perfused with a rinse solution (20°C), and then with a digestion solution (37°C) containing collagenase (250 U/ml) and fatty acid-free bovine albumin (0.1%). After digestion, the heart was cut above the atria, and the ventricles were removed and rinsed in a calcium-containing solution ($CaCl_2$ 1.5 mM, fatty acid-free bovine albumin 1%). Two incubations with the digestion solution achieved almost complete dispersion of cardiac myocytes. Dispersed cells were left to sediment by gravity, rinsed ($CaCl_2$ 50 μM, fatty acid-free bovine albumin 1%), pooled and filtered. Cell viability averaged 84 ± 7%. Myocytes were then incubated in Krebs-Henseleit buffer containing bovine serum albumin (1%) for receptor assay. The normal Na buffer contained (mM): NaCl 121.2, choline chloride 25, KCl 2.3, KH_2PO_4 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 19.8, glucose 5.5 and $CaCl_2$ 0.05 ($[Na^+]_o$ 141 mM). The LNa and HNa solutions contained 166 and 116 mM sodium and 0 and 50 mM choline chloride, respectively.

Radioligand binding assay. Myocytes (6×10^5/assay) were incubated in 50 μl of incubation buffer with 1.3 μM ATX II, 13 nM [^{3}H] BTXB (50 Ci/mmol) and 0.13 mM TTX as previously described (Sheldon *et al.*, 1986). Flecainide was added at concentrations ranging from 0.1 to 300 μM, and nonspecific binding was determined by adding 1.08 mM aconitine. After incubation for 55 min, filtration and rinse manipulations were performed as previously described (Sheldon *et al.*, 1986, 1987). In three experiments, the volume of isolated myocytes was large enough to obtain complete displacement curves in the presence of all three concentrations of sodium. In four experiments, data were obtained at normal and high $[Na^+]_o$, whereas in two experiments, binding was studied in the presence of high $[Na^+]_o$ only.

Statistical Analysis

Results are presented as the mean ± S.D. Student's *t* test was used for comparison with the Bonferroni correction for multiple comparisons (Sachs, 1984). Data fitting was obtained with least squares regression analysis, and Marquardt's procedure was used for nonlinear curve fitting. [^{3}H]BTXB binding displacement curves were analyzed with commercial software (Ligand, Biosoft Co., Milton, NJ).

Results

Modulation of flecainide's cellular electrophysiologic actions by $[Na^+]_o$. Flecainide decreased APA, APD_{95} and $\dot{V}_{max}$ (table 2). The depressant effects of the drug on APA and $\dot{V}_{max}$ were enhanced as cycle length decreased. APA, APD_{95} and $\dot{V}_{max}$ all tended to increase as $[Na^+]_o$ increased. APD_{95} in the presence of flecainide was similar in LNa and NNa solutions (fig. 1). HNa superfusate increased APD_{95} in the presence of flecainide at various cycle lengths by 3 to 16%, with the magnitude of change in APD_{95} increasing at longer cycle lengths.

The effects of flecainide on $\dot{V}_{max}$ were modulated by $[Na^+]_o$.

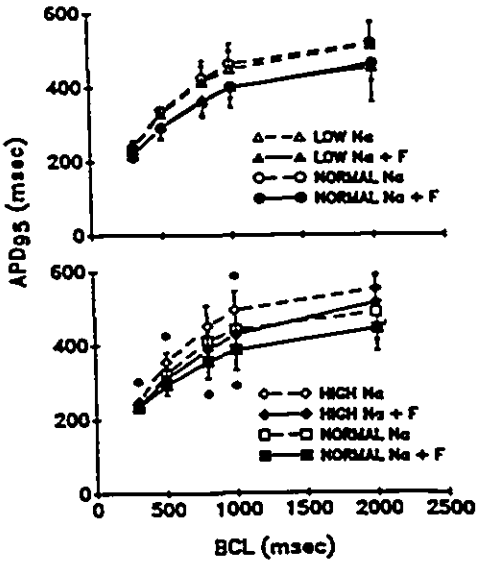

Fig. 1. APD_{95} in the presence and absence of flecainide (3.2 μM) at low and normal $[Na^+]_o$ (top) or high and normal $[Na^+]_o$ (bottom). * $P < .05$ for statistical significance of differences between APD_{95} resulting from changing $[Na^+]_o$ with comparisons made either under drug-free conditions (*i.e.*, NNa *vs.* HNa) or in the presence of flecainide (*i.e.*, HNa + F *vs.* NNa + F). Results are mean ± S.D. from five cells (top) and seven cells (bottom).

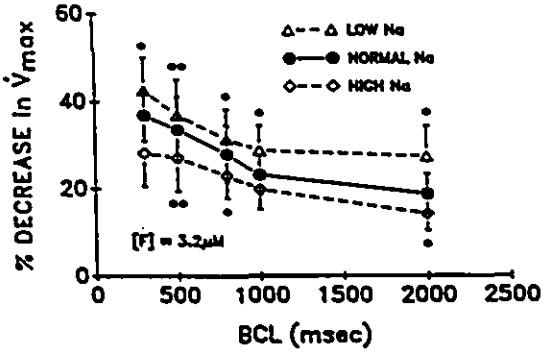

Fig. 2. Cycle length-dependent effects of 3.2 μM flecainide on $\dot{V}_{max}$, as measured at three different extracellular sodium concentrations. Results are mean ± S.D. * $P < .05$, ** $P < .01$ compared to drug effect at NNa concentration (141 mM) at the same cycle length; statistics based on comparisons between values during continuous impalement of the same cell under both conditions ($N = 5$ for LNa experiments, $N = 7$ for HNa).

(fig. 2). HNa concentration decreased and LNa increased flecainide's ability to reduce $\dot{V}_{max}$. The modulating properties of sodium did not depend on cycle length because the change in effect on $\dot{V}_{max}$ produced by altering $[Na^+]_o$ was similar at all cycle lengths studied.

Modulation of the flecainide concentration-response relation by extracellular sodium. Figure 3 shows flecainide concentration-response curves for depression of $\dot{V}_{max}$ at various $[Na^+]_o$. LNa concentrations shifted the flecainide concentration-response curve to the left, indicating an enhancement of the drug's actions. HNa concentrations resulted in a smaller drug effect for any given concentration, shifting the concentration-response curve to the right. The concentration required for a 50% reduction of $\dot{V}_{max}$ (EC_{50}) was 14.6 μM in the presence of 141 mM sodium, decreased to 10.8 μM in the presence of 116 mM sodium and increased to 19.0 μM in the presence of 166 mM sodium. The concentration-response curves do not

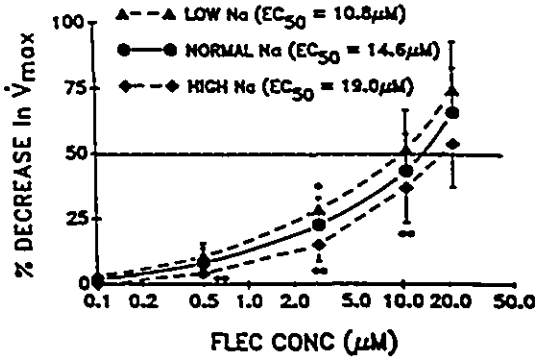

Fig. 3. Concentration dependence of flecainide-induced decreases in $\dot{V}_{max}$. Results are mean ± S.D. Horizontal dashed line = concentration for 50% decrease in $\dot{V}_{max}$ (EC_{50}). * $P < .05$, ** $P < .01$ compared to drug effect at the same concentration in the presence of NNa concentration ($N = 7$ for LNa experiments, $N = 10$ for HNa).

TABLE 3

Effects of $[Na^+]_o$ on the voltage dependence of $\dot{V}_{max}$

$\dot{V}_{max}$ inactivation curves (of the type shown in fig. 4) were fitted by nonlinear least squares regression to the Boltzmann equation $\dot{V}_{max} = \dot{V}_{max,max}/[1 + \exp((V_{50}-MAP)/S)]$, where $\dot{V}_{max,max}$, V_{50} and S are constants, and $\dot{V}_{max}$ is a function of MAP. V_{50} and $\dot{V}_{max,max}$ are the voltage for 50% reduction in $\dot{V}_{max}$ and the maximum value of $\dot{V}_{max}$, respectively. r is the correlation coefficient of nonlinear curve fit. All values are mean ± S.D.

	V_{50}	$\dot{V}_{max,max}$	r
Control ($n = 7$)			
NNa	73.7 ± 2.8	626 ± 114	0.99 ± 0.01
HNa	72.4 ± 2.4	643 ± 100	0.99 ± 0.01
Flecainide 3.2 μM ($n = 9$)			
NNa	77.4 ± 3.5†	471 ± 55‡	0.99 ± 0.01
HNa	75.8 ± 3.1*	535 ± 56*†	0.99 ± 0.01

* $P < .05$, value in the presence of flecainide and HNa *vs.* value in the presence of flecainide and NNa.

† $P < .05$ for value in the presence of flecainide compared to control value at the same sodium concentration.

‡ $P < .01$ for value in the presence of flecainide compared to control value at the same sodium concentration.

include responses close to 100% depression of $\dot{V}_{max}$. High concentrations of flecainide (≥42.8 μM) were able to reduce $\dot{V}_{max}$ to zero at all $[Na^+]_o$; however, the resulting inexcitability made it technically very difficult to maintain a stable impalement while washing out flecainide. We therefore studied a maximum drug concentration of 21.4 μM at all $[Na^+]_o$.

Effects of $[Na^+]_o$ on the $\%\dot{V}_{max}$ inactivation curve. Increases in $[Na^+]_o$ did not significantly alter the $\dot{V}_{max}$ inactivation curve under control conditions (table 3). The maximum value of $\dot{V}_{max}$ was substantially reduced in the presence of flecainide compared to control at normal $[Na^+]_o$, and a slight hyperpolarizing shift in V_{50} occurred. In the presence of increased $[Na^+]_o$, flecainide's effects on both variables were attenuated. Figure 4 shows $\dot{V}_{max}$ inactivation curves obtained during continuous impalement of the same cell in the presence of normal and high $[Na^+]_o$ under control conditions and after flecainide superfusion. Changing $[Na^+]_o$ did not significantly alter the curve under control conditions. Flecainide shifted the inactivation curve downward and to the left in the presence of normal $[Na^+]_o$. Increasing $[Na^+]_o$ in the presence of flecainide shifted the inactivation curve back toward control (*i.e.*, drug-free) values.

Electrophysiologic effects of choline. The addition of choline chloride did not alter activation potential or $\dot{V}_{max}$, but produced a dose-related increase in APD (table 4). Because choline did not affect $\dot{V}_{max}$, the alterations in flecainide action

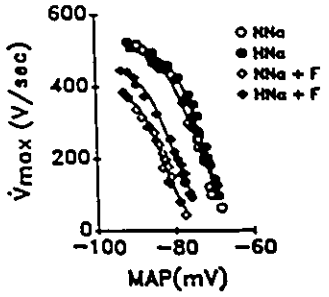

Fig. 4. Voltage dependence of $\dot{V}_{max}$ inactivation at NNa and HNa sodium concentration, in the absence (circles) and presence (diamonds) of flecainide in a representative experiment. The voltage dependence of $\dot{V}_{max}$ was determined by increasing $[K^+]_o$ to 10 mM, and values at each condition were confirmed by returning to 4 mM $[K^+]_o$. All results were obtained during continued impalement of the same cell. Best-fit curves to the Boltzmann equation $\dot{V}_{max} = \dot{V}_{max-r}/[1 + \exp\{(V_{50} - MAP)/S\}]$ are shown.

on $\dot{V}_{max}$ resulting from our modified Tyrode's solutions are not due to direct actions of choline. The effects of choline were not altered by atropine, indicating that choline was not acting by stimulating muscarinic cholinergic receptors. The addition of sucrose mimicked the effects of choline; thus, the changes in APD produced by adding choline chloride to standard Tyrode's solution were attributable to increases in osmolarity (Bailey, 1981). Because choline chloride was isotonically substituted for NaCl in our modified Tyrode's solutions, osmolarity was constant and no electrophysiologic changes would be expected.

Effects of flecainide on I_{Na}. We used $\dot{V}_{max}$ as the primary index for flecainide's action because of the possibility of measuring $\dot{V}_{max}$ in a stable fashion from the same cell for prolonged periods under physiologic conditions of ion concentration, temperature and intracellular constituents. Because of concerns about nonlinearities in the relationship between I_{Na} and $\dot{V}_{max}$, we also determined whether 25 mM increases in $[Na^+]_o$ alter the effects of flecainide on I_{Na} in isolated guinea pig myocytes. Figure 5 shows the current-voltage relation for I_{Na} under control conditions and in the presence of 3.2 μM flecainide during superfusion with solutions containing 25 or 50 mM $[Na^+]_o$ in the same cell. Flecainide reduced I_{Na} in this cell by 21% in the presence of 25 mM sodium and by 15% in the presence of 50 mM extracellular sodium. Overall, 3.2 μM flecainide reduced peak I_{Na} by 15.0 ± 3.5% at 25 mM $[Na^+]_o$, but only by 9.2 ± 3.5% at 50 mM $[Na^+]_o$ (N = 10 cells for 25 mM, 6 cells for 50 mM sodium, $P < .001$).

Extracellular sodium and flecainide's interaction with [^{3}H]BTXB binding. Figure 6 shows mean data for [^{3}H]BTXB displacement by flecainide in all experiments. HNa concentration shifted the displacement curve to the right, indicating that larger flecainide concentrations were necessary to displace [^{3}H]BTXB. The opposite effect was seen in the presence of LNa concentrations, indicating that [^{3}H]BTXB displacement occurred at smaller flecainide concentrations. The IC_{50} for flecainide displacement of [^{3}H]BTXB averaged 4.4 ± 2.6 μM in the presence of LNa, 6.0 ± 3.7 μM at NNa and 9.5 ± 4.6 μM with HNa concentration. The Hill coefficient averaged 0.51 ± 0.10, 1.00 ± 0.28 and 0.88 ± 0.18 in the presence of LNa, NNa and HNa concentrations, respectively, consistent with a single binding site for flecainide. There was a strong linear correlation (r = 0.99) between the IC_{50} for flecainide displacement of [^{3}H]BTXB at various $[Na^+]_o$ and the EC_{50} for reduction of $\dot{V}_{max}$ in electrophysiologic experiments (fig. 7).

Discussion

We have shown that changes in $[Na^+]_o$ over the clinically relevant range of 116 to 166 mM are capable of altering the effects of flecainide on $\dot{V}_{max}$ in canine cardiac Purkinje fibers, and that increasing $[Na^+]_o$ opposes the drug's action on I_{Na} in guinea pig myocytes. This interaction appears to be due to an effect of $[Na^+]_o$ on the binding of flecainide to its receptor in the cardiac sodium channel, resulting in a reversal of flecainide's action on net phase 0 inward current.

Underlying mechanisms. Cahalan and Almers have shown that increases in $[Na^+]_o$ antagonize Na^+ channel blockade by the quaternary lidocaine derivative QX-314 in squid giant axons (Cahalan and Almers, 1979). They postulated electrostatic repulsion by sodium ions at a cationic binding site of ionized drug molecules bound to an adjacent site in the channel. A similar explanation was put forward by Hille and Schwarz (1978) to explain previous observations by Armstrong (1971) of the ability of increased $[K^+]_o$ to relieve K^+ channel block by tetraethylammonium. An allosteric effect of external sodium on an internal QX-314 receptor is an alternative possibility which Cahalan and Almers felt less likely. Kohlhardt (1982) postulated a role of the magnitude of I_{Na} as a determinant of Na^+ channel blockade.

The displacement of [^{3}H]BTXB is well established as an indicator of local anesthetic drug binding to Na^+ channel receptors in neural tissue (Postma and Catterall, 1983), and more

TABLE 4
Effects of choline chloride and sucrose on Purkinje fiber action potentials

	MAP	APD_{50}	Change	APD_{90}	Change	$\dot{V}_{max}$
	mV	msec	%	msec	%	V/sec
Control (n = 6)	88.6 ± 1.7	284 ± 38		372 ± 48		547 ± 67
Choline (25 mM)	87.3 ± 2.6	336 ± 53**	18.3 ± 6.9	413 ± 55**	11.4 ± 4.2	547 ± 56
Atropine (10^{-7} M)	87.8 ± 2.4	347 ± 54**	22.2 ± 8.4	429 ± 57**	15.6 ± 6.8	544 ± 52
Control (n = 4)	87.8 ± 2.6	278 ± 17		361 ± 29		471 ± 18
Sucrose (25 mM)	88.8 ± 2.9	319 ± 27	14.5 ± 5.8	401 ± 40*	11.1 ± 4.3	463 ± 16
Control (n = 6)	88.7 ± 1.7	318 ± 47		405 ± 55		536 ± 45
Choline (50 mM)	87.9 ± 1.9	395 ± 65**	24.0 ± 4.2	486 ± 77**	19.8 ± 9.0	537 ± 52
Atropine (10^{-7} M)	88.2 ± 2.6	404 ± 69**	26.7 ± 5.8	487 ± 72**	20.1 ± 4.6	532 ± 60
Control (n = 4)	88.1 ± 2.9	279 ± 17		360 ± 29		471 ± 18
Sucrose (50 mM)	88.1 ± 1.9	343 ± 41	23.0 ± 9.8	429 ± 56*	18.9 ± 8.1	472 ± 32

* $P < .05$ compared to corresponding control value.
** $P < .01$ compared to corresponding control value.

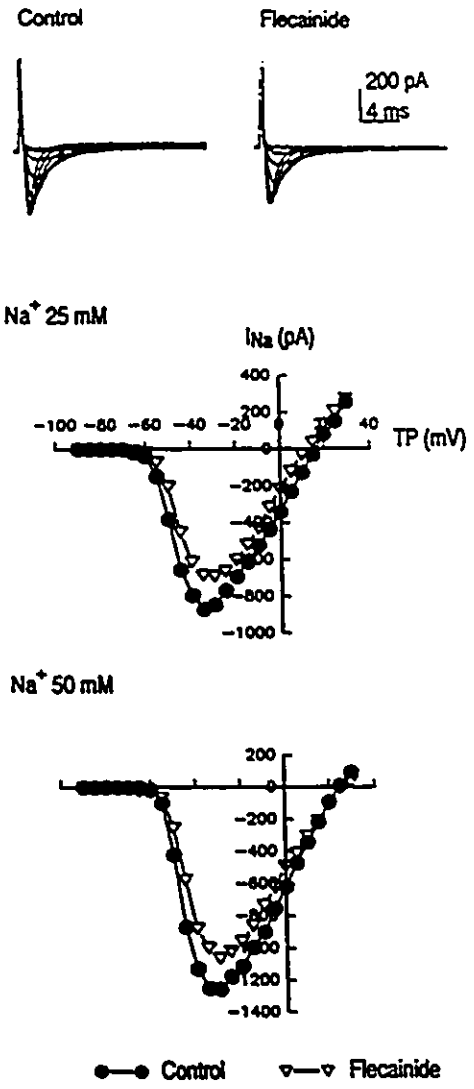

Fig. 5. Top: Representative recordings of I_{Na} from a single cell superfused with 25 mM $[Na^+]_o$ under control conditions (left) and in the presence of flecainide (right). Current tracings are shown for 20-msec depolarizations from −100 mV to −90, −60, −55, −50, −45, −40, −35 and −30 mV. Bottom: Representative current-voltage relations recorded in the absence (●) and presence (Δ) of flecainide with 25 and 50 mM $[Na^+]_o$. Peak I_{Na} was reduced by 21% by flecainide in the presence of 25 mM $[Na^+]_o$ and by 15% in the presence of 50 mM $[Na^+]_o$. All results shown in the figure were recorded from the same cell.

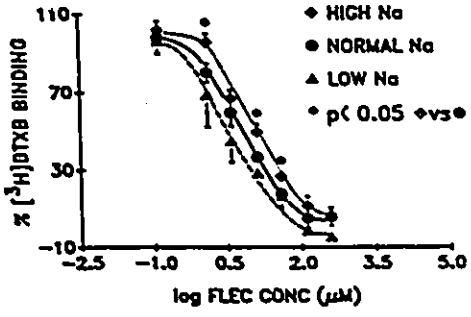

Fig. 6. Competition of specific [^{3}H]BTXB binding by flecainide. Values are normalized to specific [^{3}H]BTXB binding in the absence of drug (100%). Results are mean ± S.D. from all experiments (N = 9 for HNa, 7 for NNa, 3 for LNa).

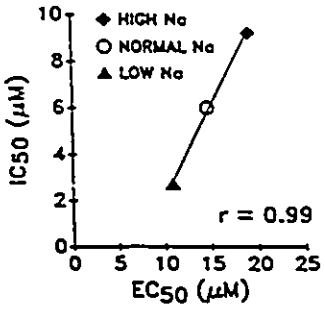

Fig. 7. Relationship between IC_{50} for flecainide displacement of [^{3}H] BTXB binding and EC_{50} for $\dot{V}_{max}$ reduction at each of three sodium concentrations studied. Values were obtained from fits of mean data shown in figures 5 and 9.

recently, has been shown to provide similar information in cardiac tissue (Sheldon *et al.*, 1987, 1988). Our binding data showing that $[Na^+]_o$ modulates flecainide displacement of [^{3}H] BTXB provides the first biochemical evidence for the suggestion of Cahalan and Almers that $[Na^+]_o$ can modulate the binding of a sodium channel blocker to its receptor site. In addition, because binding experiments were performed in the presence of substantial quantities of TTX to block I_{Na}, our results indicate that a change in I_{Na} *per se* is not necessary for the modulating action of $[Na^+]_o$. A similar conclusion was reached in recent electrophysiologic studies of batrachotoxin-activated Na^+ channels in planar lipid bilayers, which showed that the calculated K_D for cocaine binding is modulated by $[Na^+]_o$ (Wang, 1988). Because Na^+ appeared to block the association and accelerate the dissociation of cocaine from the channel, a straightforward competitive interaction was felt to be less likely than an electrostatic "knock-off" phenomenon (Wang, 1988). Whether extracellular sodium ions are altering drug binding by interacting electrostatically with a drug binding site just inside the selectivity filter or whether an external local anesthetic receptor site (Alpert *et al.*, 1989; Carmeliet *et al.*, 1989) is involved remains to be determined.

Barber *et al.* (1990) have presented preliminary data suggesting that $[Na^+]_o$ does not alter the kinetics or magnitude of lidocaine block of rabbit atrial Na^+ channels. It is possible that $[Na^+]_o$ modulation of the action of Na^+ channel blockers is variable among drugs and depends on factors such as lipophilicity, kinetics of drug action, charge/size ratio, drug binding site, etc.

Relevance to the effect of sodium salts in clinical toxicity with class 1c drugs. A variety of investigators have reported beneficial actions of molar sodium lactate (Chouty *et al.*, 1987; Camous *et al.*, 1987) or sodium bicarbonate (Pentel *et al.*, 1986; Winkelmann and Leinberger, 1987) in patients intoxicated with class 1c antiarrhythmic drugs. Changes in serum sodium concentration resulting from sodium lactate in one patient consisted of a 19 mM increase (Camous *et al.*, 1987). Bajaj *et al.* found that 10 mEq/kg sodium chloride increased the serum sodium concentration by 27 mM and reduced QRS prolongation by *O*-desmethylencainide by 48% (Bajaj *et al.*, 1989).

We studied 25 mM changes in $[Na^+]_o$ above and below normal values, similar to the range reported in prior clinical and experimental studies. Assuming a squared relationship between conduction velocity and $\dot{V}_{max}$ (Hunter *et al.*, 1975; Walton and Fozzard, 1983; Buchanan *et al.*, 1985; Nattel, 1987; Nattel and Jing, 1989), one can estimate the degree of conduction slowing

for a given change in $\dot{V}_{max}$. The changes in $\dot{V}_{max}$ that we observed in the presence of flecainide after a 25 mM increase in $[Na^+]_o$ should decrease drug-induced conduction slowing by about 33%. The latter result is in the same range as that reported by Bajaj *et al.* (1989), but is nonetheless somewhat smaller. The discrepancy may be due to contributions from hyperosmolarity, alkalinization, hemodynamic improvement, differences in drugs studied or simply to the difficulty of making quantitative comparisons between such widely differing models. Although our work does not prove that modulation of flecainide binding to the sodium channel by $[Na^+]_o$ is responsible for all of the beneficial actions of sodium salts on flecainide toxicity, it suggests that this mechanism contributes significantly. The extent to which the same mechanism applies to the interactions between $[Na^+]_o$ and other local anesthetic drugs remains to be determined.

Limitations. One limitation is our use of $\dot{V}_{max}$ as a primary index of flecainide action. The relationship between $\dot{V}_{max}$ and I_{Na} is nonlinear (Cohen *et al.*, 1984; Sheets *et al.*, 1988). The degree of nonlinearity decreases with increasing temperature over the range at which I_{Na} can be measured accurately (7–27°C) (Sheets *et al.*, 1988), suggesting that at 37°C the nonlinearity is likely small. The use of standard microelectrode techniques avoids the problems of nonphysiologic ionic conditions, hypothermia and cell rundown that are common with current voltage-clamp methods. Sodium channel blocking drug action can be qualitatively altered by lowering temperature (Johns *et al.*, 1989). Prolonged, stable microelectrode impalements allow for the precise measurement of $\dot{V}_{max}$ in the presence of varying drug and $[Na^+]_o$ in the same cell. Finally, we have confirmed, using voltage-clamp techniques, that changes in $[Na^+]_o$ influence the sodium current blocking effects of flecainide in a fashion qualitatively similar to the interaction observed for effects on $\dot{V}_{max}$.

For free-running action potentials, factors such as resting potential and APD, which can influence sodium channel blockade, are not controlled. However, the improvement in $\dot{V}_{max}$ caused by increasing $[Na^+]_o$ in the presence of flecainide occurs when the role of activation potential is controlled (fig. 4). Lowering $[Na^+]_o$ did not alter APD, and although increasing $[Na^+]_o$ increased APD slightly, this should have augmented the action of flecainide, in contrast to the antagonism of flecainide effect actually observed. Finally, under voltage-clamp conditions, which controlled holding and test potential and depolarizing pulse duration, increased $[Na^+]_o$ reduced the I_{Na} blocking effect of flecainide.

We studied flecainide's interaction with its receptor in an indirect fashion, by evaluating flecainide displacement of [^{3}H] BTXB. It would be preferable to evaluate directly radiolabeled flecainide binding to the sodium channel, but this is currently impossible. On the other hand, [^{3}H]BTXB displacement is a well-established approach to study drug-receptor interactions of local anesthetic drugs in both neural (Postma and Catterall, 1983) and cardiac (Sheldon *et al.*, 1986, 1987, 1988; Hill *et al.*, 1988) tissues.

We did not evaluate the changes in flecainide's sodium channel blocking kinetics that might result from changes in $[Na^+]_o$. Although kinetic analysis can be obtained with the techniques applied in the present study (Packer *et al.*, 1989), the pacing protocols necessary would have increased the likelihood of loss of impalement and made it difficult to complete experimental protocols in the same cell. Theoretical analysis suggests that the steady-state rate dependence of drug effects can be used to estimate the kinetics of drug action (Starmer, 1986). If the reciprocal of drug-induced changes in $\dot{V}_{max}$ are plotted as a function of cycle length, a linear relation with a slope proportional to the rate constant of drug action should be obtained (Starmer, 1986), an expectation we have evaluated previously (Wang *et al.*, 1990). When the effects of flecainide in the present study are plotted in this fashion, a linear relation similarly results ($r = 0.98 \pm 0.02$). The rate constants average 1.7 ± 0.6 at LNa and 2.1 ± 1.0 at NNa in the same experiments (P = N.S.); and 2.4 ± 0.8 at HNa *vs.* 3.1 ± 0.9 at NNa (P = N.S.) in a different set of experiments. These results suggest that $[Na^+]_o$ does not influence flecainide's action on the Na^+ channel by altering its kinetics.

Potential significance. Our findings give insights into the mechanisms by which sodium salts may reverse the toxic effects of potent sodium channel blocking drugs. Such therapy is relevant to the treatment of drug intoxication and possibly of serious proarrhythmic reactions. Although we studied the interactions between flecainide and extracellular sodium ions, our results may relate to the mechanism by which sodium salts reverse the cardiotoxic actions of a variety of local anesthetics including other class 1 antiarrhythmic drugs, tricyclic antidepressants and cocaine. In a broader sense, similar mechanisms may underlie the interactions between blockers of a variety of cation channels and permeant cations.

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Publication 5

In Publications 1 and 2 our approach to study the mechanism by which class IC antiarrhythmic drugs may cause ventricular arrhythmias was clinical. In Publication 5, in addition to our clinical approach we used the microelectrode technique to determine the possible cellular mechanism of a proarrhythmic action observed with a combination of erythromycin and quinidine. The long QT syndrome represents a specific form of arrhythmia associated with drugs which delay repolarization (Roden et al, 1984; Jackman et al, 1984). Quinidine is the antiarrhythmic drug most commonly associated with proarrhythmic actions manifest as polymorphic ventricular tachycardia and torsades de pointes (Roden et al, 1986). This syndrome has also been reported as a complication during treatment with other antiarrhythmic drugs (Jackman et al, 1988) as well as with antidepressant drugs (Hermann et al, 1983). Based on a clinical case report of a patient experiencing ventricular tachyarrhythmia (torsades de pointes) in association with the acquired long QT syndrome, we investigated the cellular electrophysiologic mechanism by which erythromycin may cause a long QT syndrome.

Erythromycin-Induced Long QT Syndrome: Concordance with Quinidine and Underlying Cellular Electrophysiologic Mechanism

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Life-threatening ventricular tachyarrhythmias can be caused by a variety of therapeutic agents [1]. The drug-induced long QT syndrome is a specific form of tachyarrhythmia that may complicate therapy with a range of compounds [2,3]. This syndrome consists of a marked QT-interval prolongation on the electrocardiogram, often accompanied by an exaggerated U wave, associated with polymorphic ventricular tachycardias of a characteristic undulating morphology called "torsades de pointes" [4]. These tachyarrhythmias are usually self-terminating [1] but frequently result in syncope, and they can degenerate to ventricular fibrillation [2,3].

The drugs that most commonly cause an acquired long QT syndrome are antiarrhythmic agents that delay repolarization [2,3]. Delayed repolarization is believed to lead, in predisposed persons, to abnormal early afterdepolarizations, triggered activity, and ventricular tachyarrhythmias [5,6]. Over the last several years, several cases of the acquired long QT syndrome caused by the macrolide antibiotic, erythromycin, have been reported [7–10]. We recently managed a patient who developed torsades de pointes arrhythmias as an apparent complication of quinidine therapy, and then experienced the same adverse effect after erythromycin treatment. This is, to our knowledge, the first reported case of cross-sensitivity for the long QT syndrome to both class IA agents and erythromycin. In order to explore the potential mechanism by which erythromycin causes a long QT syndrome, we conducted microelectrode experiments to study the cellular effects of the compound, as we could find no reports of the cellular electrophysiologic actions of erythromycin in the literature.

MATERIAL AND METHODS

Mongrel dogs were anesthetized with pentobarbital, 30 mg/kg intravenously, and their hearts were removed through a right thoracotomy. Free-running Purkinje fiber false tendons were dissected along with subjacent ventricular muscle and pinned to the Sylgard-covered floor of a Lucite tissue chamber. The tissue was superfused with a modified Tyrode's solution containing (in mM): sodium 141; bicarbonate 22; dextrose 10; potassium 4; biphosphate (monobasic) 0.9; magnesium 0.5; calcium 1; and chloride 125. The superfusate was aerated with a mixture of 95% oxygen/5% carbon dioxide and kept at 37°C by a feedback-controlled heater (Hanna Instruments Co., Philadelphia, Pennsylvania). Standard microelectrode and computer analysis techniques were used, as previously described [11], to measure action potential characteristics at a pacing cycle length of 800 msec. Control observations were made after at least 60 minutes of equilibration time in the bath, and measurements were repeated at 30, 60, and (when possible) 120 minutes of erythromycin superfusion. Since erythromycin's effects approached steady state by 60 minutes, and continuous impalement could not be maintained for 120 minutes in all experiments, results are presented in this manuscript as measured after 60 minutes of drug superfusion. Continuous impalement of the same cell was required throughout both control and drug superfusion periods in each experiment.

Results of the *in vitro* experiments are presented as the mean ± SD. Student's paired t-test was used to compare control values with those after superfusion with erythromycin [12]. Erythromycin (Sigma Chemicals Co., St. Louis, Missouri) was superfused as the pure base dissolved in Tyrode's solution, because of the possible direct effects of the preservative (benzyl alcohol) used in the commercial parenteral erythromycin preparation.

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CASE REPORT

The patient was a 76-year-old man admitted on July 16, 1987, to the Montreal Heart Institute for the investigation of syncope. His past medical history included an inferior myocardial infarction complicated by a left ventricular aneurysm, mitral insufficiency, and ventricular tachyarrhythmias. He had moderately severe left ventricular dysfunction, with a radionuclide ejection fraction of 29%. His treatment prior to admission included slow potassium tablets, three times a day; quinidine sulphate, 200 mg four times a day; and digoxin, 0.125 mg, furosemide 40 mg, and amiodarone 200 mg, each once a day.

The admission was precipitated by three episodes of loss of consciousness resulting in mild head injury. Physical examination on admission showed an alert male with a blood pressure of 100/46 mm Hg and a pulse rate of 58/minute. He had bilateral carotid bruits and scattered rhonchi over both lungs. The jugular venous pressure was normal, and he had an S_3 gallop and a grade 3/6 apical pansystolic murmur radiating to the axilla. Apart from a systolic bruit over the

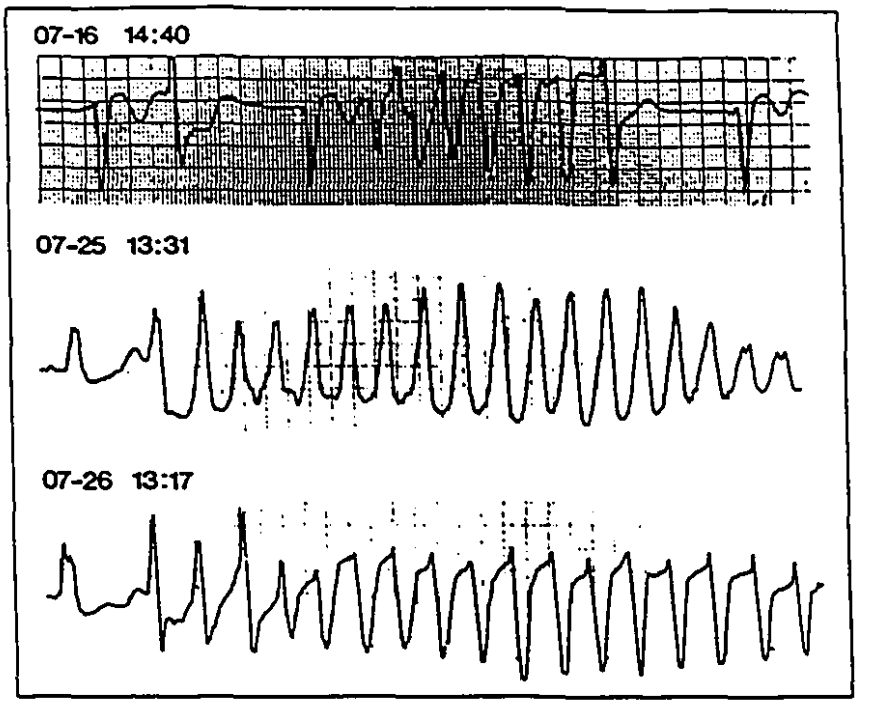

Figure 1. Rhythm strips showing ventricular tachyarrhythmias. Top, strip recorded on the day of admission while the patient was receiving quinidine. Numerous episodes of ventricular tachycardia were recorded, tending to begin after a pause and terminating spontaneously. Longer episodes all had the characteristic torsades de pointes morphology. Middle, torsades de pointes recorded after a syncopal episode during erythromycin therapy. Bottom, torsades de pointes occurring immediately after rechallenge with intravenous erythromycin.

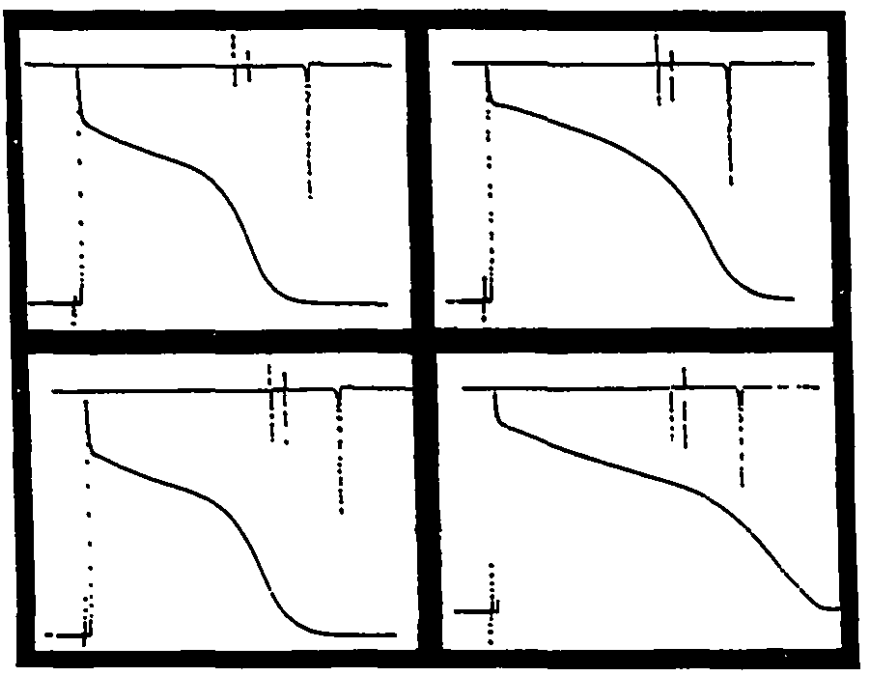

Figure 2. Response of canine Purkinje fiber action potentials to superfusion with erythromycin. Top left, control; top right, after 60 minutes of erythromycin, 50 mg/L; bottom left, return to control after washout of erythromycin; bottom right, after 60 minutes of subsequent superfusion with erythromycin, 100 mg/L (scale: one division = 50 msec [horizontal] or 20 mV [vertical] for action potential; 5 msec [horizontal] or 200 V/sec [vertical] for differentiated signal).

aorta and both femoral arteries, the abdominal examination was normal. The rest of the physical examination was unremarkable.

The admission electrocardiogram showed a sinus rhythm at 58/minute, with a left bundle branch block, prominent U waves, and a QT interval of 0.60 (QT_c = 0.59) second. Shortly after the initial electrocardiogram was obtained, episodes of polymorphic ventricular tachycardia of "torsades de pointes" morphology (Figure 1, top) were noted. These episodes were always self-terminating and tended to occur after post-extrasystolic pauses. Serum electrolyte concentrations on admission included a potassium of 3.8 mEq/L, sodium of 142 mEq/L, chloride of 108 mEq/L, carbon dioxide combining power of 26 mEq/L, calcium of 8.6 mg/dL, and magnesium of 1.7 mg/dL. After correction for the serum albumin (3.6 g/dL), all electrolyte values were within normal limits. Other pertinent laboratory data included a serum creatinine of 2.1 mg/dL, a glucose of 135 mg/dL, a blood area nitrogen of 32 mg/dL, and normal values for glutamate pyruvate and oxalate transaminases, lactic dehydrogenase, and creatine kinase. Serum concentrations of digoxin (1.3 mg/mL) and quinidine (3.2 mg/L) were within the therapeutic range. Plasma amiodarone and desethylamiodarone concentrations were 0.5 and 0.6 mg/L, respectively.

TABLE I

Effects of Erythromycin on Action Potential Characteristics

	MAP	APA	APD_{50}	APD_{95}	$\dot{V}_{max}$
Control	−86 ± 3	126 ± 3	227 ± 26	326 ± 31	609 ± 97
Erythromycin 10 mg/L	−86 ± 2	126 ± 3	233 ± 20	333 ± 31	603 ± 89
Control	−89 ± 4	126 ± 6	215 ± 32	301 ± 34	596 ± 72
Erythromycin 50 mg/L	−88 ± 4	125 ± 5	232 ± 35*	339 ± 44*	542 ± 85†
Control	−88 ± 5	124 ± 7	226 ± 26	329 ± 45	624 ± 47
Erythromycin 100 mg/L	−86 ± 6*	121 ± 8†	281 ± 32†	428 ± 49†	532 ± 54†

MAP = membrane activation potential (in mV); APA = action potential amplitude (in mV); APD_{50}, APD_{95} = action potential duration to 50% and 95% repolarization, respectively (in msec); V_{max} = maximum rate of voltage rise during phase 0 (in V/sec).
* $p < 0.05$, † $p < 0.01$ compared with corresponding control value, by paired t-test.
Results are shown as mean ± SD, for 8, 11, and 6 experiments, respectively, with 10, 50, and 100 mg/L erythromycin. All results were obtained at a basic cycle length of 800 msec.

Quinidine administration was discontinued and the patient's ventricular tachyarrhythmias stopped. Two days later, the patient became confused, tachypneic, and febrile to 39.5°C. The QT interval had decreased to 0.48 (QT_c = 0.52) second. A chest radiogram showed interstitial pulmonary edema with possible superinfection and cardiomegaly. The pulmonary edema resolved in response to intravenous furosemide, but pulmonary infiltrates compatible with either widespread bronchopneumonia or amiodarone-induced pulmonary toxicity remained.

Empiric treatment of a possible pneumonia was begun on July 20, using piperacillin 2 g intravenously every 4 hours and tobramycin, 70 mg intravenously twice daily. Erythromycin lactobionate, 1 g intravenously every 6 hours, was added on July 22, and tobramycin was discontinued on July 23. Within 24 hours of the onset of erythromycin therapy, the QT_c on the monitor lead increased to 0.60 second (QT = 0.55 second). The corrected QT interval remained in this range throughout the period of erythromycin therapy. On July 25, the patient had a syncopal episode in the early afternoon and a torsades de pointes form of ventricular tachycardia was recorded (Figure 1, middle). This was the first episode of such a rhythm since the discontinuation of quinidine. A temporary transvenous pacemaker was inserted, and during ventricular demand pacing at 70/minute no further episodes of ventricular tachycardia were observed. Because of the temporal relationship between intravenous erythromycin administration (ending at 1:00 PM) and the syncopal episode (at 1:30 PM), a possible causal relationship was considered. Erythromycin administration was withheld, and the pacemaker was turned off in the late afternoon. No further episodes of torsades occurred, until the patient was re-challenged with erythromycin at noon the next day. Approximately 15 minutes after the end of the erythromycin infusion, torsades de pointes arrhythmias began to recur (Figure 1, bottom). These responded to the reactivation of the ventricular demand pacemaker. No further erythromycin was given, and no more ventricular tachyarrhythmias were noted for the rest of the hospitalization despite the removal of the pacemaker the next day. The patient remained tachypneic and intermittently febrile, and died on July 31, 1987. Autopsy showed triple vessel coronary artery disease, with inferior and lateral wall myocardial infarction and a left ventricular aneurysm. Pulmonary edema and a right-sided bronchopneumonia were also noted.

RESULTS

Erythromycin caused dose-related, reversible changes in the Purkinje fiber action potential. Figure 2 shows the drug's effects on action potentials in one preparation. The control action potential is at the upper left. After 60 minutes of erythromycin (50 mg/L), $\dot{V}_{max}$ (maximum rate of voltage rise during phase 0) was slightly reduced and the action potential duration increased (upper right). These effects were completely reversed after 1 hour of drug washout (lower left). A further 60 minutes of erythromycin at a higher dose (100 mg/L) produced larger changes in both $\dot{V}_{max}$ and action potential duration, as shown on the lower right. Mean data for all experiments are shown in Table I. While 10 mg/L of erythromycin had no significant effects, at higher concentrations erythromycin produced dose-related increases in action potential duration and reductions in $\dot{V}_{max}$. At the highest concentration (100 mg/L), erythromycin also slightly depolarized the tissues and reduced action potential amplitude. In one experiment at the highest concentration, erythromycin produced sporadic early afterdepolarizations, none of which we were able to photograph.

COMMENTS

Our patient experienced torsades de pointes arrhythmias reproducibly upon provocation with erythromycin. This is the fifth reported case of erythromycin-induced long QT syndrome in the literature. A unique aspect of our case is the apparent concordance between quinidine and erythromycin in the initiation of torsades de pointes arrhythmias. Concordance among class IA agents and amiodarone in causing torsades de pointes has been described [13]. Our observation suggests that erythromycin shares this concordance and should be avoided in patients with a history of the drug-induced long QT syndrome.

The precise precipitating factors of the initial presenting tachyarrhythmias are not clear. An important role for quinidine is suggested by the absence of ventricular tachyarrhythmias for 1 week between the discontinuation of quinidine and the erythromycin-induced arrhythmia. Amiodarone, continued throughout the hospitalization, may have played an important contributory role in the occurrence of torsades de pointes arrhythmias during both quinidine and erythromycin therapy. Although amiodarone is generally thought to be an unusual cause of torsades

de pointes, Jackman *et al* [14] have observed this arrhythmia among amiodarone-treated patients with an incidence (4%) similar to the occurrence rate of the quinidine-induced long QT syndrome [2]. That amiodarone therapy alone did not cause torsades de pointes in our patient is indicated by the absence of such arrhythmias when he was not receiving quinidine and erythromycin.

Our *in vitro* experiments show that erythromycin increases action potential duration and reduces V_{max} in a concentration-related fashion. Its electrophysiologic effects, therefore, place it in the class IA category of drug action [15]. This may explain its ability to produce a long QT syndrome, and its concordance with the class IA drug, quinidine, in our patient. The cellular effects of erythromycin differ from those of quinidine in that quinidine abbreviates the early phases of the Purkinje fiber action potential, reducing APD_{50} (potential duration to 50% repolarization) and producing a "triangularization" of the action potential [5,16]. In contrast, erythromycin prolonged all phases of the action potential to a similar extent. The ability of erythromycin to delay repolarization could explain its propensity to produce an acquired long QT syndrome, just as in the case of other drugs implicated in this phenomenon [14]. Early afterdepolarizations are thought to be an important initiating mechanism of torsades de pointes arrhythmias [2,3,5,6,14], and are caused by agents that delay repolarization. Although we rarely observed early afterdepolarizations during erythromycin superfusion *in vitro*, the experimental induction of such afterdepolarizations often requires altered extracellular solutions, with reduced bicarbonate [5,17], potassium [5,17], or magnesium concentrations [17], or other precipitating factors. This is not necessarily surprising, since drugs that cause the acquired long QT syndrome in humans do so infrequently and often in association with other factors delaying repolarization.

The concentration dependence of erythromycin's cellular actions was such that it had no effect at 10 mg/L, produced moderate changes at 50 mg/L, and caused substantial alterations in the Purkinje fiber action potential at 100 mg/L. Peak serum erythromycin concentrations average about 30 mg/L after 900 mg of intravenous erythromycin [18]. Thus, our results suggest that significant changes in repolarization could result from a 1-g intravenous dose of the compound. On the other hand, a 500-mg oral dose of erythromycin produces concentrations in the range of 2 to 4 mg/L [19], and a marked increase in sensitivity would have to be involved to explain a long QT syndrome resulting from oral erythromycin. Of the five cases of erythromycin-induced torsades de pointes arrhythmias reported, four patients were receiving intravenous erythromycin and only one [10] was receiving oral erythromycin therapy.

In conclusion, our results indicate that erythromycin can produce a long QT syndrome as a result of its direct electrophysiologic actions, and that cross-sensitivity may exist with class IA antiarrhythmic agents. It is probable that intravenous erythromycin is much more likely than oral erythromycin to produce this syndrome, because of the higher concentrations achieved. Erythromycin should be avoided in patients with a history of the long QT syndrome. It may also be wise to monitor the QT interval periodically when using intravenous erythromycin in patients with predisposing factors for a long QT syndrome, such as treatment with type IA antiarrhythmic agents or amiodarone, hypokalemia, and hypomagnesia.

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CHAPTER III

GENERAL DISCUSSION

1. SUMMARY OF NOVEL FINDINGS AND THEIR IMPORTANCE

Class I antiarrhythmic drugs produce use-dependent sodium channel blockade. Experimentally, this property manifests *in vitro* by a frequency-dependent reduction of V_{max} (phase 0 sodium current) in the presence of class I drugs (Campbell and Vaughan Williams, 1983; Courtney, 1980; Nattel, 1987a and 1987b). Quantitative studies have shown kinetics of action of sodium blockers on conduction *in vivo* in animal models (Nattel, 1985; Davies et al, 1987; Anderson et al, 1990) paralleling their kinetics on phase 0 of cardiac action potential and conduction *in vitro* (Nattel, 1985; Nattel, 1987a and 1987b). The characteristic kinetics of rate-dependent block of various class I antiarrhythmic agents has been used for classification (Campbell, 1983). Clinically, the ECG represents a non-invasive and readily available instrument, and the QRS duration has been used as an index of intraventricular conduction (Gang et al, 1985). Antiarrhythmic drug-induced conduction slowing has been assessed at rest by the degree of QRS prolongation and represents a clinical indicator of pharmacologic action (Cascio et al, 1988). Although Cascio et al (1988) have shown that exercise prolonged QRS duration, their study did not provide insight into underlying mechanisms.

The work described in this thesis was the first to show that the sinus tachycardia of exercise produces an amplification of class IC drug rate-dependent effects on ventricular conduction slowing. We evaluated the onset rate kinetics of the changes in QRS duration produced by flecainide and found that they were similar to flecainide's rate constant for use-dependent changes in V_{max} *in vitro*. The QRS changes either during exercise or electrophysiologic studies, using programmed stimulation, were similar for comparable heart rate changes. We therefore demonstrated that the underlying mechanism of flecainide-induced slowing of conduction during exercise is rate-dependent sodium channel blockade. Subsequently, we confirmed this hypothesized mechanism by demonstrating that a variety of class I drugs with sodium channel blocking properties produced use-dependent QRS prolongation in man with characteristic kinetics for each agent which are similar to the kinetics of V_{max} depression *in vitro*. This study represents the first quantitative analysis of the kinetics of use-dependent conduction slowing by antiarrhythmic drugs in humans. We demonstrated that this use-dependent blocking action is responsible for conduction slowing by flecainide, propafenone, quinidine, and amiodarone. Our work provided a basis for subsequent studies, for example the clinical

investigation of frequency-dependent actions of class I agents in human atrial tissue reported by Sakai et al (1995). Kidwell et al studied the rate-related changes of myocardial conduction associated with an abrupt onset of tachycardia. During tachycardia, the ventricular rhythm and heart rate may become very fast and lead to an amplification of use-dependent conduction slowing with a similar underlying mechanism to the one we observed during the sinus tachycardia of exercise. As expected on the basis of our work, Kidwell et al showed that a use-dependent prolongation of ventricular tachycardia cycle length was associated with various class I antiarrhythmic drugs in humans (Kidwell et al, 1993). QRS prolongation during exercise was recently used to assess the degree of rate-dependent conduction slowing produced by a class IC drug (pilsicainide) during ischemic episodes, using a design based on the one we developed (Sadanaga and Ogawa, 1994).

It has been recognized that sodium channel blockers which produce significant conduction slowing may predispose to the development of ventricular arrhythmias or are often associated with proarrhythmia (Rikenberger et al, 1982; Podrig et al, 1993; Levine et al, 1989). Reentry has been suggested to be a mechanism of the proarrhythmic effects associated with class IC drugs based on their strong effect to slow conduction and minor effect on refractoriness (Coromilas, 1988; Brugada et al, 1991b). However, these studies did not involve detailed mapping of proarrhythmic events which would permit the elucidation of the underlying mechanism. As mentioned in the Introduction, important factors predisposing to proarrhythmia include structural heart disease, large antiarrhythmic drug concentrations, ischemia and myocardial infarction, increased heart rate and altered autonomic activity. Of these factors, structural heart disease, ischemia and myocardial infarction may provide a substrate that can support reentry and increase proarrhythmic risk. Publication 3 represents the first systematic comparison of the occurrence and mechanisms of flecainide proarrhythmia between normal dogs and dogs with myocardial infarction. We demonstrated the role of previous myocardial infarction (MI) in flecainide-induced proarrhythmia. We were the first to produce a detailed analysis of rate- and concentration-dependent effects caused by flecainide in both normal and MI dogs. We found that although proarrhythmia was associated with significant rate-dependent conduction slowing, it did not appear to be sufficient to cause proarrhythmia in a large percent of hearts. Our detailed analysis of conduction velocity in local regions represents an original approach and demonstrated that an abrupt conduction block appeared at rapid rates and was related to the rate-dependence of drug-induced proarrhythmia.

Activation maps during ventricular arrhythmias revealed that anisotropic reentry occurred in the infarct zone around an arc of rate-dependent conduction block in the transverse direction, which played a central role in the occurrence of proarrhythmia. In similar experiments, the direction in which the block of conduction occurs preferentially has been variable; some studies have reported a consistant block in the longitudinal direction (Turgeon et al, 1992), transverse direction (Dillon et al, 1988; Cardinal et al, 1988; Zuanetti et al, 1990; Restivo et al, 1995) and both directions (Brugada et al, 1991; El-Sherif et al, 1985; Restivo et al, 1990; Coromilas et al, 1995). In normal myocardium the requirements for active propagation are lower in the transverse direction (Spach and Kootsey, 1983); in other words, safety factor for impulse propagation is greater in the transverse direction. When active membrane properties are impaired, or when sodium current current is depressed, conduction block is predicted to occur preferentially in the transverse direction (Spach et al, 1988; Delmar et al, 1987; Saffitz et al, 1993; Delgado et al, 1990). The anisotropic properties of myocardial coupling are thought to be an important determinant of the reentrant arrhythmias in patients with previous myocardial infarction (Saffitz et al, 1992; Spear et al, 1992; Saffitz et al, 1993). The evidence presented in our publication suggests that the anisotropic properties of myocardial coupling are an important determinant of flecainide-induced proarrhythmia in MI dogs and that when sodium current is depressed, flecainide caused conduction to fail in the the direction of weaker cell-to-cell coupling.

A strength of our approach is that we analyzed and compared conduction velocity within the same area of the heart in normal and MI dogs, during control and drug conditions. We favored this approach over using a (presumed) normal area within a heart with a zone of infarction (Restivo et al, 1995), since different regions within the heart may have a different population of cells with various ionic components resulting in variations in electrophysiological properties. We studied the dose-response relation for flecainide-induced proarrhythmia and evaluated the changes in electrophysiological properties associated with flecainide in the presence of steady-state drug concentrations. We found that drug-induced conduction slowing was the same in the longitudinal and transverse direction, a finding similar to those reported in other closely-related studies (Cardinal et al, 1988; Brugada et al, 1991; Coromilas et al, 1995; Restivo et al, 1995). Other experimental studies have found that sodium channel blocker effects were larger on longitudinal than transverse conduction velocity (Kadish et al, 1986; Bajaj et al, 1987; Anderson et al, 1989 and 1990; Turgeon et al, 1992). The difference between these

results may be due to the drug concentrations studied or to a difference in drug action between flecainide and procainamide (Kadish et al, 1986), mexiletine (Bajaj et al, 1987), quinidine (Bajaj et al, 1987), amiodarone (Anderson et al, 1989), lidocaine (Anderson et al, 1990) and encainide (Turgeon et al, 1992). The variability between drug-induced effects may also be due to differences between the mode or site of induction, and whether the experimental model used involved normal or infarcted hearts. We found that conduction block was due to an interaction between rate and the underlying substrate and not to a generalized susceptibility to drug action. We concluded that in our model, flecainide-induced proarrhythmia is due to an interaction determined by rate-dependency, the underlying substrate and drug-induced slowing of conduction.

Sodium salts are used to treat class IC antiarrhythmic drug toxicity, but underlying mechanisms have been poorly understood. In Publication 4 we demonstrated the mechanism for the reversal of flecainide cardiotoxicity by sodium salts. Our experimental studies of the modulation of flecainide's cardiotoxicity used different technical approaches including the microelectrode technique, biochemical methods and whole-cell voltage-clamp experiments. While maintaining osmolarity constant, we showed that high extracellular sodium concentration *per se* modulated antiarrhythmic drug effects on phase 0 of the action potential. Our binding study showed that changes in extracellular sodium concentration modulated flecainide displacement of tritiated batrachotoxin A benzoate ([^{3}H]-BTXB). Our results are consistent with those of Calahan and Almers (1979), who reported that external sodium modulated the block of sodium current by lidocaine (QX-314). They suggested that the Na^+ ions were acting by a mechanism of electrostatic repulsion ("knock-out" phenomenon) at a cationic binding site adjacent to a drug binding site in the channel. This type of mechanism was favored over an allosteric effect of external sodium on an external drug receptor. Our conclusions are also consistent with studies conducted on batrachotoxin-activated Na^+ channels in planar lipids bilayers showing that both the cocaine association and dissociation rate constants are altered with changes of external Na^+ ions concentration (Wang, 1988). In these experiments, changes of internal Na^+ ions concentration produced little effect. The modulation of cocaine binding affinity by external Na^+ ions suggests that either electrostatic repulsion ("knock-out" phenomenon) or some indirect interactions occur between Na^+ ions and the cocaine binding site (Wang, 1988). A similar modulation of K^+ channel block by tetraethylammonium has been reported with changes in K^+ ions (Armstrong, 1971) and electrostatic repulsion was suggested to occur

(Hille and Schwartz, 1978). The magnitude of the sodium inward current has been presumed to play a role in the modulation of sodium channel blockade by changes in extracellular [Na^+] (Kohlhardt, 1982). Our biochemical experiments were conducted in the presence of TTX, and suggest that the mechanism of reversal of cardiotoxic effects associated with class IC antiarrhythmic drugs by Na^+ does not require changes in sodium current as would be necessary for the mechanism proposed by Kohlhardt.

Our results explain how sodium salts reverse the toxic effects of a potent class IC agent such as flecainide, and this mechanism of action may apply to a variety of sodium channel blockers, including class I antiarrhythmic agents, tricyclic antidepressants, local anesthetics and cocaine. Similar mechanisms may occur in interactions between drugs with blocking properties on other cation channels and permeant cations.

2. DIRECTION OF FUTURE RESEARCH

Ion channels as targets for antiarrhythmic agents

The cardiac action potential results from a delicate balance between inward and outward currents generated by ion channels, pump currents and exchange current. In large part, ion channels are responsible for the generation and maintenance of the cardiac action potential, and block of specific channel(s) is the major mode of action of many antiarrhythmic drugs.

Possibilities for improved antiarrhythmic drugs

Sodium channel blockers

Before the results of CAST became known, the sodium channel represented a common target for antiarrhythmic drug development (Grant, 1990; Grant and Wendt, 1991). In CAST, class I antiarrhythmic drugs that effectively suppressed ventricular ectopic activity were associated with a higher rate of sudden death and total mortality compared to the placebo group (Echt et al, 1991). Rate-dependent conduction slowing, which we showed to be due to use-dependent I_{Na} blockade, represents a major effect of class I antiarrhythmic agents, and has clinical implications which are important regarding the mechanisms of action of antiarrhythmic agents *in vivo*. We and others have reported class I drug-induced proarrhythmia which was associated with escalating drug concentration and rate-dependent effects (Ranger et al, 1989; Ranger and Nattel, 1995; Winkle et al, 1981; Winkelman and Leinberger, 1987; Anastasiou-Nana et al, 1987; Morganroth, 1987; Rikenberger, 1982). Lower antiarrhythmic drug concentrations may not slow conduction enough to suppress serious arrhythmia, but concentrations high enough to terminate a tachyarrhythmia may lead to frequency-dependent proarrhythmia. Paradoxically, class I antiarrhythmic drug therapy aiming to slow conduction during an episode of ventricular tachyarrhythmia may cause enhanced use-dependent conduction slowing and proarrhythmic events. Experimental work in this thesis has demonstrated the determinants and mechanisms of proarrhythmia associated with class I antiarrhythmic agents. In patients with structural heart disease or poor left ventricular function, the potential proarrhythmic risk associated with class I drugs may become more important than beneficial therapeutic potential. Therefore, long-term treatment of chronic arrhythmias with sodium channel blockers may represent greater risk than benefit. Sodium channel blockers may still be useful in specific conditions, for example short-term in-hospital treatment of reperfusion arrhythmias with lidocaine in post-infarction patients. Flecainide lengthens atrial action potentials more at

fast rates than at normal heart rates (Wang et al, 1990) and may be useful in the treatment of supraventricular tachyarrhythmias.

Findings of this thesis may not entirely explain the mechanism responsible for the flecainide-encainide mortality in CAST. Limitations of our clinical and experimental *in vivo* studies include the possibility of other intervening factors, such as myocardial ischemia, being partly responsible for some of the response observed in the presence of antiarrhythmic drugs. What is the possible contribution of acute myocardial ischemia to flecainide-induced proarrhythmia? Myocardial ischemia represents another risk factor for proarrhythmic actions and could have a potentially arrhythmogenic interaction with antiarrhythmic drugs. During ischemia there is heterogeneity between ischemic and normal tissue, resulting in a difference of pH and extracellular potassium concentrations locally. These changes result in local alterations in the electrophysiologic properties of the myocardium (Hill and Gettes, 1980). Ischemia produces an increase of extracellular K^+ levels which in turn result in changes in resting membrane potential, conduction velocity and activation times in the myocardium.

A potentially arrhythmogenic interaction has been reported between ischemia and antiarrhythmic drugs. Nattel et al (1981) demonstrated that nonuniform reduction of blood flow produced by coronary occlusion may result in alterations in the regional myocardial distribution of antiarrhythmic drug (aprindine) and on its arrhythmogenic effects. These results were supported by Elharrar et al (1977) who showed that in the presence of ischemia, aprindine produced a greater conduction slowing and activation delay than in myocardium with ischemia alone. Both of these studies, along with subsequent work with class I drugs (Ireda et al, 1985; Lynch et al, 1987; Ye et al, 1993; Sadanaga and Ogawa, 1994; Podrid et al, 1992), suggest that ventricular tachyarrhythmia associated with therapeutic concentrations of class I drug may be due to proarrhythmia due to acute ischemia in the presence of the drug. Preliminary work in our laboratory compared the concentration-dependence and prevalence of ventricular proarrhythmia in the presence of flecainide in conditions of myocardial infarction and acute myocardial ischemia. The results were presented in abstract form (Nattel et al, 1994) and showed that (1) myocardial ischemia increased the occurrence of lethal flecainide-induced proarrhythmia, and (2) flecainide strongly increased the incidence of ventricular fibrillation during acute ischemia at lower concentrations than the ones in our chronic infarct model (Ranger and Nattel, 1995). The mechanism of action prevailing during episodes of acute ischemia may

represent a transient factor responsible for the mortality encountered with class IC drugs in CAST. Section 2 of the Introduction described a large variety of mechanisms of cardiac arrhythmias. Acute myocardial ischemia produces changes in membrane potential and in inward and outward currents during the action potential which will lead to changes in conduction velocity, refractoriness and automaticity. Conduction velocity is reduced both in the longitudinal and the transverse direction after the onset of ischemia before fibrillation (Kleber et al, 1986). It has been proposed that drugs may act by sensitizing the recently infarcted myocardium to factors promoting arrhythmias (Ruskin 1989; Roden, 1991). Ischemia and the slow healing process after recent myocardial infarction represent risk factors for arrhythmias.

Electrophysiological studies (EPS) with programmed stimulation techniques have been used to determine drug efficacy for arrhythmia suppression (Ruskin et al, 1980). However, the assessment of antiarrhythmic drug therapy is performed in a nonischemic state and drugs that suppress arrhythmias in a nonischemic state may become proarrhythmic during ischemia. Using programmed stimulation during EPS, Morady et al (1987) reported that myocardial ischemia may be an important contributor to successful induction of ventricular arrhythmia in patients. The precise mechanisms of drug-induced proarrhythmia during ischemia still remain to be determined.

Na^+ channel blockers have been used in conjunction with ß blockers which prevented ventricular tachyarrhythmias in a canine model of acute myocardial infarction (Hope et al, 1974). ß blockers were found to decrease the incidence of sudden death in patients with previous myocardial infarction (ß-blocker Heart Attack Trial Group, 1982). Changes in autonomic activity may predispose to proarrhythmia (Podrid et al, 1990) and the autonomic nervous system modulates cardiac electrophysiological properties and influences arrhythmogenesis associated with myocardial ischemia and infarction (Malliani et al, 1980; Lombardi et al, 1983). Drugs affecting autonomic nervous system function are likely to have a different proarrhythmic potential. ß-adrenergic blocking drugs increase the ventricular refractory period (Brodsky et al, 1989) and can prevent the induction of ventricular arrhythmias. Propranolol appears to be effective antiarrhythmic therapy both in ischemic and nonischemic states (Pfisterer et al, 1992). ß-adrenergic blockers may partially limit ischemic effects because of their negative chronotropic and inotropic mechanisms (reduction of myocardial oxygen demand). Some ß-blockers can increase the ventricular fibrillation threshold in ischemic animals

(Anderson et al, 1983) and decrease the frequency of ventricular ectopy in postmyocardial infarction patients (Friedman et al, 1986). Our experimental work described the use-dependent behavior of class IC drug-induced conduction block in the infarcted zone. Sodium channel blocking drugs possessing ß-adrenergic properties would combine effects on conduction velocity and refractoriness intended for antiarrhythmic action in the ventricles and might prevent use-dependent toxicity associated tachycardia.

Nevertheless, the clearly documented proarrhythmic properties of Na^+ channel blockers makes it unlikely that such drugs, even in conjunction with ß-blockers, can be used very safely to treat arrhythmias. Current knowledge does not permit the development of new Na^+ channel blockers which would be safer to use.

I_{Na} agonists that could bind preferentially to the receptor during the open state would increase plateau I_{Na} and result in prolongation of APD and ERP. State-dependent actions with high affinity to the open state would imply that dissociation from its receptor would occur during the plateau phase or during diastole. The ideal kinetics of recovery from inactivation and reactivation of an open-state I_{Na} agonist should permit a tachycardia-selective increase of cardiac action potential duration. In other words, at normal heart rates, dissociation time would be long enough to allow the drug to unbind; during tachycardia, rate-dependent drug association would occur concomitantly with a reduced diastolic interval and less drug dissociation. Useful therapeutic agents modulating I_{Na} should produce use-dependent prolongation of cardiac action potential duration and refractoriness without conduction slowing. Because the limitations to the value of Na^+ channel blocking antiarrhythmic drugs studied in this thesis, it is useful to consider the potential value of antiarrhythmic drugs acting on other channels.

Calcium channels

Various Ca^{++} channel blockers have been shown to suppress spontaneous ventricular arrhythmias and ventricular fibrillation in experimental models of acute myocardial ischemia produced by transient coronary occlusion (Kaumann and Aramendia, 1968; Clusin et al, 1982 and 1984; Muller et al, 1988). In models of ischemic injury (Patterson et al, 1983; Lynch et al, 1985) and in the setting of recent myocardial infarction (Lynch et al, 1985 and 1986; Billman, 1989), the efficacy of Ca^{++} channel blocker therapy to prevent ventricular arrhythmias has been variable. It

was debated whether the potential antiarrhythmic and antifibrillatory effects of Ca^{++} channel blocker therapy was attributable to direct electrophysiologic actions or indirect results of anti-ischemic effects (Katz and Reuter, 1979; Lynch et al, 1986; Peter et al, 1983; Patterson et al, 1983; Watanabe et al, 1989).

In post-myocardial infarct patients, a Ca^{++} channel blocker (verapamil) did not show the potential beneficial effects seen in experimental studies (Danish Study Group Study, 1984). A study of Ca^{++} channel blocker therapy with diltiazem in survivors of acute MI did not demonstrate an overall beneficial effect on mortality, although there was a possible benefit with drug therapy when left ventricular function was intact but worsened outcome with left ventricular function dysfunction (Multicenter Diltiazem Postinfarction Research Trial Group, 1988).

An increase in inward current during the plateau phase of the cardiac action potential could result in a more positive plateau phase. Depending on the magnitude of the increase of inward current, the overall effect of a Ca^{++} channel agonist could be a lengthening or shortening of action potential duration, because repolarizing currents could be activated more quickly and fully at positive voltages (Bennett et al, 1985). For example, the block of I_{to} results in a positive shift in plateau potential and shortening of action potential duration (Litovsky and Antzelevitch, 1988). Substantial rate-dependent increase of inward current could be achieved by a Ca^{++} agonist binding to its receptor during the upstroke of the cardiac action potential, while state-dependent augmentation could occur during the plateau phase. The Ca^{++} channel agonist Bay K 8644 lengthens APD within the plateau voltage range (January et al, 1988). If binding of an inward current agonist was achieved by high-affinity association during the plateau phase, this could result in an even greater receptor occupancy, possibly leading to arrhythmogenesis such as EADs and torsades de pointes. Theoretically, the kinetics of recovery from inactivation and reactivation of a Ca^{++} agonist could be modified so that early afterdepolarizations would be avoided. A Ca^{++} agonist should then preferentially bind to its receptor during the upstroke of cardiac action potential so that dissociation from its receptor occurs during the plateau phase or during diastole. The kinetics of recovery from inactivation and reactivation should be adequate so that the increase of cardiac action potential duration would be tachycardia-selective. During normal heart rates, dissociation would predominate and the drug would unbind, and during tachycardia rate-dependent drug association would occur concomitantly with

reduced diastolic interval and less drug dissociation. As for sodium channel blockers, a very delicate balance between Ca^{++} agonist concentration and ideal drug kinetics would be prerequisites for antiarrhythmic efficacy and avoidance of possible arrhythmogenesis, if such drug design was feasible.

Besides antiarrhythmic drug therapy, nonpharmacologic approaches to arrhythmia control have been developed as alternatives (eg, catheter ablation, fulguration and implantable cardiac defibrillator (Tchou et al, 1988),

Potassium channels

Class III agents lengthen action potential duration (usually via a reduction of repolarizing outward currents). A number of antiarrhythmic agents which prolong action potential without blocking sodium channels have been tested in clinical trials (Roden, 1993). Sotalol blocks I_K (delayed rectifier potassium current) (Carmeliet, 1985) and prolongs APD and ERP (Hayward and Taggart, 1986). Sotalol represents an effective treatment of some supraventricular arrhythmias (Daubert et al, 1993). Class III agents are antiarrhythmic because of their prolongation of cardiac APD during tachyarrhythmias, but may be proarrhythmic at normal heart rates. Some class III agents produce reverse use-dependent prolongation of ERP: on one hand, prolongation of repolarization is reduced at fast heart rates and this may limit their therapeutic potential (Hondeghem and Snyders, 1990; Hondeghem, 1993), and on the other hand, prolongation of repolarization at normal and slow heart rates is increased and may lead to proarrhythmia. Proarrhythmic risk manifests as polymorphic ventricular tachycardia and torsades de pointes (Carlsson et al, 1990).

Two different components have been identified for the delayed rectifier potassium current, a fast component I_{Kr} and a slow component I_{Ks}, possessing different kinetics (Noble and Tsien, 1969; Sanguinetti and Jurkiewicz, 1990 and 1991; Zeng et al, 1995). I_{Ks} possesses slower deactivation kinetics compared to I_{Kr}, and at fast stimulation rate there is an incomplete deactivation and accumulation of I_{Ks} (Sanguinetti et al, 1991). Because of extracellular K^+ accumulation, there is an increase of I_{K1} in addition to I_{Ks}. The increase of both outward currents reduces the impact of I_{Kr} blockade at fast stimulation rates in multicellular preparations (Sanguinetti et al, 1991).

Almokalant blocks I_{Kr} in a use-dependent fashion (Carmeliet, 1992) but

produces reverse use-dependent prolongation of the cardiac action potential (Carlsson et al, 1990; Duker et al, 1992). As mentioned above the reverse use-dependent repolarization changes induced by almokalant and other class III drugs (Carmeliet, 1992; Follmer and Colatsky, 1990; Sanguinetti et al, 1991), may limit their therapeutic benefit and lead to proarrhythmia at slow rates (Snyders and Hondeghem, 1990).

In principle, specific I_{Ks} blockers should produce rate-dependent prolongation of APD as opposed to the observed reverse use-dependency associated with I_{Kr} blockers (Jurkiewicz and Sanguinetti, 1993). At fast heart rates the I_{Ks} relative contribution to the net repolarizing current is increased compared to I_{Kr}. Specific I_{Ks} blockers could represent tachycardia selective antiarrhythmic agents causing rate-dependent APD prolongation without conduction slowing associated with Na^+ channel blockers.

I_{Kur} is a novel delayed rectifier current with rapid activation kinetics and noninactivating properties characterized in our laboratory by Wang et al (1993). Experimental data suggests that I_{Kur} may be the natural expression in the human heart of the Kv1.5 genes (Wang et al, 1993). Selective I_{Kur} blockade with 50 μM 4-aminopyridine prolonged human atrial APD, suggesting a significant contribution of I_{Kur} to the net repolarizing current in atrial cells (Wang et al, 1993; Snyders et al, 1992). Based on this evidence, specific I_{Kur} blockers have been suggested as candidates for novel antiarrhythmic drugs.

Cl^- channels

Recent work has identified at least five distinct chloride conduction pathways in cardiac cells (Ackerman and Clapham, 1993). These chloride currents may play a role in the modulation of the cardiac action potential and may represent new targets for antiarrhythmic therapy.

1) Cyclic AMP-regulated Cl^- current ($I_{Cl,cAMP}$) is a time-independent chloride current which is elicited upon ß-adrenergic stimulation or direct activation of adenylyl cyclase (Harvey and Hume, 1989). Experimental data on single channels suggest that the protein kinase A-regulated cardiac Cl^- channel resembles the conductance of cystic fibrosis transmembrane regulator (CFTR) (Nagel et al, 1992). $I_{Cl,cAMP}$ may participate in shortening APD and depolarizing membrane resting potential by isoproterenol in guinea pig ventricular myocytes (Harvey et al, 1990) and may participate in arrhythmogenic actions (Yamawake et al, 1992).

2) Zygmunt and Gibbons (1991 and 1992) have reported that the 4-aminopyridine-resistant transient outward current (I_{to2}) of rabbit ventricular myocytes is a calcium-activated chloride current ($I_{Cl,Ca}$).

3) Swelling-induced Cl^- current ($I_{Cl,swell}$): cell swelling, resulting from an osmotic gradient, can activate a chloride-sensitive conductance. $I_{Cl,swell}$, the current associated with cell swelling, has been observed in atrial and ventricular cells (Sorota, 1992; Tseng, 1992). However, Sorota (1992) reported that swelling and chloride-sensitive current activation was produced with more ease in canine atrial cells than in ventricular cells. During myocardial ischemia, cell swelling may occur and adrenergic tone may be elevated. Consequently $I_{Cl,swell}$ may appear in response to cell swelling and since $I_{Cl,swell}$ is enhanced by isoproterenol (Sorota, 1992), a similar effect may take place during ischemic conditions. Another possible effect is the shortening of APD and ERP resulting from outward Cl^- current during the plateau phase of cardiac action potential. Oz and Sorota (1995) reported that human myocardium does express a swelling-induced current that can be stimulated by forskolin. $I_{Cl,swell}$ blockers may represent novel drugs in treating atrial arrhythmias.

4) In guinea pig ventricular cells phorbol esters activate another Cl^- current ($I_{Cl,PKC}$) by stimulating protein kinase C (PKC) (Walsh, 1991; Walsh and Long, 1994). This Cl^- current resembles $I_{Cl,cAMP}$ current which is activated by phosphorylation via protein kinase A.

5) An ATP-activated Cl^- current has been reported in guinea pig atrial myocytes ($I_{Cl,purinergic}$) (Matsuura and Ehara, 1992). The pathway for activation of this current still remains to be clarified.

Although Cl^- currents are present in some cardiac tissues and experimental studies indicate that Cl^- currents may play a physiological role, their functional role still remains poorly understood. Before designing drugs acting specifically on Cl^- channels, the identification of these channels at the molecular level is needed, as well as a better understanding of the contribution Cl^- current in controlling APD and depolarization under physiologic, ischemic and arrhythmogenic conditions.

Molecular techniques for studying cardiac ion channels

The nucleotide and predicted amino sequence of complementary DNA encoding many cardiac channels have been identified and functional domains have been studied using mutagenesis approaches (Chiamvimonvat et al, 1995;

Tomaselli et al, 1995).

Recent molecular biology techniques and biophysical techniques will allow definition of the precise molecular structure of various ion channels, the location of the binding site(s) for antiarrhythmic drugs (Snyders et al, 1992), and whether multiple binding sites reside in distinct regions of different ion channel structures. These studies may help to understand antiarrhythmic drug interactions with specific or multiple receptors of ion channels and lead to the development of antiarrhythmic drugs with improved selectivity and affinity for specific ion channels, or for specific ion channel states.

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