ANTIARRHYTHMIC AND ARRHYTHMOGENIC PROFILES OF QUINIDINE AND THEIR MODULATION BY CLASS IB ANTIARRHYTHMIC DRUGS

by

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CHAPTER I

MODIFICATION OF THE FREQUENCY AND VOLTAGE DEPENDENT EFFECTS OF QUINIDINE WHEN ADMINISTERED IN COMBINATION WITH TOCAINIDE IN CANINE PURKINJE FIBERS

PREFACE

In accordance with the "Guidelines Concerning Thesis Preparation" of the Faculty of Graduate Studies and Research of McGill University, the candidate has chosen the option of including, as part of the present thesis, the text of an original paper already published by a peer-reviewed journal, and original papers submitted, or suitable for submission to learned journals. The text pertaining to this option is as follows:

> The Candidate has the option, subject to the approval of the Department, of including as part of the thesis the text, or duplicated published text, of an original paper, or papers. In this case, the thesis must still conform to all other requirements explained in Guidelines Concerning Thesis Preparation. Additional material (e.g. procedural and design data as well as descriptions of equipment) must be provided in sufficient detail (e.g. appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported. The thesis should be more than a mere collection of manuscripts published or to be published. It must include a general abstract, a full introduction and literature review and a final overall conclusion. Connecting texts which provide logical bridges between different manuscripts are usually desirable in the interests of cohesion.

The "Results" section of this thesis consists of three manuscripts. The first manuscript has been published in *Circulation*; the second one is under review with *Journal of Cardiovascular Pharmacology*; the third has been submitted to *Journal of Cardiovascular Electrophysiology*. The experimental studies were performed by the candidate. The initial versions of the manuscripts were written by the candidate. These were all revised by the supervisor to make them suitable for publication.

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ABSTRACT

The present study investigates *in vitro* electrophysiological effects of therapeutic concentrations of quinidine (5 - 10 μM) and its combination with the Class Ib agents mexiletine and tocainide in canine Purkinje fibers with the use of standard microelectrode techniques. The frequency- and voltage-dependent depression of $\dot{V}_{max}$, used as an index of peak sodium conductance, by quinidine and the combination of quinidine and tocainide (50 μM) were assessed with the kinetics of onset of, and recovery from, rate-dependent block, and the curve relating $\dot{V}_{max}$ to membrane potential. The frequency-dependence of quinidine-induced repolarization abnormalities arising from early after-depolarizations (EADs) was characterized in the presence of low $[K^+]_o$ (2.7 mM) and mild acidosis (pH = 7.06 ± 0.08). Acidosis was found to favor triggered activity by directly prolonging action potential duration. Quinidine induced two types of EAD-induced triggered activity, viz. arising from phase 2 and arising from phase 3, which differed in the frequency-dependence of their characteristics (activation voltage, amplitude, and coupling interval), and their sensitivities to abolition by mexiletine. Adrenaline (1 μM) decreased the minimum cycle length for triggered activity, shortened the coupling interval of triggered responses, and transformed single triggered responses into multiple. It also induced rapid activity resembling sustained triggered activity, induced triggered responses arising from phase 2, and facilitated their transmission to ventricular muscle. The combination of quinidine with a Class Ib drug proved to be beneficial by improving its antiarrhythmic effect and preventing its bradycardia-related excessive QT prolongation. Triggered activity was found to originate only in Purkinje fibers, but electrotonic influence from ventricular muscle strongly affected its manifestation. These results provide the evidence that EAD-induced triggered activity may be a relevant *in vitro* model for the arrhythmia torsade de pointes.

RÉSUMÉ

Cette étude utilise la technique d'électrodes intracellulaires pour l'investigation des effets électrophysiologiques *in vitro* de la quinidine (5 - 10 μM) et de sa combinaison avec les agents de la Classe Ib, mexiletine et tocainide, dans les fibres de Purkinje canines. $\dot{V}_{max}$ a été utilisé pour l'évaluation de l'importance du blocage des canaux sodiques. La dépendance de la réduction de $\dot{V}_{max}$ sur la fréquence cardiaque et le voltage par la quinidine et la combinaison de la quinidine avec la tocainide, a été déterminé par les cinétiques (constantes de temps) de sa diminution et de son rétablissement, et par la courbe de la relation entre $\dot{V}_{max}$ et le potentiel de membrane. La dépendance sur la fréquence cardiaque des anormalités de repolarization induites par la quinidine a été caracterisée en présence d'une concentration basse de potassium (2.7 mM) et de l'acidose (pH = 7.06 ± 0.08). L'acidose contribue à la génération de l'activité déclenchée par une prolongation directe de la durée du potentiel d'action. La quinidine peut produire deux types d'activité déclenchée provenant des *early afterdepolarizations* (*EADs*), viz. naissant de la phase 2 ou de la phase 3 qui diffèrent par la dépendance sur la fréquence cardiaque de leurs caractéristiques (voltage d'activation, amplitude, et interval d'association), et leur sensibilité à l'abolition par la mexiletine. L'adrénaline (1 μM) a diminué la longueur de cycle minimum pour la manifestation de l'activité déclenchée, a réduit l'interval d'association des réponses déclenchées, et a transformé les réponses déclenchées simples en multiples. Elle a aussi produit une activité rapide ressemblant à l'activité déclenchée soutenue, induit des réponses déclenchées emergeant de la phase 2, et facilité leur transmission au muscle ventriculaire. La combination de la quinidine avec un agent de la Class Ib est donc bénéfique par l'amélioration de ses effets antiarythmiques et la prévention

de la prolongation excessive de l'interval QT associée à la bradycardie. L'activité déclenchée prend naissance que dans le système de Purkinje, mais est très influencée par les interactions électrotoniques du muscle ventriculaire. Ces résultats fournissent aussi l'évidence que l'activité déclenchée naissant des *EADs* pourrait être un modèle *in vitro* approprié pour l'arythmie torsade de pointes.

LIST OF ABBREVIATIONS

A M P: **Amp**litude of an action potential

A P D: Action Potential Duration

A V: Activation Voltage of a triggered action potential

B C L: Basic Cycle Length

C I: Coupling Interval of a triggered action potential

D A D: Delayed AfterDepolarization

E A D: Early AfterDepolarization

E C G: ElectroCardioGram

E D B: Early Diastolic Block

E R P: Effective Refractory Period

H M P: High Membrane Potential

L M P: Low Membrane Potential

M D P: Maximum Diastolic Potential

R D B: Rate-Dependent Block

S D: Standaid Deviation

T A P: Triggered Action Potential

T O P: Take-Off Potential

$\bar{V}$: Voltage at which $\dot{V}_{max}$ occurs

V_{max}: Maximum upstroke (phase 0) velocity of the action potential

τ_{rec}: Time constant of recovery

GENERAL INTRODUCTION

1. ACTION OF ANTIARRHYTHMIC DRUGS

1.1. Preamble

Cardiac arrhythmias represent a major problem in cardiovascular diseases today. Disturbances of heart rhythm are a threat to the population at all age categories and remain an important cause of mortality and morbidity in the industrialized countries.

Effective pharmacological treatment of cardiac arrhythmias remains a challenge despite the many advances in the understanding of these pathologies. Accurate prediction of the response to drugs is based on the identification of the cardiac mechanism involved. Even though technological tools have substantially helped to realize this goal, improvement in arrhythmia management awaits more drug development, or at least new approaches which will hopefully add precision to the therapeutic function of the agents already in use.

Limitations in the use of conventional antiarrhythmic drugs for the treatment of ventricular arrhythmias still interfere with optimal therapy. Successful control of arrhythmias is limited, among others, by an incomplete suppression of abnormal rhythms achieved at the maximal tolerated doses and by arrhythmogenic side effects. Whenever single agents prove ineffective or proarrhythmic, combination therapy could improve antiarrhythmic efficacy with fewer additional adverse effects. This approach has become a widely accepted way to improve the treatment of cardiac arrhythmias.

1.2. Antiarrhythmic and Arrhythmogenic Mechanisms

One of the most important modes of suppression of arrhythmias by

antiarrhythmic drugs is through an alteration of transmembrane ionic current flow. At the macroscopic level, ionic changes are ultimately expressed as mechanisms that can depress abnormal rhythms. Among others, prolongation of the ratio of effective refractory period to action potential duration aims to prevent the initiation or perpetuation of reentrant circuits. Antiarrhythmic drugs may also prevent tachyarrhythmias due to enhanced automaticity by depressing the pacemaker current in the sino-atrial and atrioventricular nodes, or in the His-Purkinje system. They may alternatively act to alter autonomic influence on the heart.

However, it is not unusual that the effect of a particular drug is beneficial under some circumstances and proarrhythmic under other circumstances. Arrhythmogenicity of antiarrhythmic drugs is regarded as an important complication of antiarrhythmic therapy. For instance, a slowing of conduction may indirectly facilitate reentry and prolongation of the action potential duration may also promote reentry through extreme dispersion of repolarization. The possibility of these opposing effects emphasizes how the identification of the mechanism of arrhythmia is crucial to proper selection of drugs.

1.3. Classification of Antiarrhythmic Drugs

1.3.1. The Vaughan Williams scheme

Based on their electrophysiological effects, Vaughan Williams proposed a classification some two decades ago (Vaughan Williams, 1970, 1975, 1984a, 1985a), which still remains to this day the most widely accepted scheme (Singh & Hauswirth, 1974; Gettes, 1979; Singh *et al*, 1983).

Briefly, the Vaugham Williams classification groups antiarrhythmic drugs by their assumed major mechanism of action into four groups, viz. Class I, the sodium channel blockers; Class II, the β-blockers; Class III, drugs which prolong the action potential duration; Class IV, the calcium channel blockers. However, most antiarrhythmic drugs have a so-called "mixed profile" of activity, and consequently should belong to more than one Class (Harrison, 1985).

1.3.2. The Subclassification of Class I

Agents grouped in Class I are those with local anesthetic activity; they all exert their antiarrhythmic effect by blocking sodium channels, thus reducing the fast inward current carried by sodium ions. The so-called "Class I antiarrhythmic action" produces a slowing of the maximum upstroke velocity of the action potential (first derivative of the phase zero, termed $\dot{V}_{max}$, which is used experimentally as an index of the maximum sodium conductance) leading to a slowing of conduction and widening of the QRS complex on the surface electrocardiogram. Because block of sodium channels by antiarrhythmic agents is a function of membrane potential and time, these drugs are said to exhibit voltage- and use-dependent actions. Clinically useful Class I antiarrhythmic agents have a profile of interaction with the sodium channel such that at normal heart rates and membrane potentials, they have less depressant effect, while altering abnormal rhythms to a greater extent, especially in depolarized tissues.

Although all Class I drugs share the common point of inducing a progressive enhancement of their depressant effects on the fast inward current with faster rates of activity, they nevertheless exert a wide spectrum of electrophysiological effects on conduction velocity at normal heart rate (QRS complex), action potential duration (QT interval), and refractoriness. These

differences in effects have led Harrison and co-workers to propose a new subclassification in 1981. Subsequently, *in vitro* studies on the electrophysiological effects of these drugs correlated the difference in the kinetics of onset of their use-dependence with the difference in their clinical effects (Campbell, 1983a).

1.3.2.1. Drug affinity as a mean of subclassification

Class I antiarrhythmic drugs are subdivided into three groups according to the rate at which $\dot{V}_{max}$ declines to a new steady state following an increase in stimulation frequency, namely fast (Ib), intermediate (Ia), and slow (Ic) kinetics (Campbell, 1983a). Kinetics of onset of frequency-dependent block vary with drug affinity (binding and unbinding) for the sodium channel receptor.

Class Ia comprises the quinidine-like drugs which slow down conduction at normal heart rates, slow repolarization, and prolong the effective refractory period relative to the action potential duration, the ERP/APD ratio (Estes *et al*, 1985; Franz & Costard, 1988). Class Ib comprises the lidocaine-like drugs which slow down conduction velocity minimally in normal tissues and more at depolarized membrane potentials, shorten repolarization, but prolong the ERP/APD ratio (Kupersmith *et al*, 1975; Estes *et al*, 1985). Class Ic comprises lorcainide, flecainide, encainide, and propafenone. These drugs induce profound slowing of conduction, have minimal effect on repolarization, and only moderately affect the ratio of effective refractory period to action potential duration (Hellestrand *et al*, 1982; Estes *et al*, 1985).

Drugs with slow offset kinetics will exhibit little recovery from block over the period of one diastolic interval at heart rates in the physiological range. If

an early extra-systole is viewed as an increase in rate that lasts for only one beat, it then becomes evident why drugs with the fastest onset of rate-dependent block (Class Ib) produce the greatest increases in refractoriness (Campbell, 1983a). Thus, the speed at which a particular Class I drug responds to a sudden increase in pacing rate by further depressing $\dot{V}_{max}$ seems to play a key role in its ability to prolong the ratio of the effective refractory period relative to the action potential duration. This difference among drugs of the various subclasses may well be a consequence of their distinct abilities to prolong recovery of the sodium channel from inactivation (Campbell, 1983a). Thus, the interaction of drugs with the sodium channel receptor will also be a determinant of their effect on refractoriness.

The Harrison subclassification, often referred to as the modified Vaugham Williams classification (Vaughan Williams, 1984b, 1985b), provides a link between the electrophysiological actions of Class I antiarrhythmic drugs *in vitro* and their clinical effects. According to Harrison (1985, 1986), this system has enabled the clinician to predict the effects (therapeutic and adverse) of antiarrhythmic drugs on the electrocardiogram; it has permitted a rational choice of drugs for a specific disorder; it has encouraged the use of rational drug combinations. Nevertheless, its limitations cannot be ignored (Frumin *et al*, 1989): this scheme could be viewed as an oversimplification of a complex situation as very often, efficacy of antiarrhythmic drugs may not be necessarily due to a single electrophysiologic effect, but rather are the result of an interaction of their primary, secondary and tertiary effects. Such effects may include alterations of passive membrane properties which have not been taken into account in the Vaughan Williams scheme. Moreover, it is mostly based on laboratory results run on normal tissue and therefore does not take into account differential effects on ischemic or otherwise altered tissue. However, it should be pointed out that no attempt for

classification so far has given a complete rationale for the differences in all aspects of the effects of these drugs.

1.3.2.2. Models of drug interaction with the sodium channel

Quantitative description of sodium channel kinetics has been used as a basis to quantify drug interaction with its receptor. One such model, which has been proposed by Hondeghem & Katzung (heart) and by Hille (nerve) in 1977, is called the "modulated receptor hypothesis". This model is based on a set of differential equations which allow quantitatively testable predictions of drug action. The equations and parameters of the model have the function of estimating a predicted sodium conductance ($\bar{G}_{Na}$), which in turn is directly proportional to the fraction of channels in the activated state. In their review of 1984, Hondeghem & Katzung quoted at that time over one hundred laboratory studies that confirmed at least one of the predictions of their model (see below). The "modulated receptor hypothesis" has also been considered in reviews concerning sodium channel blocking drugs (Sasyniuk & Nattel, 1982; Kendig, 1985) and calcium channel blocking drugs (Hondeghem & Katzung, 1984; Chin, 1986).

An alternative model is the "guarded receptor hypothesis" proposed by Starmer *et al* (1984). This model postulates that there is a single, fixed affinity receptor site for Class I antiarrhythmic drugs that is guarded by the gates of the sodium channel. The accessibility of the receptor is therefore determined by the channel conformation. The receptor is then said to be "periodically (or transiently) accessible" (Starmer, 1986). Moreover, it states, in contrast to the modulated receptor hypothesis, that drug binding has no effect on the rate constants for channel gating. Ion channel blockade is thereby viewed as a two-stage process: diffusion of drug to a region near the channel binding site and coupling of drug to

the binding site resulting in a non-conducting, drug-complexed channel. Therefore, the ligand diffusion path is regulated by the gating process of the channel.

1.4. The Modulated Receptor Hypothesis

The "modulated receptor hypothesis" of Hondeghem & Katzung (1977, 1980) stipulates that Class I antiarrhythmic drugs interact (bind and unbind) with the sodium channel in the activated, inactivated, or resting state with different affinities. These interactions are each governed by their own kinetics, association and dissociation constants. That is to say, the affinity of the channel receptor for the drug changes with its conformation. Upon binding, drugs alter the voltage dependence of inactivation. Even though the transitions between the different states (activated, inactivated, resting) are still governed by the standard Hodgkin-Huxley equations (1952), the inactivation parameters of the drug-associated channels are shifted to more negative potentials. Moreover, drug-bound channels do not conduct, even in the activated state, i.e. they are blocked.

The affinity of the channel receptor for the drug is modulated by the channel state, such that resting channels usually have a low affinity, while activated and/or inactivated channels have a high affinity. Two main pathways of access to the receptor site of the channel can be used by a drug molecule. Charged drugs can take the hydrophilic route (the channel pore) only while the channel is open, whereas neutral drugs could take the lipophilic pathway (the lipid layer of the membrane), even when the channel is closed (resting or inactivated states). Most antiarrhythmic agents of the Class I are weak bases, with a pK_a ranging from 7.5 to 10. Therefore, they exist in both the neutral and cationic form at physiological pH. This implies that changes in pH could drastically alter the overall effect of a drug

through the modulation of its access pathway to the receptor of the sodium channel.

An additional determinant of the effect that a drug will exert is whether this drug has affinity for the open state of the sodium channel only, equal affinity for both the open and the inactivated states, or alternatively, for the inactivated state only. This property will determine whether action potential duration will influence block development. A prolonged action potential duration will promote binding of a drug with high affinity for the inactivated state of the sodium channel by prolonging the time spent by the sodium channel in the inactivated state. This could well explain how two drugs administered in combination can influence each other's action beyond a simple additive effect: a drug that prolongs action potential duration will indirectly promote binding by another sodium blocker with high affinity for the inactivated state simply by prolonging the time the sodium channel spends in this state (Katzung *et al*, 1985; Stroobandt & Kestleloot, 1986). On the other hand, a drug with high affinity to the open state only is not very likely to be influenced by the duration of the action potential since the time spent by the sodium channel in the open state is very short regardless of the duration of the action potential.

The modulated receptor hypothesis has evolved quite substantially since its original proposal in 1977. Data from patch clamping has helped in this respect by providing a better insight of the mechanism of alteration of the sodium current by drugs. A recent version of the modulated receptor hypothesis (Hondeghem & Bennett, 1989) aims to incorporate accurate description and reproduction of macroscopic currents, single channels currents, and drug effects.

At the single channel level, the main mechanisms for the depression

of the sodium current are now interpreted as a use-dependent reduction of the probability of opening and shorter open times (Hondeghem & Bennett, 1989). Sodium channels are less likely to open and may open for a shorter time. Drug occupation would also increase the energy barriers separating the rested and the inactivated pools (Johns *et al*, 1989). It ensues that drug-associated channels are more likely to be trapped in the inactivated pool in the presence of a drug with affinity for the inactivated state of the sodium channel.

1.4.1. Experimental basis of the model

Several crucial experimental observations led to the formulation of the "modulated receptor hypothesis" for antiarrhythmic drug action (Hondeghem & Katzung, 1984). The first one is derived from the classic work of Weidmann (1955). His results presented, with the use of steady state inactivation curves of $\dot{V}_{max}$ of the action potential, the evidence that in the presence of sodium channel blockers, cardiac sodium channels behave as if their voltage dependent inactivation is shifted towards more negative membrane potentials. Moreover, he made the observation that the depressing effect of cocaine on the sodium current can be reduced by increasing membrane potential. In his discussion, he questioned the then-accepted view of considering local anesthetics as "membrane stabilizers", saying that his results did not suggest that these drugs act simply by blocking a fixed proportion of sodium channels.

A second observation was that the depression of $\dot{V}_{max}$ by quinidine progressively increases with each successive action potential in a train of pulses (Johnson & McKinnon, 1957). Moreover, the steady-state effect is more pronounced with faster driving rates. Along the same line, Heistracher (1971) showed that $\dot{V}_{max}$ recovers exponentially between trains of stimuli, while the

preparation is at rest. He made the observation as well that the first beat of the train following a quiescent period was not depressed in quinidine-treated fibers.

A third observation came from the study of Chen *et al* (1975). They showed that during diastole, $\dot{V}_{max}$ recovers from lidocaine-induced use-dependent block faster at more negative membrane potentials than at depolarized membrane potentials. On the basis of the shift of the membrane responsiveness curve by lidocaine, they predicted that lidocaine will depress premature responses without altering non-premature responses. However, several points in their methodology resulted in misinterpretation of some of the data. An accurate measurement of the recovery of the sodium current from drug-induced rate-dependent block implies that $\dot{V}_{max}$ has reached a new depressed steady state resulting from this block. In this particular study, block was induced with conditioning pulses at a rate of .2 Hz. This frequency of stimulation is too slow to induce any rate-dependent block by lidocaine since this drug has very fast kinetics of interaction with the receptor of the sodium channel. This experimental imprecision probably led to the dose-dependence of recovery kinetics seen with lidocaine. This rate of stimulation of .2 Hz is insufficient to allow any significant rate-dependent block from quinidine as well. On the other hand, the stimulus train used to induce block lasted eight basic beats, and each recovery curve covered only the first 500 msec of the diastolic interval. This is an insufficiently long period to properly assess the recovery from quinidine-induced block. The authors nevertheless correctly concluded that the recovery kinetics from lidocaine-induced block differed from those of quinidine-induced block in their voltage dependence, based on the observation that the recovery kinetics of $\dot{V}_{max}$ were not affected by quinidine in the range of concentrations used (17.2 to 68.7 μM). Therefore, previously reported rate-dependent effect of quinidine in mammalian fibers could not be attributed to a

change in reactivation kinetics.

1.4.2. Predictions of the model

Prior to the modulated receptor hypothesis, Strichartz (1973) and Courtney (1975) had proposed that drugs could interact with the sodium channel only while it is in the activated conformation. The combination of the three key observations mentioned above led Hondeghem & Katzung (1977) to consider the possibility that drugs are able to interact with the sodium channel in each of its three states. Class I antiarrhythmic drugs depress the sodium current in a dose-related fashion, but for any given concentration, this block is markedly influenced by factors such as frequency of stimulation, membrane potential, number of action potentials at any particular rate. The rate constants of interaction with the sodium channel of lidocaine and quinidine, as calculated in their original model (Hondeghem & Katzung, 1977), could relatively accurately describe the actions of these drugs over a wide range of cycle lengths and voltages.

On the basis of the calculation of such rate constants, the modulated receptor hypothesis predicts the voltage- and frequency-dependent behavior of Class I antiarrhythmic drugs under various experimental conditions, which present the advantage to be quantitatively testable (Hondeghem, 1987). These predictions are based on the assumption that all Class I antiarrhythmic drugs interact with one common receptor type within the sodium channel.

1.4.2.1. The rate of onset of frequency-dependent depression of $\dot{V}_{max}$

The time required for the use-dependent depression of the sodium current by a drug to reach steady state depends on the rapidity of dissociation of

this drug from the sodium channel.

This has been tested in studies using protocols designed to determine the rate of onset of frequency-dependent block by a drug (which reflects both its binding and unbinding processes to and from the receptor of the sodium channel) with trains of stimuli at rates rapid enough to induce depression of the sodium current after a quiescent period long enough to ensure complete recovery from any rate-dependent block so that the first response of the train truly represents 0% rate-dependent block. Other protocols include measurements of the kinetics of recovery from frequency-dependent block (reflecting only the unbinding process from the receptor of the sodium channel) with single stimuli at diastolic intervals of increasing duration following a train of stimuli. This has been confirmed, among others, for lidocaine, classified as a drug with "fast" kinetics (Hondeghem & Katzung, 1980; Bean *et al*, 1983; Campbell, 1983a; Sánchez-Chapula *et al*, 1983; Varró *et al*, 1985; Matsubara *et al*, 1987) and for quinidine, classified as a drug with "intermediate" kinetics (Hondeghem & Katzung, 1980; Grant *et al*, 1982; Weld *et al*, 1982; Campbell, 1983a; Varró *et al*, 1985).

1.4.2.2. Synergistic combinations

Combinations of drugs with different kinetics of interaction with the sodium channel (e.g. one drug with fast kinetics and one drug with slower kinetics) can achieve more block of early premature responses than either drug alone while not affecting normal conduction beyond what is seen with the drug with slower kinetics.

This prediction is based on the possibility of synergism between Class

I drugs to achieve a degree of diastolic block not attainable with either agent alone. Increased block of extra-systoles during early diastole is based on additive interaction of the two drugs in depressing the sodium current.

The principle of combining two drugs with dissimilar kinetics of interaction with the sodium channel receptor also involves minimal probability for additional toxicity through excessive depression of normal beats, which would be expected from a combination of two drugs from the same subclass. Since the block by the fast drug would recover during the course of diastole, only the drug with slower kinetics would contribute to the block of extra-systoles during late diastole.

Confirmation of this prediction may also be viewed as an evidence arguing in favor of the presence of an unique specific binding site for all Class I antiarrhythmic drugs, functionally associated with the sodium channel.

1.4.2.3. Antagonistic combinations

Agents with fast kinetics can competitively displace a slower agent, at appropriate cycle lengths of activity.

This prediction implies the possibility that Class I antiarrhythmic drugs can alternatively interact in a competitive manner under conditions where the drug with fast kinetics is present in high enough concentration to block a significant fraction of the sodium channel population. When drug concentrations are low, mixing two drugs is only expected to increase the degree of block, since the probability that the two agents interact simultaneously with the same receptor is relatively low. However, if drug concentrations are raised to levels at which each drug occupies a large fraction of channels, then each of the two drugs may interfere

with the other's binding. If both drugs have similar rates of association and dissociation, then competition between them will be of little electrophysiological consequence. Displacement is facilitated if one drug binds to the receptor of the sodium channel faster than the other. The greater the difference in kinetics of interaction, the greater the ability of the fast drug to significantly alter the binding of the slow drug.

Competitive displacement has been tested with protocols involving recovery from frequency-dependent block and steady state $\dot{V}_{max}$ depression over a range of cycle lengths for the combination of bupivicaine and lidocaine plus the combination of quinidine and lidocaine (Clarkson & Hondeghem, 1985) and the combination of aprindine and lidocaine (Kodama *et al*, 1987). Sánchez-Chapula (1985b) found competitive interactions between lidocaine and benzocaine, but such that lidocaine was displacing benzocaine. This result could possibly be explained by the very high concentration of benzocaine used in his study (200 μM) and only 30 μM of lidocaine. He nevertheless concluded that the two drugs interacted with the same receptor.

1.4.3. Tonic versus phasic block

Two types of $\dot{V}_{max}$ depression have been defined by the modulated receptor hypothesis and described experimentally, viz. tonic and phasic block (Hondeghem, 1987).

Tonic block results from the block of sodium channels under rest conditions. It is experimentally measured as the reduction of sodium permeability during the first depolarization after a long rest period (Bennett, 1987). Tonic block

is not restricted to the block of channels in the resting state, although this subpopulation of channels prevails during diastole. It may include as well the block of open or inactivated channels. Block of open channels that develops before the peak sodium conductance (experimentally measured as $\dot{V}_{max}$) will appear as tonic block. However, when studying the effects of therapeutic concentrations of antiarrhythmic drugs (μM range), there is usually little tonic block at normal membrane potential, as noted by Hondeghem & Katzung themselves (1984). Affinity of the resting state of the sodium channel for the Class I antiarrhythmic drugs is often too low to be measured accurately at therapeutic concentrations.

On the other hand, phasic block refers to the additional drug-induced reduction of sodium channel availability during repetitive depolarizations, i.e. the use-dependent or frequency-dependent block. Phasic block results from accumulation of drug-associated sodium channels during a train of action potentials when the diastolic interval is too short to allow complete recovery of $\dot{V}_{max}$. It is dependent upon the activity of the channel, and therefore, reflects the block of open and/or inactivated channels. The balance between the rate of development of block during an action potential and the rate of recovery from block during diastole determines the degree of sodium current inhibition. According to Hondeghem & Katzung (1984), all Class I antiarrhythmic drugs will exhibit phasic block under appropriate conditions, i.e. when tonic block is maintained at a low level and diastolic interval is shorter than the recovery time (4-5 time constants).

Phasic block by antiarrhythmic drugs can be directly influenced by the extent to which they also induce tonic block. For example, one study (Lee *et al*, 1981) reports very minimal phasic block by lidocaine and quinidine (20 μM) at 5 Hz, i.e. 10% and 8% respectively and concluded that phasic block contributes very

little to the overall sodium current blockade by these drugs. These results, which depart from those from the modulated receptor hypothesis (Hondeghem & Katzung, 1977), could be explained in terms of their experimental conditions which induced large amounts of tonic block, 40% and 60% respectively. This reinforces the claim of Hondeghem & Katzung (1984) that under conditions of great tonic block only limited use-dependent block can occur.

2. *DRUG COMBINATIONS*

In the treatment of arrhythmias, combination of antiarrhythmic drugs are often used when single agents prove to be ineffective. This concept is not new (Bigger & Giardina, 1974) and is now a common clinical practice (Coumel *et al*, 1985a; Lévy, 1988). However, a lot of inconsistency remains regarding the rationales invoked for using drug combinations as well as for the selection of drugs to be used in combinations. In addition, while the benefit of such an approach has been established in the treatment of supraventricular arrhythmias, the advantages of combination therapy for ventricular arrhythmias are somewhat more controversial (Greenspan *et al*, 1986).

2.1. Use of Combination Therapy in Clinical Medicine

Some of this controversy about the value of combination therapy arises from discrepancies among investigators about the concept of efficacy of treatment. The studies comparing the efficacy of combination therapy versus that of monotherapy can be divided into two main groups. First, there are the studies quantifying the decrease in the number of premature ventricular contractions and arrhythmia runs (defined as more than three consecutive premature ventricular contractions), either by 24-48 hour ambulatory monitoring or electrocardiogram

during hospitalization (Duff *et al*, 1983; Kim *et al*, 1985; Kim *et al*, 1987; Klein *et al*, 1987; Barbey *et al*, 1988; Patt *et al*, 1988; Tanabe *et al*, 1988; Kim *et al*, 1989). For most of these authors, success of the treatment implies a reduction in the number of premature ventricular contractions by at least 70%. Secondly, there are studies using the induction of arrhythmias with electrical programmed stimulation (Ross *et al*, 1982; Duffy *et al*, 1983; Greenspan *et al*, 1985; Kim *et al*, 1986; Duff *et al*, 1987; Whitford *et al*, 1988). For these authors, success of the treatment resides in a greater difficulty in induction of ventricular arrhythmias, either through an increase in the number of stimuli required for the induction, or a lower incidence of sustained ventricular arrhythmias. Different electrophysiologic measurements are used by the two groups, viz QTc, QT interval prolongation, effective refractory period, QRS prolongation, cycle length of arrhythmia runs, coupling interval of extra-systoles. Furthermore, heterogeneity in patient populations (normal people versus those with cardiovascular disease) makes it even more difficult to get a clear picture from these studies.

With such a wide spectrum of parameters to describe response to combination therapy, it is not surprising that conclusions of these studies range from poor, or no better than monotherapy (Ross *et al*, 1982; Duffy *et al*, 1983), to more effective than monotherapy (Duff *et al*, 1983; Greenspan *et al*, 1985; Kim *et al*, 1987; Klein *et al*, 1987; Patt *et al*, 1988; Kim *et al*, 1989).

The mechanisms of drug interactions are diverse and complex. As in other situations, drugs, when administered simultaneously for the treatment of arrhythmias, can interact by modifying each other's pharmacokinetic or pharmacodynamic profile of action (Bigger & Giardina, 1984).

2.1.1. Pharmacokinetic interactions

Pharmacokinetic interactions between antiarrhythmic drugs are not the main rationale for selecting the best candidates for combination therapy. These interactions nevertheless have to be taken into consideration as any change in absorption, distribution, or metabolism will affect the overall effect of a drug.

One aspect of particular pharmacokinetic relevance in antiarrhythmic therapy is the formation of active metabolites. Indeed, the formation of pharmacologically active metabolites is known to occur frequently with antiarrhythmic drugs (Woosley & Roden, 1983). Metabolic alteration of these drugs may produce compounds with enhanced, or different, pharmacological properties. Determination of the modulatory role of metabolites on the effect of a drug requires knowledge of any interaction between a metabolite and its parent drug. A metabolite may modify the effect of its parent drug (cardiac and noncardiac effect) or alternatively, may directly contribute to its clinical efficacy (Kates, 1984). In many cases, metabolites are closely related structurally to the parent compound. In these cases, they can be expected to interact with the same receptor as the parent drug and possibly compete for receptor occupancy. Such situation would alter the efficacy of the parent drug (Bennett, 1987).

Two important factors in the assessment of the clinical importance of antiarrhythmic drug metabolites are their intrinsic pharmacologic activity and the extent of their accumulation (Kates, 1986). Differences in the disposition kinetics between the parent drug and metabolite will influence the degree to which each accumulates. An extremely variable relation between plasma drug concentration and effect should raise the possibility that active metabolites may be present (Woosley & Roden, 1983).

The formation of active metabolites is one important factor that may deviate the outcome of combination therapy from the simple extrapolation of superimposed individual drug effects. Awareness of the possibility of such alteration (whether beneficial or detrimental) is essential for an adequate selection of drugs to be used in combination.

2.1.2. Pharmacodynamic interactions

Antiarrhythmic drugs have been used in combination for the benefit of their pharmacodynamic interactions. These effects do not necessarily depend on a significant change of the plasma concentration of a drug and are often the result of an altered membrane activity (Bigger & Giardina, 1984).

First, complementarity of electrophysiologic effects led to the practice of combining antiarrhythmic drugs from different classes. Most frequently used are the combinations of a Class I drug with a Class II drug (a β-blocker). The success of treatment in such instances is well established and has been reported safe and effective (Deedwania *et al*, 1987; Lévy, 1988).

Also, whenever the development of dose-related side effects prevented the maintenance of plasma levels adequate for arrhythmia suppression with one particular agent, the combination of this agent with another antiarrhythmic drug associated with different side effects could enable additive therapeutic effects without additive adverse effects. The success of the therapy therefore depends primarily on whether a therapeutic control of arrhythmias can be achieved with smaller and better tolerated doses of each individual drug: the aim is

to decrease the effective dose (Coumel *et al*, 1985a). In such cases, studies using two Class I antiarrhythmic drugs of the same subclass have claimed better therapeutic effects and less adverse effects than either drug alone (Baker *et al*, 1983; Kim *et al*, 1985). The selection of drugs is based on avoiding similar adverse effects that could possibly be additive when used in combination (Kim *et al*, 1987).

2.2. *The Modulated Receptor Hypothesis as a Rationale for Combination Therapy*

I am not aware of any *in vitro* study that investigated a combination of two sodium blockers from different sub-classes for the purpose of an antiarrhythmic benefit published prior to Hondeghem & Katzung (1980). Huang & Ehrenstein (1981) & Ehrenstein & Huang (1981) used combinations of local anesthetics (QX 572 and benzocaine) for the principle of decreasing side effects by reducing the required dose, while maintaining an optimal level of desired effect. However, this hypothesis was based on a model of two separate binding sites within the sodium channel (one for charged molecules and one for neutral molecules).

The work of Hondeghem & Katzung (1977, 1980) and the Harrison modification of the Vaughan Williams classification (Harrison *et al*, 1981; Campbell, 1983a) offered a new and more rational approach in justifying the use of drug combinations, by predicting that some combinations of Class I antiarrhythmic drugs might have synergistic antiarrhythmic activity. It is the joint theoretical and experimental work of these investigators that led to the proposal of a new theoretical basis for the selection of agents to be combined in therapy.

Following Hondeghem & Katzung (1980), some *in vitro* studies tested the interaction of Class I antiarrhythmic drugs when used in combination. In a study on guinea-pig papillary muscle, Kohlhart & Seifert (1985) concluded that

simultaneous application of drugs with similar kinetics of interaction with the sodium channel (quinidine and propafenone) intensified $\dot{V}_{max}$ blockade as would an increase in drug concentration and that combining kinetically different Class I compounds (lidocaine and propafenone) modified $\dot{V}_{max}$ depression. This modification was strongly dependent on the concentration of lidocaine and the inter-stimulus interval between the conditioning and the test pulses. A low concentration of lidocaine seemed to act additively with propafenone whereas in the presence of a high concentration of lidocaine, the propafenone block fraction declined. Fransen *et al* (1984) and Sánchez-Chapula (1985b) found a competitive interaction between lidocaine and q.. nidine on guinea-pig papillary muscle, but used high concentrations of the two drugs that would be expected to show competitive displacement. All these studies concluded that their results were compatible with the modulated receptor hypothesis of Hondeghem & Katzung (1977, 1980), and confirmed the probability of a common receptor site for Class I antiarrhythmic drugs within the cardiac sodium channel.

Carmeliet (1985) unexpectedly found competitive interaction between quinidine and lidocaine with rather low concentrations (2 mg/l) in a voltage clamp study on rabbit Purkinje fibers. Lidocaine apparently prevented quinidine from retarding the recovery of blocked channels to the same extent as quinidine alone. Although the author recognized that his preliminary results differed from those of Hondeghem & Katzung (1980), no hypothesis was offered for this discrepancy.

These studies support the concept that some combinations of Class I agents can be antagonistic or additive under appropriate circumstances. Identification of the conditions under which the interaction between two drugs

could be of either nature can help to make full use of their potential usefulness in antiarrhythmic therapy. Clear recognition of the cellular basis for interaction of Class I antiarrhythmic agents is mandatory if rationale is ever to replace empirism in the selection of drugs to be used in combination therapy for the management of cardiac arrhythmias.

3. TRIGGERED ACTIVITY

The usefulness of antiarrhythmic drugs is often limited by their proarrhythmic effects. Although most of the side effects that are related to plasma blood levels are predictable, those that are not related to drug level are rather idiosyncratic and unpredictable (Podrid, 1984). Such proarrhythmic effects seen under quinidine therapy are usually due to its ability to delay repolarization, thereby prolonging the QT interval. Abnormalities of ventricular repolarization are associated with polymorphic arrhythmias called "torsade de pointes". The mechanism responsible for torsade de pointes has not been established with certainty, but one model is triggered activity due to early afterdepolarizations. While information linking drug-induced torsade de pointes to prolonged repolarization is well documented, the links between early afterdepolarizations and torsade de pointes are somewhat more tenuous (Rosen & Wit, 1987).

3.1. Description

Triggered activity is a mechanism of cardiac arrhythmias based on abnormal impulse generation. It is dependent on the formation of afterpotentials in the heart. Afterpotentials are "non-driven" potentials that arise from an afterdepolarization following a preceding action potential. The features and behavior of triggered activity are distinct from the two other, more classical,

arrhythmia mechanisms of reentry and enhanced automaticity. Triggered afterpotentials differ from abnormal repetitive activity arising from enhanced automaticity in that they never arise spontaneously. They are dependent upon a preceding action potential and can give rise to "non-driven" action potentials (Cranefield, 1975; Cranefield, 1977).

Triggered activity can be divided into two main subclasses: 1) early afterdepolarizations (EADs), which occur before membrane potential has normally returned to the same level as prior to the upstroke of the preceding action potential and therefore, interrupt the repolarization phase of this action potential, and 2) delayed afterdepolarizations (DADs), which occur only once membrane potential has normally returned to the same level as prior to the upstroke of the preceding action potential, and therefore follow the repolarization phase of this action potential.

The main distinction (and probably the most relevant in arrhythmogenesis) between these two types of afterdepolarizations is their response to frequency of activity. The manifestation of EADs is bradycardia-dependent, i.e. they are suppressed by rapid activity whereas that of DADs is tachycardia-dependent, i.e. they are enhanced following rapid activity.

Initially, triggered activity attracted attention mostly through the study of DADs. Until more recently (Wit & Rosen, 1986; Cranefield & Aronson, 1988b), reviews on triggered activity would briefly describe EADs, while concentrating on DADs (Wit *et al*, 1980; Rosen & Reder, 1981). Indeed, DADs have been more extensively studied; they are presumed to be the underlying cellular mechanism for cardiac glycoside toxicity (Ferrier, 1977). The ionic

mechanism of digitalis-induced DADs has been the subject of intense investigation (January & Fozzard, 1988). Briefly, digitalis inhibits the electrogenic pump through blockade of the enzyme Na^+-K^+ ATPase, which leads to an accumulation of sodium inside the cell. This influences the Na^+-Ca^{2+} exchange mechanism to raise the intracellular calcium concentration. The calcium concentration is further increased by calcium release from the sarcoplasmic reticulum and mitochondria. The increase in intracellular calcium triggers a transient inward current carried mainly by Na^+, leading to DADs. An essential feature of this mechanism is the requirement for intracellular calcium overload for the induction of DADs. However, more study is needed to solve whether sarcolemmal calcium entry or intracellular calcium release plays the central role (Marban *et al*, 1986).

On the other hand, the understanding of EADs is still far from being complete. Although these could be experimentally induced *in vitro* and *in vivo* by several means (see below), their specific ionic mechanism has not been characterized. Confusion concerning their mechanism and ionic nature still remains. The types of arrhythmias that might be explained by EADs are those that arise as a result of the prolongation of the action potential and bradycardia. EAD-induced triggered activity has been implicated as the cellular basis of the acquired long QT syndrome leading to the bradycardia-dependent polymorphic arrhythmia called torsade de pointes (Brachman *et al*, 1983; Roden & Hoffman, 1985).

EADs share some of their properties with DADs and some others with activity generated from enhanced automaticity. EADs usually exhibit a different response to overdrive and premature stimulation than DADs. Their ability to be suppressed by overdrive and to be reset is a behavior more like that of automatic arrhythmias. However, unlike automaticity, once terminated, they

cannot reinitiate arrhythmias *de novo*. In their dependence on a preceding action potential to induce them, these are more similar to DADs than to automaticity. However, as of today, we are still missing an unique characteristic that could positively distinguish an EAD-induced triggered rhythm *in vivo* from any other arrhythmia mechanism.

While a DAD represents a membrane activity with a precisely characterized appearance, an EAD in turn, may include a full spectrum of protean events.

Cranefield (1975) first described EADs as "a depolarizing afterpotential that begins prior to the completion of repolarization and causes (or constitutes) an interruption or retardation of normal repolarization". EADs can evoke or be followed by either an arrest of repolarization at a relatively positive stable level of resting potential, or a second upstroke, or a burst of rhythmic activity in the same range of membrane potentials. Later, Coulombe *et al* (1985) have described EADs as "alterations of the repolarization phase of the Purkinje fiber action potential, corresponding to humps of variable size occurring on the falling phase of the response, which when large enough can trigger single or multiple re-excitations".

EADs *per se* do not necessarily involve a positive slope of depolarization (upstroke) during the repolarization phase whereas the triggered activity induced by these EADs, termed triggered action potentials or triggered responses, necessarily involve an upstroke (depolarizing phase).

3.2. In Vitro Models of EAD-Induced Triggered Activity

Interventions that can induce EAD-dependent triggered activity share the common property of prolonging the action potential duration, and to do so to a greater extent at slower rates of activity.

One group of compounds that is known to induce EADs and/or triggered responses *in vitro* is that of drugs of clinical use. This group includes 1) antiarrhythmic drugs with Class III action: N-acetyl-procainamide (Dangman & Hoffman, 1981), clofilium (Gough & El-Sherif, 1988), sotalol (Strauss *et al*, 1970; Lathrop, 1985), bretylium (Gough & El-Sherif, 1989); 2) Class Ia antiarrhythmic drugs with Class III action: quinidine (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Davidenko *et al*, 1989; Kaseda *et al*, 1989) and disopyramide (Sasyniuk *et al*, 1989); 3) Class IV antiarrhythmic drugs with Class III action: bepridil (Campbell *et al*, 1985); 4) diuretics: amiloride (Curtis Marchese *et al*, 1985); 5) serotonergic blocking drugs: ketanserin (Zaza *et al*, 1989); 6) catecholamines (Brooks *et al*, 1955; Hoffman & Cranefield, 1960; Kupersmith & Hoff, 1985; Mendez & Delmar, 1985).

A second group of drugs comprises the non-clinical substances. In this group are found 1) veratrin (Goldenberg & Rothberger, 1936); 2) tetraethylammonium (Ito & Surawicz, 1977; Aronson, 1981); 3) aconitine (Leichter *et al*, 1986; 4) Bay K 8644 (January *et al*, 1988; January & Riddle, 1989); 5) batrachotoxin (Brown, 1983); 6) anthopleurin-A (El-Sherif *et al*, 1988); 7) 4-aminopyridine (Graham *et al*, 1989; Kaseda *et al*, 1989); 8) caffeine (DiGennaro & Vassalle, 1985; Cranefield & Aronson, 1988a).

A third group of agents are the inorganic ions. These comprise 1)

cesium (Brachman *et al*, 1983; Damiano & Rosen, 1984; Marban *et al*, 1986; Bailie *et al*, 1988); 2) barium (Mugelli *et al*, 1983; Ino et al, 1988); 3) nickel (Kupersmith & Hoff, 1985).

Other changes in experimental conditions can also facilitate the emergence of triggered activity. These are 1) acidosis through high levels of carbon dioxide (Coraboeuf *et al*, 1980; Coulombe *et al*, 1985); 2) cooling (Kupersmith *et al*, 1986); 3) low potassium concentration (Carmeliet, 1961; Liu, 1989); 4) low calcium concentration (Sano & Sawanobori, 1972; Li *et al*, 1988); 5) low oxygen levels (Trautwein *et al*, 1954); 6) injury (Arnsdorf, 1977).

Favoring conditions for triggered activity caused by EADs *in vitro* are identical to the conditions that predispose to arrhythmias occurring in the setting of the acquired long QT syndrome: bradycardia, hypokalemia, hypomagnesemia, hypocalcemia, drugs that prolong the action potential duration. Similarly, both EAD-induced triggered activity and these arrhythmias are abolished by the same interventions: high potassium concentration, high magnesium concentration, pacing, drugs that shorten the action potential duration.

The contribution of EAD-induced triggered activity in the mechanism of arrhythmias related to the long QT syndrome is still poorly understood. Exactly how the occurrence of a triggered action potential at the cellular level may initiate and/or sustain these arrhythmias remains to be elucidated.

3.3. In Vivo Models of EAD-Induced Triggered Activity

Experimental studies in which monophasic action potentials were obtained from endocardial and the epicardial surfaces of the heart *in situ*, have

shown triggered activity which bears some resemblance to that recorded with intracellular microelectrode recordings from *in vitro* preparations (Brachman *et al*, 1983; Levine *et al*, 1985; Bailie *et al*, 1988; Ben-David & Zipes, 1988; El-Sherif *et al*, 1988; Hanich *et al*, 1988; Ino *et al*, 1988; Graham *et al*, 1989).

More particularly, Levine and collaborators (1985) performed extensive measurements to support the validity of monophasic action potentials in the study of triggered activity with a model of cesium-induced arrhythmia in the anesthetized dog. Their most convincing evidence is the correlation between the coupling interval of the afterdepolarizations on the monophasic action potential and the coupling interval of the premature beat on the electrocardiogram (see their Figure 10). Ino *et al* (1988) concluded that abnormal activity induced by barium superfusion can be accurately detected on monophasic action potentials and that their extracellular recordings with a contact electrode reflect "with reasonable accuracy" the activity of the preponderant cell population underlying this electrode.

Brachman *et al* (1983) and El-Sherif *et al* (1988) reported experimental arrhythmias of some similarity with the drug-induced clinical arrhythmias; however, instead of therapeutic agents, these investigators used cesium (Brachman *et al*, 1983) and anthopleurin-A (El-Sherif *et al*, 1988). These studies, combining *in vitro* and *in vivo* experiments of similar conditions, have shown that the same interventions will induce or abolish both the *in vivo* and the *in vitro* triggered activity. Furthermore, EADs and polymorphic arrhythmias are abolished with the same time sequence by the usual interventions that are used in the clinical setting. El-Sherif *et al* (1988) have used three different types of recordings to study anthopleurin-A induced arrhythmias *in vivo*: transmembrane epicardial recording, endocardial and epicardial monophasic action potential

recordings, and the surface electrocardiogram. EADs and triggered action potentials on transmembrane or monophasic action potentials correlated with the U waves on the ECG. Moreover, they were always of bigger size on the endocardial than on the epicardial surface, suggesting a possible importance of the subendocardial system. However, the arrhythmias induced with this model were not necessarily bradycardia-dependent, sometimes occurring without the classic slowing of heart rate that had become the clinical marker of torsade de pointes.

Even though monophasic action potentials are considered to be a fairly good measure of the duration of ventricular activity, the reliability of this extracellular recording technique is in question for both the *in vitro* and the *in vivo* preparations. Monophasic action potentials, recorded with either contact unipolar electrodes or suction electrodes, have been reported to present motion artifacts, which could interfere with proper interpretation of experimental results (Hoffman *et al*, 1959; Olsson *et al*, 1985). Moreover, Gough & Henkin (1989) recently concluded that artifact EADs may appear on monophasic action potential records whether or not actual EADs are present. Suction electrodes are known to induce injury at the point of contact and the activity recorded with this technique is produced by this injury current. Monophasic action potentials will last only as long as this injury; at the point necrosis is starting, recording with monophasic action potentials is no longer possible. Therefore, the feasibility of this technique is very transitory.

Another problem with this technique arises if triggered activity only occurs *in vivo* in isolated foci, rather than the overall cardiac mass. The ability of EADs to be expressed on the surface ECG will be significant only if they are to occur in a fairly large mass of tissue rather than in isolated foci (Cranefield &

Aronson, 1988b). Therefore, if they originate in isolated foci, they have to have the ability to reexcite the myocardial fibers if they are to cause arrhythmias and be identified on surface recordings (Wit & Rosen, 1986). If this is the case, a proper use of extracellular electrodes would involve the identification of the location of such foci (Wit & Rosen, 1986). A recent study by El-Sherif *et al* (1988) reports that the amplitude of EADs on monophasic action potentials recorded from the endocardial surface of a patient varies from one recording site to another. They claim that the most prominent EADs were recorded from the posterior paraseptal region. On the other hand, Levine *et al* (1985) reported that the location of the electrode did not make any difference in the recording with their cesium model.

One argument against the validity of the cesium *in vivo* model has arisen with the study by Nayebpour *et al* (1989) who reported that the characteristics of cesium-induced arrhythmias, whether administered by bolus or infusion, could be very different from those induced by the acquired long QT syndrome. These disparities include different profiles of serum electrolyte concentrations (potassium levels being much higher in their study), different response to overdrive pacing and the different frequency of occurrence of ventricular fibrillation. Therefore, they recommended caution in the extrapolation of conclusions from the cesium-induced arrhythmia models to the clinical arrhythmias secondary to the acquired long QT syndrome.

Some clinical studies have also implicated EADs in the initiation and occurrence of polymorphic arrhythmias (Luca, 1977; Gavrilescu et Luca, 1978; Bonatti *et al*, 1979; Jackman *et al*, 1984; Bonatti *et al*, 1985; El-Sherif *et al*, 1989). In the study from the Bonatti group (1985), repetitive torsade de pointes activity recorded on surface ECGs of patients with a long QT syndrome of various etiology

"started in very close synchrony" with repetitive activity associated with an EAD on the monophasic action potential recording.

In summary, the major contribution of *in vivo* models in this field has been to show emergence of deflections on the surface electrocardiogram and extracellular recording, which could be EADs, under conditions which induce polymorphic ventricular arrhythmias as well as disappearance of such deflections under conditions which abolish the same arrhythmias.

3.4. Possible Ionic Mechanisms of EAD-Induced Triggered Activity

Little is known about the ionic mechanisms that cause EADs and triggered responses. Several current components are involved in the repolarization phase of the cardiac action potential and it is likely that they all contribute in combination to different degrees of importance.

One of the determinants of the level of membrane potential at which a triggered action potential takes off is the contribution of the ionic current(s) involved in its upstroke. This parameter has been termed the activation voltage. The activation voltage is determined indirectly by the blockade of all the outward currents whose balance of time- and voltage-dependence determines the shape of repolarization and also by the inward current(s) directly involved in the depolarizing phase of the triggered response.

The net depolarizing current of the upstroke of the triggered response has to result from imbalance between outward and inward membrane currents during the course of the repolarization phase of the action potential. Any

intervention that will produce an inward shift of the current-voltage relationship over the proper range of membrane potentials will favor the appearance of EADs. Because of the high membrane resistance over this crucial range of membrane potentials, little changes in current could produce a large effect.

As stated previously, not all substances that induce EADs and the triggered responses they induce do so through the same ionic mechanism; these substances nevertheless give rise to EAD-induced triggered activity which all share some common basic characteristics. Substances with very specific effects on different currents can induce repolarization abnormalities. This shows that, with the diversity of ionic currents in activity during repolarization, each of them can individually affect the repolarizing process to a great extent.

The block of the inwardly-rectifying potassium current i_{K1} by cesium has been shown to induce EADs and triggered action potentials (Damiano & Rosen, 1984; Marban *et al*, 1986). The specific block of the delayed rectifier potassium current i_k by tetraethylammonium (Ito & Surawicz, 1977) or WY-48986 (Sasyniuk *et al*, 1989) has also been shown to induce EADs and triggered activity.

Likewise, substances which enhance inward currents flowing during repolarization are known to produce EAD-dependent triggered activity in Purkinje fibers. Anthopleurin-A most likely acts by delaying sodium inactivation as it does in nerve (Low *et al*, 1979). Bay K 8644 is a calcium agonist and likely induces EAD-induced triggered activity by producing a voltage-dependent increase in macroscopic calcium current through a prolongation of the mean open time of L-type calcium channels (Kokubun & Reuter, 1984).

3.5. Features of Torsade de Pointes Bearing a Mechanistic Relevance

Torsade de pointes is a ventricular arrhythmia that could be caused by many different drugs and pathological states (Keren *et al*, 1981; Stern *et al*, 1984; Roden *et al*, 1986b; Stratmann & Kennedy, 1987). It has been reported at all ages (Dessertenne, 1966; Finley *et al*, 1978), and equally in both sexes (Smith & Gallagher, 1980).

The name "torsade de pointes" comes from the main electrocardiographic feature of this arrhythmia (Dessertenne, 1966): during the acute episodes, the QRS complexes twist around the isoelectric baseline in an undulating sinusoidal fashion; their polarity and amplitude constantly change, which confers its polymorphic nature. It may either terminate spontaneously or degenerate into ventricular fibrillation. The most striking features of torsade de pointes are that it is bradycardia-dependent and that it usually occurs in the setting of a long QT interval, which could be either acquired or congenital. The QRS complexes are usually within the normal range. Its initiating electrocardiographic sequence is also very peculiar and has been described by Kay *et al* (1983), Jackman *et al* (1984, 1988), Roden *et al* (1986a), and Cranefield & Aronson (1988a,b). The common point of all these descriptions consists of a series of short-long-short R-R intervals, the last of which is the first beat of the tachycardia.

The clinical conditions most commonly associated with torsade de pointes are slow heart rates, electrolyte imbalance (mainly potassium, magnesium, and calcium), and antiarrhythmic drugs which delay repolarization. These conditions all share the property of prolonging the QT interval. Despite the variety of conditions that may lead to the manifestation of torsade de pointes in an individual, quinidine is recognized as the most common culprit (Roden *et al*, 1986b;

Stratmann & Kennedy, 1987; Jackman *et al*, 1988).

The cellular mechanism of polymorphic arrhythmias has been a matter of debate for a long time. Part of this debate comes from its very definition: on one side, a long QT interval is as a mandatory criterion for the diagnosis of the arrhythmia (Ranquin & Parizel, 1977; Smith & Gallagher, 1980; Keren *et al*, 1981; Soffer *et al*, 1982; Kim & Chung, 1983; Tzivoni *et al*, 1983; Stern *et al*, 1984; Lévy, 1985) whereas on one other side, whatever looks polymorphous on the surface electrocardiogram could simply be diagnosed as torsade de pointes, regardless of the QT interval (Sclarovsky *et al*, 1979; Zilcher *et al*, 1980; Coumel *et al*, 1985b; Horowitz, 1985; Nguyen *et al*, 1986). This controversy has complicated the interpretation of many clinical and laboratory studies which have tried to solve the mechanistic issue.

In the early years following the identification of torsade de pointes as a separate electrocardiographic entity, the very original thinking was toward two ectopic foci alternatively competing for the control of the ventricles (Dessertenne, 1966). Later on, as people linked the major disturbances of repolarization (regardless of the etiology) with the risk of developing torsade de pointes, reentry was considered more seriously as a potential candidate (Brochier & Fauchier, 1978; Sclarovksy *et al*, 1979; Smith & Gallagher, 1980; Aliot *et al*, 1982; Stern *et al*, 1984). Fontaine *et al* (1982) have noticed that torsade de pointes is a very short-lived phenomenon when it occurs in the absence of concomitant pathologies susceptible to producing reentry. No study has been able yet to rule out the involvement of reentry, even if their results strongly support multiple ectopic foci (Zilcher *et al*, 1980; D'Alnoncourt *et al*, 1982) or triggered activity (Brachman *et al*, 1983).

However, arrhythmogenic mechanisms are not necessarily mutually exclusive. Malik & Camm (1986) have shown with a computer model that the presence of one or several ectopic foci, whether or not accompanied by reentry, could produce an electrocardiographic pattern similar to that of torsade de pointes. Whatever is the mechanism of induction, the features of torsade de pointes undoubtedly suggest an interaction between two (or more) different arrhythmogenic areas (Coumel *et al*, 1985b). Two laboratory studies (Bardy *et al*, 1983; Inoue *et al*, 1986) have shown that the twisting of the QRS complexes that is typical of torsade de pointes correlates with a change in the earliest site of ectopic activity.

The most serious drawbacks to this day to the reentry hypothesis are the difficulty of inducing torsade de pointes with programmed electrical stimulation (Wellens & Lie, 1975; Wellens, 1978; Coumel *et al*, 1985b) and its well known abolition by pacing. The few reports who claimed success in inducing torsade de pointes with programmed electrical stimulation (Evans *et al*, 1976; Horowitz *et al*, 1981) have been criticized (Stern *et al*, 1981; Fontaine *et al*, 1982) for using the term "torsade de pointes" inconsistently and too loosely, that is in the absence of the complete list of symptoms that characterize the full syndrome.

According to Coumel *et al* (1985b), torsade de pointes is a puzzling arrhythmia not only by its unique response to treatment, but also because it shares some of its features with the reentry-induced arrhythmias as well as with the triggered activity-induced arrhythmias. Other authors have as well discarded enhanced automaticity as a possibility and focus their discussion to oppose reentry to triggered activity (Cranefield & Aronson, 1989b; Surawicz, 1989).

The prerequisite to implicate triggered activity as the underlying mechanism of an arrhythmia is the presence of a critically prolonged cellular action potential. Bradycardia-dependent arrhythmias certainly fulfill this requirement. However, a link between triggered activity arising from EADs and torsade de pointes, no matter how well documented, would be insufficient in itself to explain the polymorphic appearance of the electrocardiogram during torsade de pointes. Cranefield & Aronson (1988b) and Jackman *et al* (1988) have pointed out that there is no compelling reason why rhythmic activity arising from an EAD would necessarily give rise to a polymorphic pattern of ventricular tachycardia. A "twisting of the points" would likely require that there are more than a single competing focus present. The main characteristics of an arrhythmia caused by EAD-induced triggered activity would be 1) the presence of the short-long-short sequence of cardiac activity just prior to its initiation and 2) overdrive suppression.

Nevertheless, the most recent view of the mechanistic understanding has been focusing on the initiating sequence of arrhythmias from the acquired long QT syndrome (Jackman *et al*, 1984, 1988). This new approach emphasizes that the crucial feature of these arrhythmias is their mode of initiation more than their polymorphic nature. Polymorphic electrocardiographic patterns are more likely to depend on the number or rates of tachycardia generators (reentrant, automatic, or triggered) than on the initiating mechanism. The authors claim that understanding the bradycardia-dependence of TU wave aberrations is the basis to elucidate their etiology. The salient sign announcing impending drug-induced torsade de pointes is either a pause or an abrupt slowing of ventricular rhythm and the first complex of the tachycardia is late coupled, emerging from a large post-pause U wave. Therefore, the important feature of the mechanistic issue has shifted from the polymorphic character of these arrhythmias to their pause-dependence.

According to this view, if laboratory studies (both *in vivo* and *in vitro*) are to successfully address the question of cellular mechanism, they should focus on the initiating sequence of these arrhythmias. This hypothesis of work may confer to EAD-dependent triggered activity an etiological role that goes beyond pause-dependent arrhythmias to include the syndrome described by Coumel *et al* (1985c) as repetitive monomorphic idiopathic ventricular tachycardia. The progression of laboratory work (clinical and experimental) on this phenomenon may well disclose its importance as a major arrhythmia mechanism shared by many subsets of arrhythmias once thought to be completely disparate.

4. STATEMENT OF THE PROBLEM

The first aim of the present thesis project was to study, in canine Purkinje fibers, the frequency and voltage dependent effects of clinically relevant concentrations of quinidine, a Class Ia agent and tocainide, an orally active Class Ib drug, singly and in combination in order to provide a rationale for the increased antiarrhythmic efficacy of a combination of two Class I drugs which interact with the sodium channel with different kinetics. According to the prediction of Hondeghem & Katzung (1980), such a combination should provide a better protection against early premature responses or rapid heart rates than either drug alone, while not depressing normal heart rates beyond the level produced by the drug with slower kinetics. This study is reported in Chapter I.

The second aim was to evaluate the mechanism by which Class Ib drugs prevent quinidine-induced triggered activity. In order to do this, we needed to characterize the precise conditions under which quinidine induced repolarization abnormalities. Roden & Hoffman (1985) had shown that in the presence of

bradycardia and hypokalemia, quinidine induced triggered activity. However, the description of experimental conditions was incomplete. We explored the role of acidosis which had been shown previously to induce triggered activity in the presence of hypokalemia (Coraboeuf *et al*, 1980). This led us to develop a consistent and reproducible *in vitro* model of triggered activity with a combination of therapeutic concentrations of quinidine, slow rates of stimulation, low $[K^+]_o$, and mild acidosis. Such a model allowed us to study the mechanism by which Class Ib drugs abolish triggered activity, in order to assess their usefulness in reversing this arrhythmogenic aspect of quinidine's action. This study constitutes Chapter II.

In the second study, we showed that quinidine-induced triggered activity was highly dependent upon frequency of stimulation. The frequency required to induce the phenomenon would be unlikely to occur clinically. Moreover, the coupling interval of the triggered responses were extremely prolonged. In order to explain these discrepancies from the clinical observations pertaining to the arrhythmia torsade de pointes, we postulated that either triggered activity generated *in vivo* is restricted to a protected parasystolic focus or alternatively, that the presence of catecholamines may allow the phenomenon to be more readily induced *in vivo*. Thus, the aim of the third study was to assess the modulation of quinidine-induced triggered activity by catecholamines. According to this hypothesis, catecholamines should allow *in vitro* triggered activity to occur under conditions more likely to be observed clinically. This study comprises Chapter III.

Triggered activity has been demonstrated to occur in Purkinje fibers (Coraboeuf *et al*, 1980; El-Sherif *et al*, 1988). The ability of EADs to generate arrhythmias therefore relies on re-excitation of the surrounding myocardial tissue.

The study was also designed to investigate the characteristics of the propagation of EADs to ventricular muscle.

These specific aims served the objective to define a meaningful *in vitro* model for the cellular mechanism of quinidine-induced long QT related arrhythmias and to provide further support for the involvement of EAD-induced triggered activity in their initiation and occurrence.

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CHAPTER I

MODIFICATION OF THE FREQUENCY AND VOLTAGE DEPENDENT EFFECTS OF QUINIDINE WHEN ADMINISTERED IN COMBINATION WITH TOCAINIDE IN CANINE PURKINJE FIBERS

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ABSTRACT

Frequency and voltage dependent modification of drug-induced $\dot{V}_{max}$ inhibition by the combined administration of two Class I antiarrhythmic drugs was studied in canine Purkinje fibers, taking depression of upstroke velocity as an indicator of sodium channel blockade. The kinetics of onset of drug-induced $\dot{V}_{max}$ depression and the time course of $\dot{V}_{max}$ recovery were studied following exposure to therapeutic concentrations of tocainide (50 μM) and quinidine (5 μM) both singly and in combination. The rate constant for onset of block during a drive train at a cycle length of 600 msec was 0.95 ± 0.32 pulses in the presence of tocainide and 5.61 ± 0.50 pulses in the presence of quinidine. The magnitude of block was three times greater with quinidine than with tocainide. The magnitude of block produced by the combination was no greater than that produced by quinidine alone and may be partly due to abbreviation of action potential duration by tocainide. Onset of block in the presence of the combination was best fitted by a double exponential with rate constants of 0.88 ± 0.19 and 6.47 ± 1.36 pulses. $\dot{V}_{max}$ recovery following termination of a rapid train of impulses was delayed by both drugs. Post-stimulation recovery from either tocainide or quinidine induced block was characterized by a single time constant (1.04 ± 0.49 and 4.81 ± 0.76 sec, respectively) while that of the combination was characterized by two time constants (0.43 ± 0.22 and 5.94 ± 0.56 sec) presumably corresponding to dissociation of each drug from the sodium channel receptor. The mixture of the two drugs produced a large depression of $\dot{V}_{max}$ of early diastolic premature responses without producing much further depression of $\dot{V}_{max}$ than that produced by quinidine alone at longer coupling intervals. The time constant of recovery from tocainide induced block was greatly dependent upon membrane potential. Following steady-state changes in

frequency, the combination produced a greater depression of $\dot{V}_{max}$ at rapid heart rates compared to quinidine alone but abbreviated action potential duration more at slower heart rates. Addition of tocainide to fibers equilibrated with quinidine shifted the $\dot{V}_{max}$-membrane potential relationship to more hyperpolarized potentials resulting in greater depression of $\dot{V}_{max}$ at more depolarized membrane potentials with little or no additional depression of $\dot{V}_{max}$ at more negative membrane potentials. The results provide a rationale for a possible enhanced antiarrhythmic efficacy of a combination of two Class I drugs which have different kinetics of interaction with the sodium channel.

INTRODUCTION

A decrease in the maximal upstroke velocity of the action potential ($\dot{V}_{max}$) leading to a decrease in conduction velocity is characteristic of the action of Class I antiarrhythmic drugs. It is now recognized that these drugs have fairly complex effects on myocardial conduction via their modulated effects on sodium channels (Hondeghem & Katzung, 1977; Grant *et al*, 1984; Hondeghem & Katzung, 1984). Sodium channel block by Class I antiarrhythmic drugs is modulated by the rate of use of the channel. Rapid trains of action potentials or depolarizing pulses will enhance channel block above the basal (resting) level. After such conditioning trains channel block relaxes back to the basal level with widely differing time constants. This rate of unblocking, which is dependent upon drug structure and its associated size and lipid solubility, helps determine what action potential frequencies an excitable cell can follow during drug treatment (Courtney, 1980a; Campbell, 1983a). Drugs of low molecular weight and high lipid solubility (e.g.

lidocaine) tend to have rapid rates of unblocking (Courtney, 1980a; Courtney, 1984). Such drugs would tend to show relative selectivity for depressing early premature impulses or those elicited at rapid rates. Drugs with slower rates of unblocking (e.g. quinidine) would tend to be more nonselective in their depressant effects over a relatively wide range of stimulation frequencies or premature intervals. Hordeghem & Katzung (1980, 1984) have suggested that a combination of two Class I agents with different rates of unblocking, such as lidocaine and quinidine, might provide a more effective depression of early premature impulses or those elicited at rapid heart rates than either drug can achieve alone while not depressing conduction at normal heart rates beyond that produced by the drug with slower kinetics. Such a combination may be desirable for the treatment of re-entrant arrhythmias. In fact, Duff *et al* (1983) showed that a combination of mexiletine and quinidine was more effective against ventricular arrhythmias than either drug alone.

Tocainide is an orally active lidocaine congener. If this drug interacts with the sodium channel in a manner similar to that of lidocaine, then a combination of tocainide and quinidine might be a rational approach for the chronic control of re-entrant ventricular arrhythmias. Furthermore, tocainide might also be beneficial in preventing some of the toxic manifestations associated with quinidine therapy by preventing quinidine induced prolongation of repolarization. To gain further insight into the interaction between these two drugs, we investigated, in canine Purkinje fibers, the kinetics of onset of drug-induced block development during the action potential and the time course of block recovery during diastole of therapeutic concentrations of tocainide and quinidine both singly and in combination. The dependence of sodium channel block upon both stimulation rate and diastolic membrane potential was also determined.

METHODS

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and their hearts removed via a left thoracotomy. Both ventricles were thoroughly flushed with chilled, oxygenated Tyrode's solution. Purkinje fibers with small pieces of attached ventricular muscle were quickly excised and pinned to a Sylgard block at the bottom of a tissue bath (8 ml capacity) and continuously perfused at a rate of 10-12 ml/min with modified Tyrode's solution aerated with 95%0_2 - 5%CO_2. Our standard Tyrode's solution contained (in mM): NaCl -119.0; KCl - 4.0; $CaCl_2$ - 1.8; $MgCl_2$ - 0.5; $NaHP0_4$ - 0.9; dextrose - 5.5 and $NaHCO_3$ - 25. pH of the solution was 7.33 ± 0.03. The temperature was maintained at 37 ± 0.2°C.

We used small, free-running intertrabecular Purkinje fiber bundles (length < 5 mm) with attached ventricular muscle. These preparations displayed minimal diastolic depolarization and provided stable membrane potentials more negative than -80 mV during prolonged periods of quiescence. Preparations which displayed automaticity were not used.

The preparations were stimulated with rectangular pulses through bipolar tungsten electrodes etched in sodium nitrate and coated with Formvar. Electrical stimulation was provided by a Model RS-660 Timing Simulator/Word Generator controlled by an HP9816 computer in combination with a Digitimer stimulus isolation unit (model DS2). In order to reduce errors in $\dot{V}_{max}$ measurements the recording and stimulating electrodes were positioned such that

upstroke velocities did not change more than 5% with changes in stimulus strength.

Transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl and coupled to the input of a high impedance, capacitance neutralized amplifier (Model KS-700, WPI instruments). The output of the KS-700 was displayed on a Tektronix 5113 dual-beam storage oscilloscope and simultaneously displayed in digital form on a Data 6000 waveform analyzer (Data Precision, Inc.) (Sasyniuk & Jhamandas, 1984).

During control measurements and during equilibration with drug or a combination of drugs, the preparations were stimulated at a basic cycle length of 1000 msec. Action potential parameters were recorded on line every 30 seconds until steady-state effects were obtained.

The influence of drugs on action potential parameters as a function of steady state changes in stimulation frequency was examined by varying the cycle length from 300 to 2000 msec. Sufficient time (4 minutes or more) was allowed at each cycle length to permit $\dot{V}_{max}$ to reach a new steady-state value.

To study the rate of development of block with drive, the preparations were driven by 45 beat trains at an interstimulus interval of 600 msec. Rest periods between trains of stimuli were long enough to ensure full recovery from rate-dependent effects. Rate of decline of $\dot{V}_{max}$ during the stimulus train and the magnitude of rate dependent block are dependent upon the interstimulus interval within the train. The magnitude of block was greater and the rate of onset of block was faster with decreases in the interstimulus interval with both tocainide and quinidine. This reflects the well known use dependence of drug action. We chose an interstimulus interval of 600 msec because, in the presence of quinidine,

the first action potential of the train was so prolonged (see *Figure 1.5*, p.19) that if we used shorter interstimulus intervals, the second action potential of the train would occur during the repolarization phase of the first response resulting in voltage dependent depression of $\dot{V}_{max}$. Thus, in all preparations, an interstimulus interval of 600 msec was used so that comparisons between drugs and the combination could be made.

To determine the recovery of $\dot{V}_{max}$ from block, test pulses were introduced at varying diastolic intervals after a stimulus train had produced a steady level of block. The onset of diastole was defined as when repolarization had returned to the maximum diastolic potential. After each test pulse, there was a 20 sec quiescent interval followed by another stimulus train. Test pulses were initially introduced during phase 3 of the last action potential of the train and then progressively later in the cycle. Initially, the test intervals were increased in small steps and then in progressively larger steps. The steps ranged from 10 msec to 5000 msec. Each recovery curve comprised at least 50 points.

To study drug effect on steady state sodium inactivation, preparations were stimulated at cycle lengths of 5 sec under control conditions and 10 to 20 sec in the presence of drugs. Cycle lengths longer than 20 sec could not be studied because of spontaneous activity. The fibers were then exposed to progressively higher potassium concentrations in 1-2 mM steps until the preparations stopped responding. Each potassium concentration was applied until steady state effects on $\dot{V}_{max}$ were observed. The preparations were then returned to a 2.7 mM potassium Tyrode's solution, exposed to a second intervention and the potassium concentrations were again increased in 1-2 mM steps as before. During alterations of the potassium concentration, $\dot{V}_{max}$ and membrane potential were recorded on

line every 5 seconds.

Tocainide used was tocainide hydrochloride generously supplied by Astra Pharmaceuticals. Quinidine used was quinidine sulfate dihydrate obtained from J.T. Baker Chemicals. The drugs were added to Tyrode's solution made up from refrigerated stock solutions. Concentrations refer to the concentrations of the base. Therapeutic concentrations of both drugs were used, 5 μM (1.6 μg/ml) of quinidine and 50 μM (9.6 μg/ml) of tocainide. The concentration of quinidine which we have used is lower than that used in most previous *in vitro* studies. Plasma quinidine concentrations in patients receiving therapeutic doses have been reported recently to range between 1 and 7 μg/ml (Halkin *et al*, 1979; Carliner *et al*, 1980). Since the drug is 70-80% protein bound the free drug concentration in plasma is only 20 to 30% of that measured by conventional assay techniques. Therefore when the drug is studied in the tissue bath using protein free superfusates, concentration of drug should be in the range of 0.2 to 2 μg/ml. The concentration of quinidine which we have chosen is within this range. Similar concentrations have been studied by Shen & Antzelevitch (1986). Therapeutic plasma concentrations of tocainide vary from 4 to 23 μg/ml (Winkle *et al*, 1976; Anderson *et al*, 1978). Since the drug can be as little as 20% protein bound, the concentration we have chosen is again within the therapeutic range.

It was necessary to expose the preparations to quinidine for at least one hour before a steady state effect on action potential characteristics was obtained. In the presence of tocainide, steady state effects were usually achieved within 20 minutes. Thus, in the majority of experiments, onset of and recovery from block in the presence of the combination were compared to quinidine alone since it was not possible to keep single impalements long enough to study both drugs and the combination in the same cell.

Data analysis

All action potential parameters were determined using a Data 6000 waveform analyzer. This instrument consists of a 68,000 microprocessor with a 14 bit A to D converter that can acquire data at a rate of up to 100 kHz with a resolution of 0.06 mV. The action potential waveform was digitized at two different rates. The action potential waveform at the fastest digitizing rate which was always set at 50 kHz was used to determine all phase 0 parameters. The waveform at the slower digitizing rate which ranged from 1 to 1.66 kHz was used to determine action potential duration. Take- ff potential (TOP), maximum diastolic potential (MDP), action potential amplitude (AMP), maximum upstroke velocity ($\dot{V}_{max}$), membrane potential at which $\dot{V}_{max}$ occurs ($\overline{V}$) and action potential duration (APD) at the 50%, 75% and 95% levels of repolarization were measured simultaneously on line By measuring both TOP and MDP, we were able to detect the extent of any diastolic depolarization which may have occurred. In some experiments, we measured activation time i.e. the time from the beginning of the stimulus pulse to when the maximum upstroke velocity of the action potential occurs.

Where applicable, data are expressed as mean ± S.D. Student's t-test was used to make statistical comparisons between drug effects. The time course of onset or recovery from block was defined using a least square error nonlinear exponential fitting program (Hewlett Packard Statistical Library). The time constant of $\dot{V}_{max}$ block or recovery contained either one or two distinct components. Least squares analysis of the data indicated that each component (if more than one were present) could be approximated by a single exponential function of the form $Y = A \exp(-t/\tau) + B$. No attempt was made to fit the data with more complex functions. However, when appropriate, it was determined if

double exponential functions fit the data better than single exponential functions. A double exponential was considered a better fit if the residual sum of squares for the double exponential was one third or less than that for the single exponential.

Although we have taken all necessary precautions in measuring $\dot{V}_{max}$, interpretation of our results necessarily depends on the accuracy with which $\dot{V}_{max}$ serves as an indication of the fast inward sodium current, an issue which is a matter of current controversy (Cohen *et al*, 1985; Courtney, 1985; Hondeghem, 1985). For the purposes of the present study, $\dot{V}_{max}$ only needs to be a qualitatively accurate indicator of peak inward sodium conductance so that a decrease in $\dot{V}_{max}$ reflects a decrease in peak sodium current.

RESULTS

Effects on Action Potential Characteristics

Table 1.1 summarizes the effects of tocainide (50 μM), quinidine (5 μM), and their combination on action potential characteristics in Purkinje fibers stimulated at a basic cycle length of 1000 msec. Neither drug altered the maximum diastolic potential. Quinidine consistently depressed action potential amplitude and $\dot{V}_{max}$. Effects of tocainide on $\dot{V}_{max}$ ranged from little or no depression to approximately 10% depression (average of 5%). Tocainide abbreviated all phases of the action potential while quinidine abbreviated the plateau but lengthened the terminal phase of repolarization. Addition of tocainide to fibers equilibrated with quinidine caused a small but significant further depression of $\dot{V}_{max}$, and abbreviated all phases of the action potential.

TABLE 1

Effects of tocainide (50 uM) and quinidine (5 uM) and their combination on action potential characteristics in canine Purkinje fibers

Parameter	TOP (-mv)	AMP (mv)	$\dot{V}max$ (v/sec)	APD 50% (msec)	APD 75% (msec)	APD 95% (msec)
Control	90.7±4.4	123.5±4.1	580±123	226±61	271±65	312±70
Tocainide	90.2±4.6	121.7±4.4	550±123*	168±48*	221±52*	265±56*
% change	-0.8±1.8	-1.4±1.2	-5±4*	-24±8*	-18±6*	-15±5*
n = 19						
Control	89.7±2.4	127.4±2.9	803±131	238±33	280±32	323±30
Quinidine	89.0±3.5	123.6±4.8*	692±131*	200±29*	277±31	334±27*
% change	-0.8±2.9	-2.9±2.9*	-14±6*	-16±7*	-1±5	+4±6*
n = 13						
Quinidine	89.6±3.1	122.8±4.0	630±138	189±20	274±18	340±23
Combination	88.7±2.8	121.3±4.6	604±145*	160±17*	246±9*	310±13*
% Change	0.9±3.4	-1.3±1.9	-5±4*	-15±6*	-10±5*	-8±4*
n = 9						

Fibers stimulated at a BCL of 1000 msec

TOP - take-off potential; AMP - amplitude; $\dot{V}max$ - maximum rate of depolarization.

APD 50%; APD 75%, APD 95% - action potential duration at 50%, 75% and 95% levels of repolarization.

All values expressed as Mean ± S.D. * $p < 0.05$

Comparison of the Effects of Quinidine Versus the Combination on Action Potential Characteristics During Steady State Changes in Cycle Length

Effects on phase 0 characteristics

Quinidine progressively reduced the steady state level of $\dot{V}_{max}$ in a rate-dependent manner as the basic cycle length was decreased from 2000 to 300 msec (see *Figure 1.1*). Addition of tocainide to the quinidine superfusate produced a further net decrease of $\dot{V}_{max}$ (more block) at all stimulation rates. However, the additional decrease in $\dot{V}_{max}$ was much greater at a cycle length of 300 msec than at a cycle length of 2000 msec. Similarly, there was much more further depression of amplitude at the shortest basic cycle lengths following addition of tocainide. In the presence of quinidine as well as the combination there was progressive hyperpolarization with decreases in cycle length. Similar results were obtained in two other experiments.

Effects on action potential duration

Addition of tocainide to the quinidine superfusate abbreviated action potential duration at all cycle lengths and at all levels of repolarization. However, the amount of shortening was much greater at the longest cycle lengths (see *Figure 1.2*). Similar results were obtained in two other experiments.

Comparison of the Effects of Tocainide, Quinidine and Their Combination on the Rate of Development and Magnitude of Frequency Dependent Block

There was no frequency dependent alteration in $\dot{V}_{max}$ under control conditions. The magnitude and rate of development of block for each drug and the

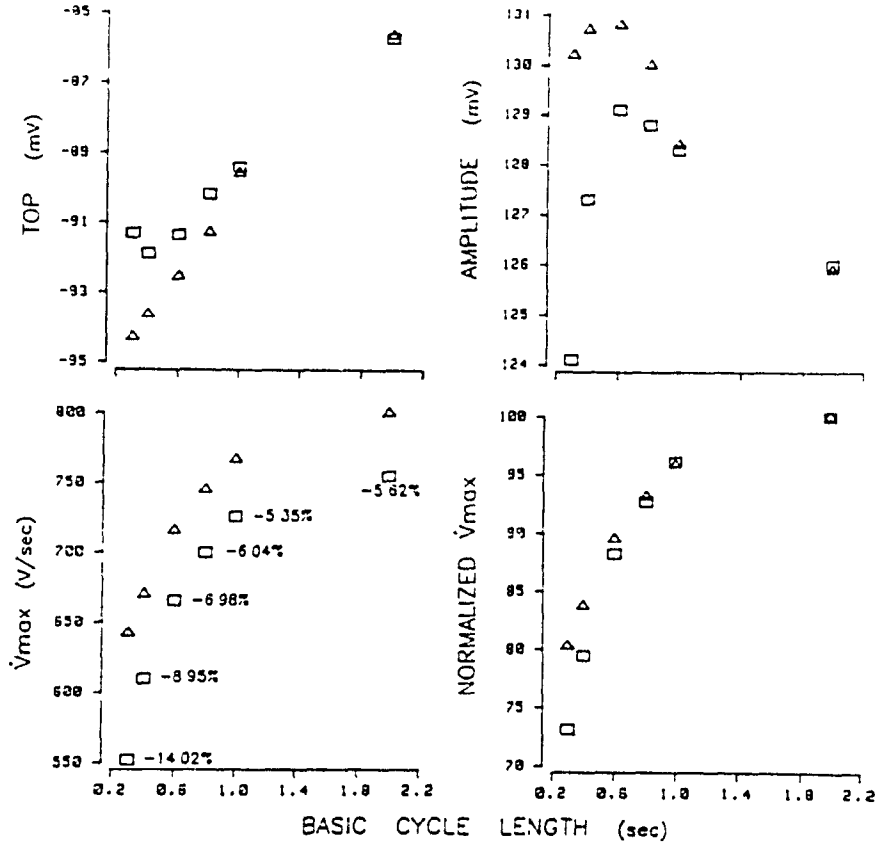

Figure 1.1. Typical example of the effects of steady state changes in basic cycle length upon take-off potential (left upper panel), action potential amplitude (right upper panel) and $\dot{V}_{max}$ (lower panels) in the presence of quinidine, 5 μM, and a combination of quinidine 5 μM, and tocainide, 50 μM. Both the actual values and the normalized values of $\dot{V}_{max}$ are plotted. In the left lower panel, the % values represent the additional depression of $\dot{V}_{max}$ at each cycle length in the presence of the combination versus quinidine alone. The amount of depression at short cycle lengths is probably underestimated because of the rate-related changes in TOP. The cycle length was varied between 2000 and 300 msec. Each cycle length was maintained until steady state effects were obtained beginning with the slowest cycle length. In this and all subsequent figures, quinidine is represented by triangles; the combination by squares.

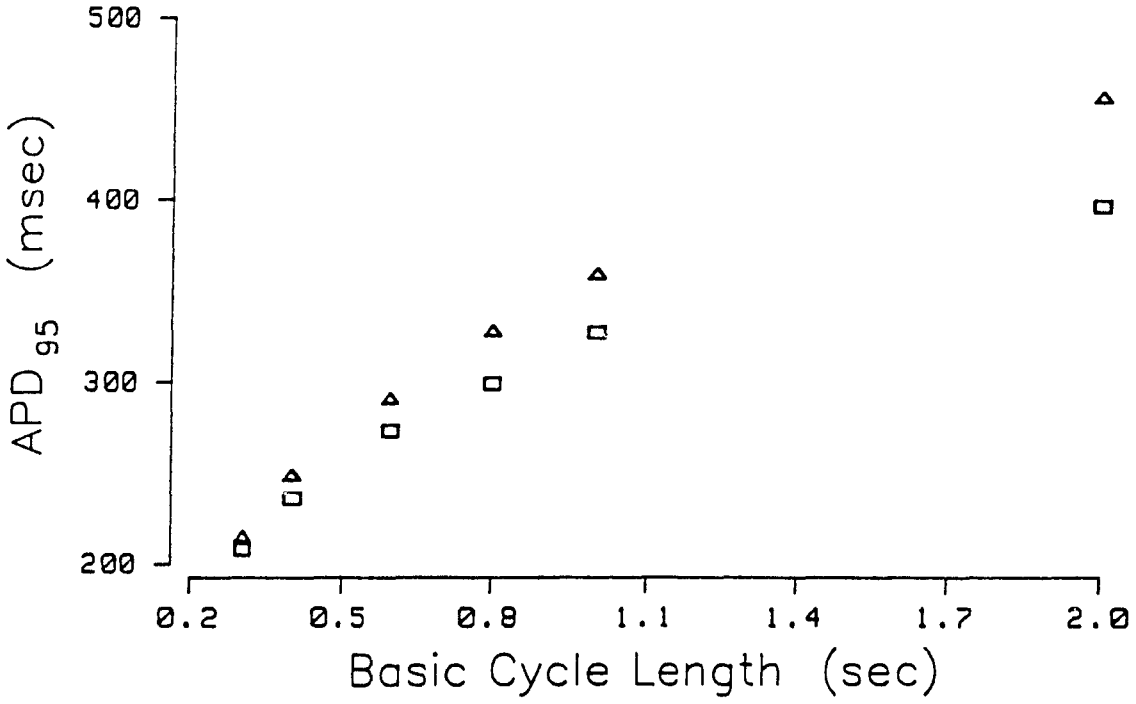

Figure 1.2. Typical example of the effects of steady state changes in basic cycle length upon action potential duration at the 95% level of repolarization in the presence of quinidine and a combination of quinidine and tocainide. Data were obtained from the same fiber as in *Figure 1.1.* APD was abbreviated at all cycle lengths in the presence of the combination. The greatest absolute difference in duration between short and long cycle lengths was apparent at the 95% level of repolarization. However, similar relative changes were apparent at the 75% and 50% levels of repolarization although these are not shown.

combination are summarized in *Table 1.2.* The rate of onset of block in the presence of tocainide was very fast. In preparations with maximum diastolic potentials greater than -90 mV, steady state block developed within one or two action potentials and the magnitude of block was small. *Figure 1.3* shows a typical example. The magnitude of rate dependent block ranged from 1.29% to 15.0% in individual preparations. The first action potential of the stimulus train is an indication of the magnitude of block after a long rest period (tonic block). Tocainide either produced no tonic block or as much as 10% tonic block in individual preparations. This variability may be related to variable degrees of depolarization during the quiescent interval.

The rate of onset of block in the presence of quinidine alone was much slower when compared to tocainide while the magnitude of block was greater (*Table 1.2*). This concentration of quinidine produces no membrane depolarization, only a small amount of tonic block, but marked use dependent block in fully polarized tissue.

Figure 1.4 compares the rate of development of block for quinidine alone versus a combination of quinidine and tocainide in a preparation in which it was possible to maintain impalement during control conditions and after exposure to the drugs. It was necessary to expose the preparation to quinidine for at least one hour before a steady state level of block was attained. Quinidine alone appeared to produce a small amount of tonic block but a much greater amount of rate dependent block. Decline of $\dot{V}_{max}$ during exposure to quinidine followed a single exponential. When the two drugs were added together, the amount of tonic block increased but the magnitude of rate dependent block was similar to that of

TABLE 1.2

Comparison of the Effects of Tocainide, Quinidine and Their Combination on the Magnitude and Kinetics of Onset of Rate Dependent Block

Drug	Rate Constant (pulses)	%RDB
Tocainide, 50 μM n = 16	0.95 ± 0.32*	5.32 ± 3.40*
Quinidine, 5 μM n = 15	5.61 ± 0.50	15.81 ± 3.68
Tocainide, 50 μM plus quinidine, 5 μM# n = 10	0.88 ± 0.19* 6.47 ± 1.36	15.64 ± 4.87

RDB - Rate dependent block

* Significantly different from quinidine alone $p < 0.05$.

Onset of block in the presence of the combination was best fitted by a double exponential.

All values expressed as mean ± S.D.

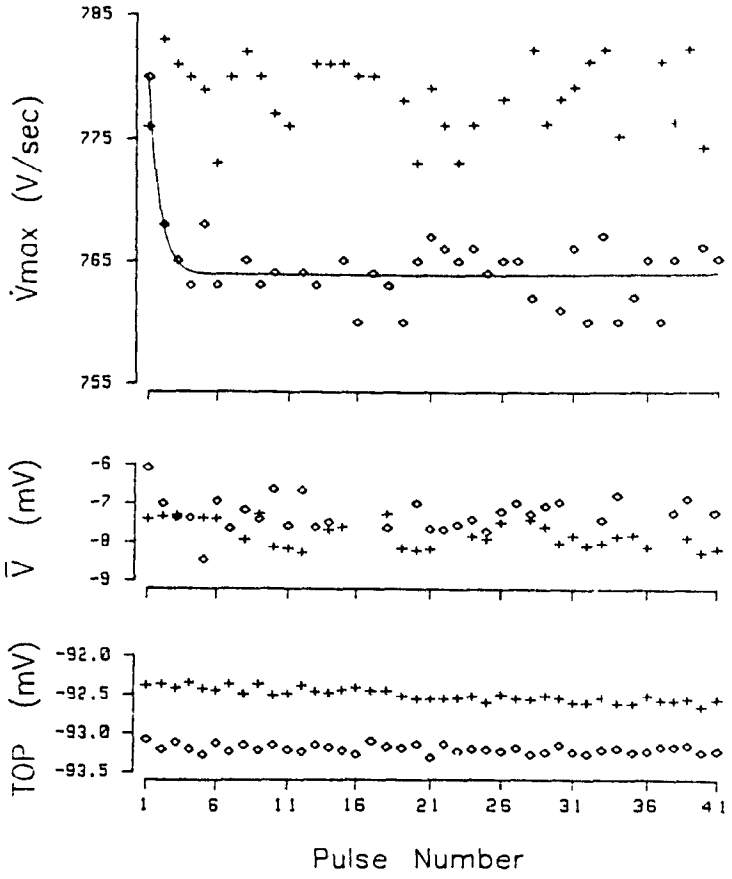

Figure 1.3. Effects of tocainide, 50 μM, on the magnitude and rate of development of frequency dependent block. $\dot{V}_{max}$ (top panel), the potential at which $\dot{V}_{max}$ occurs (middle panel) and the take-off potential (bottom panel) are plotted for each action potential during the stimulus train under control conditions and in the presence of drug. There was no frequency dependence in the absence of drug, merely a 1% variation in $\dot{V}_{max}$. The $\dot{V}_{max}$ curve in the presence of tocainide represents a nonlinear least square fit to the data points. Decline of $\dot{V}_{max}$ during the stimulus train approximated a single exponential with a rate constant of 0.75 pulses. Magnitude of block was small, 2.08%. $\bar{V}$ and TOP were essentially constant throughout the stimulus train. The 0.5 mV difference in TOP between control and drug is due to slight variations in membrane potential from one stimulus train to another. In this and all subsequent figures, control is represented by crosses; tocainide by diamonds.

quinidine alone. In all experiments the magnitude of rate dependent block with the combination was not statistically different from that of quinidine alone. The amount of tonic block with the combination was highly variable ranging from no block to about 8% block. Since there were slight variations in the degree of depolarization during each quiescent interval no conclusions can be made about the degree of tonic block. Decline of $\dot{V}_{max}$ during exposure to the combination followed a double exponential. The first rate constant was similar to that obtained with tocainide alone in other preparations, while the second one was similar to that obtained with quinidine alone (*Table 1.2*). After re-exposure to quinidine alone, the curve obtained was similar to that following initial exposure to quinidine. Experiments were also conducted using 10 μM of quinidine alone and in the combination. The results were qualitatively similar.

Rate dependent effects on action potential duration

In all preparations, action potential duration was also monitored during the stimulus train. Typical drug effects on action potential duration are shown in *Figure 1.5*. Both under control conditions and in the presence of drugs, the first action potential of the train (preceded by a quiescent interval of 30 sec) was prolonged. Shortening during the train followed a biexponential decline. In the presence of quinidine the first action potential of the train was tremendously prolonged when compared to the control action potential but shortened to below control values by the end of the train. The marked prolongation of the first action potential by quinidine is likely due to an effect on potassium channels and suggests that quinidine is able to block potassium channels in fully polarized tissue in the absence of stimulation. This is in contrast to only a small degree of block of sodium

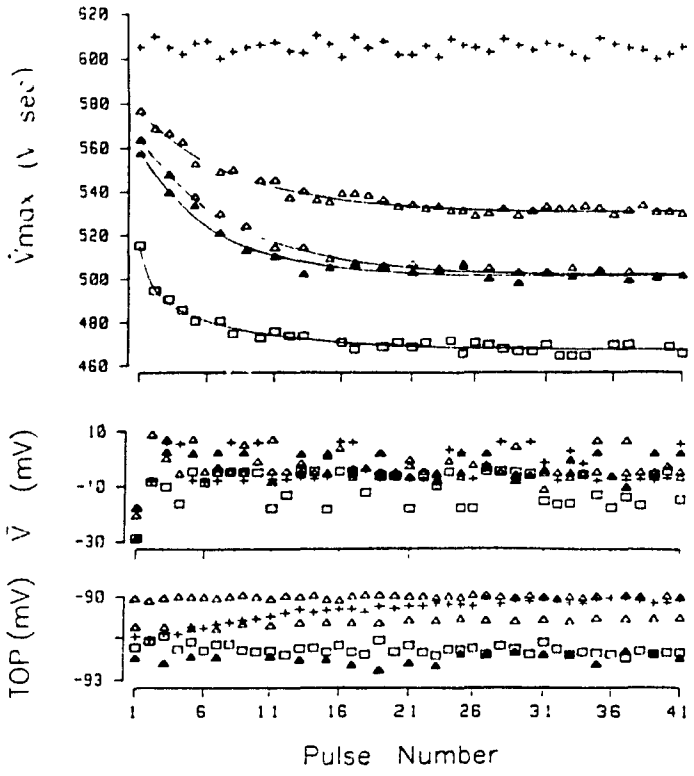

Figure 1.4. Comparison of the effects of quinidine (5 μM) and a combination of tocainide (50 μM) and quinidine (5 μM) on the magnitude and rate of development of frequency dependent block. $\dot{V}_{max}$ (top panel), the potential at which $\dot{V}_{max}$ occurs (middle panel) and the take-off potential (bottom panel) are plotted for each action potential during the stimulus train under control conditions (+), 35 and 60 min after exposure to quinidine (△), 30 min after exposure to the combination (□) and 30 min after wash-out of the combination with quinidine alone (▲). The $\dot{V}_{max}$ curves represent nonlinear least square fits to the data points. The magnitude of RDB was 8.0% after a 35 min exposure to quinidine, 12.63% after a 1 hour exposure to quinidine, 9.32% after exposure to the combination and 10.22% after re-exposure to quinidine alone. Decline of $\dot{V}_{max}$ in the presence of quinidine approximated a single exponential with rate constants of 7.41 and 7.0 beats after 35 and 60 min initial exposures to drug and 5.5 beats after re-exposure. Decline of $\dot{V}_{max}$ during exposure to the combination followed a double exponential with rate constants of 0.71 and 7.2 beats for the first and second exponential, respectively. $\overline{V}$ and TOP were essentially constant throughout the stimulus train.

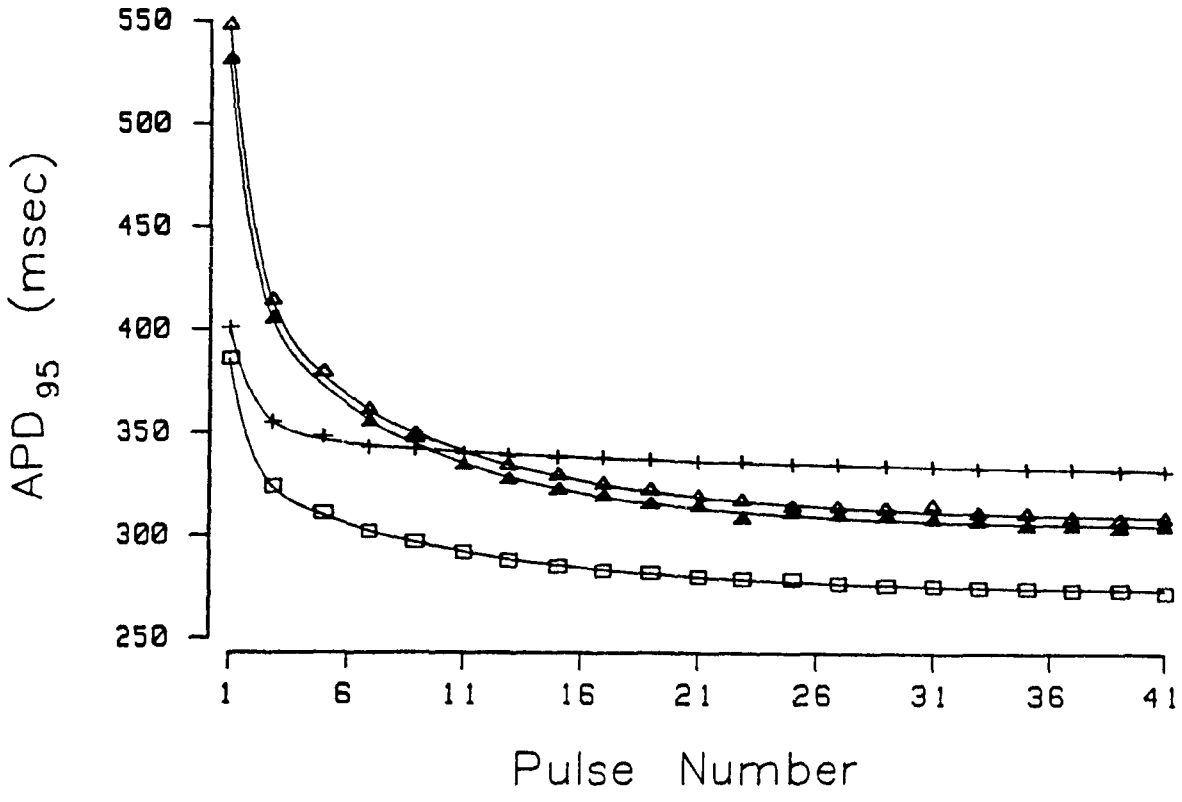

Figure 1.5. Rate dependent effects on action potential duration at the 95% level of repolarization in the presence of quinidine versus the combination. Data were obtained from the same preparation as in *Figure 1.4.* Under all conditions, the duration of the first action potential of the train was prolonged. Shortening of duration during the train followed a double exponential decline with rate constants of 0.32 and 9.1 pulses under drug free condition (+), 1.14 and 8.71 pulses after exposure to quinidine (△), 0.89 and 9.34 pulses after exposure to the combination (□) and 0.95 and 7.67 pulses after re-exposure to quinidine alone (▲). Every second point has been deleted for the sake of clarity.

channels in the absence of stimulation (*Figure 1.4*). When tocainide was added to the preparation equilibrated with quinidine, the duration of all action potentials in the train shortened. However, a striking effect was the very much greater shortening of the first action potential. Shortening of all action potentials in the train would be accompanied by a lengthening of the diastolic interval between action potentials in the presence of the combination and may partially account for a lack of enhanced rate dependent block in the presence of the combination versus quinidine alone.

Characteristics of Recovery from Frequency Dependent Block

Recovery from frequency dependent block following a train of action potentials was determined for each drug and their combination. After a steady reduction of $\dot{V}_{max}$ had been obtained following the stimulus train, rapid stimulation was terminated and $\dot{V}_{max}$ values of single action potential upstrokes elicited at various times during the ensuing rest recovery period were determined. The increasing $\dot{V}_{max}$ values obtained during the recovery period were plotted against the elapsed recovery time. The time course of $\dot{V}_{max}$ recovery was fitted to either one or more exponential curves and the time constant(s) determined.

A recovery period of 5 sec was usually sufficient for $\dot{V}_{max}$ to recover completely in the presence of tocainide. However, a recovery period of 30 sec was required in the presence of either quinidine or the combination. If there were more than a few millivolts of depolarization during the 30 sec recovery interval the results were not used.

Since it took at least one hour to achieve steady state effects with quinidine and a substantial amount of time to generate a recovery curve with each drug, it was not possible to maintain impalement in the same cell long enough to study both drugs and their combination in a given preparation. Thus, the effects of tocainide alone were assessed in one group of experiments and those of quinidine versus the combination in another group of experiments.

We could not accurately determine recovery time constants under drug free conditions because $\dot{V}_{max}$ had already attained its maximal value at the time when repolarization was complete. However, if this measurement was made during the terminal phase of repolarization a time constant of 26.14 ± 10.45 msec (mean ± S.D., n = 7) was obtained. This value for control recovery time constant (although it includes both voltage and time dependent recovery) is comparable to that obtained by other investigators in Purkinje fibers (Gettes & Reuter, 1974; Bean *et al*, 1983).

$\dot{V}_{max}$ recovery was delayed by both drugs. Unblocking following the train proceeded most rapidly with tocainide. A typical example is shown in *Figure 1.6*. The time constant for recovery of $\dot{V}_{max}$ averaged 1.04 ± 0.49 sec (mean ± S.D., n = 8). Early diastolic block, defined as the magnitude of $\dot{V}_{max}$ depression at zero diastolic interval compared to recovery $\dot{V}_{max}$, averaged 10.34 ± 3.10% (mean ± S.D., n = 8).

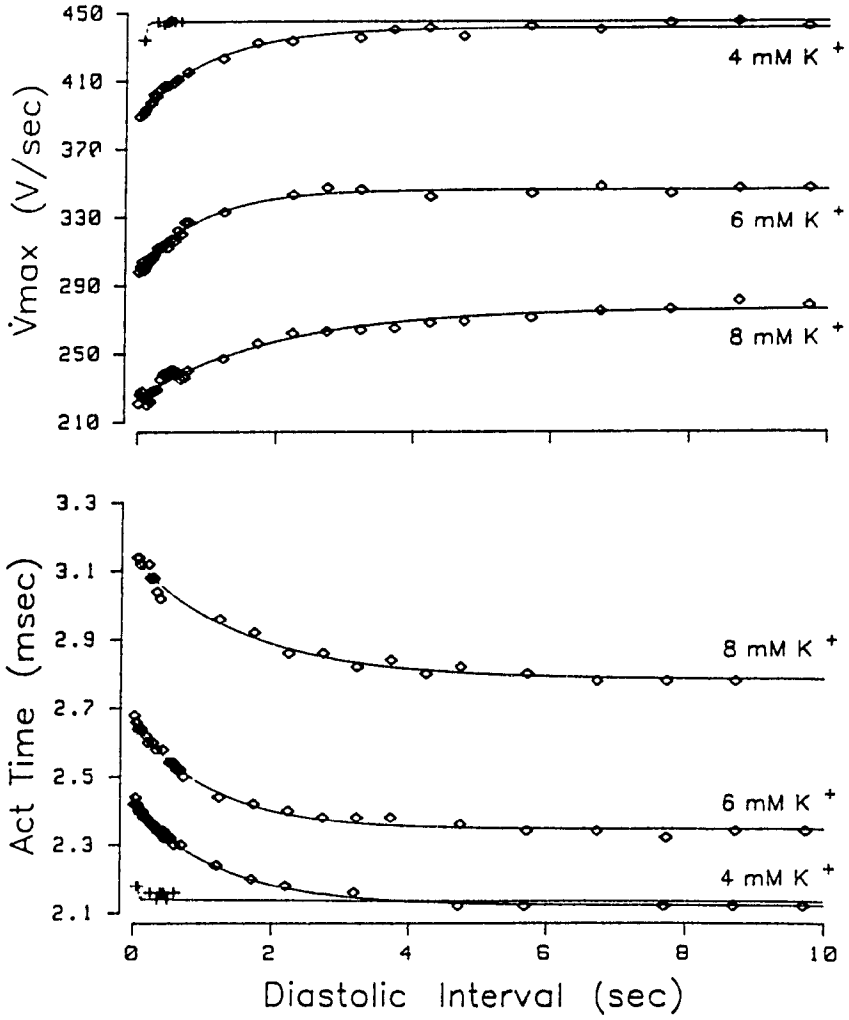

Figure 6
(Legend on the next page)

Figure 1.6. Time course of recovery from block in the presence of 50 μM of tocainide. In the upper panel, $\dot{V}_{max}$ is plotted versus diastolic interval where zero diastolic interval is defined as when repolarization has returned to the maximum diastolic potential. In the lower panel is plotted activation time i.e. the time from the beginning of the stimulus pulse to the maximum upstroke of the action potential. Although the activation time is a measure of both excitability and conduction, it does provide some approximation of drug effect on conduction. Recovery from block in the presence of drug was assessed when the extracellular potassium concentration was 4, 6 and 8 mM. The curves represent nonlinear least squares fits of the data. Under drug free conditions (+), the recovery time constant was 27 msec. Under tocainide ($\diamond$) and a 4 mM K^+ concentration, tne recovery time constant was 1.08 seconds and the membrane potential was 87.0 mV. Under 6 and 8 mM K^+ concentrations, the membrane potential decreased to 83.5 and 79.5 mV respectively, and the time constants increased to 1.28 and 2.36 sec, respectively. Early diastolic block, defined as the magnitude of $\dot{V}_{max}$ depression at zero diastolic interval compared to recovery $\dot{V}_{max}$ was 11.5, 15.7 and 20.9% at 4, 6 and 8 mM K^+, respectively. Early diastolic block was estimated by extrapolating the exponential fit back to zero time. The time constant of $\dot{V}_{max}$ recovery was reflected in a similar time course of change in activation time, namely 0.015, 1.21, 1.33 and 2.18 sec for control and tocainide at 4, 6 and 8 mM K^+, respectively, suggesting that the time course of $\dot{V}_{max}$ recovery parallels the time course of changes in conduction. At 4 mM K^+, $\dot{V}_{max}$ recovered to drug free values at long cycle lengths. However, at 6 and 8 mM K^+, $\dot{V}_{max}$ was considerably depressed even at long cycle lengths, suggesting substantial amounts of tonic block (21.6 and 37.8% respectively).

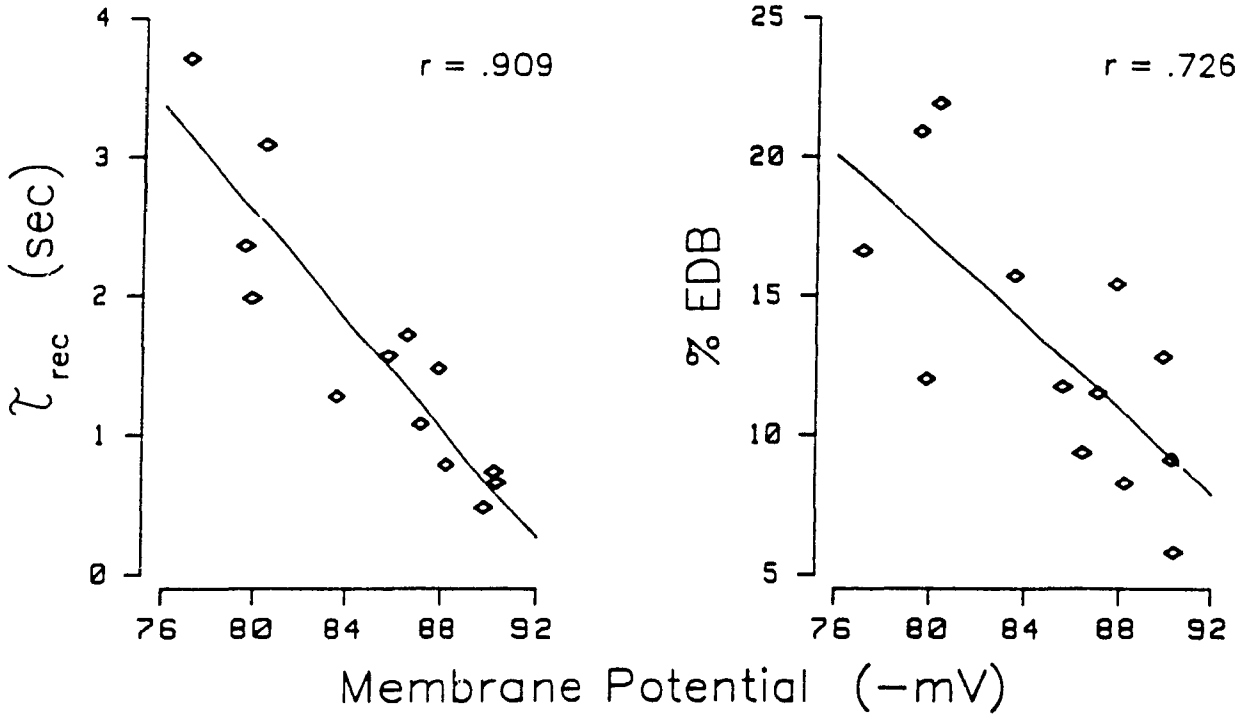

Figure 1.7. Correlation between τ_{rec} of $\dot{V}_{max}$, % early diastolic block (%EDB) and membrane potential in the presence of 50 μM of tocainide. The graph shows the results from 7 preparations. Membrane potential was varied by exposing the preparation to a Tyrode's solution in which the potassium concentration was increased to either 6 or 8 mM. Time constants of recovery were determined during superfusion with control Tyrode's solution (4 mM K^+) and re-determined after exposure to the high potassium Tyrode's solution. Membrane potential ranged from -90.4 mV to -77 mV in the 7 preparations; there was almost a 6 fold difference in τ_{rec} of $\dot{V}_{max}$ over this range of membrane potentials and approximately a 4 fold difference in the magnitude of early diastolic block. Both correlations were significant at $p < 0.05$.

Both the time constant for recovery of $\dot{V}_{max}$ and the magnitude of early diastolic block produced by tocainide was highly dependent upon membrane potential (*Figures 6 & 7*). The less negative the membrane potential the slower was the recovery time constant and the greater the early diastolic block, suggesting that at reduced membrane potentials, a greater number of sodium channels were blocked and drug unbinding was slower. There was approximately a 6 fold difference in the time constant for recovery of $\dot{V}_{max}$ and approximately a 4 fold difference in the magnitude of early diastolic block over approximately a 15 mV range of membrane potentials.

Figure 1.8 compares the recovery kinetics of $\dot{V}_{max}$ in the presence of quinidine versus a combination of quinidine and tocainide. Post stimulation recovery from quinidine induced block was characterized by a single time constant while that of the combination was characterized by two time constants. The mixture of the two drugs produced a much larger depression of $\dot{V}_{max}$ of early premature responses compared to quinidine. There was also additional depression of $\dot{V}_{max}$ (8.5%) at long coupling intervals. However, there was no additional depression of activation time at these same long intervals. The degree of depression of $\dot{V}_{max}$ at long coupling intervals following exposure to the combination was variable, ranging from minimal block to about 10% block. However, the depression of early responses was always consistently greater. During recovery from drug induced frequency dependent block changes in activation time were found to be reciprocably related to changes in $\dot{V}_{max}$. The time constant for recovery of $\dot{V}_{max}$ in the presence of quinidine averaged 4.81 ± 0.76 sec (mean ± S.D., n = 8). The two time constants for recovery of $\dot{V}_{max}$ in the presence of the combination, were 0.43 ± 0.22 and 5.94 ± 0.56 sec, respectively (mean ± S.D., n = 5). The first time constant of the combination presumably corresponds to that of

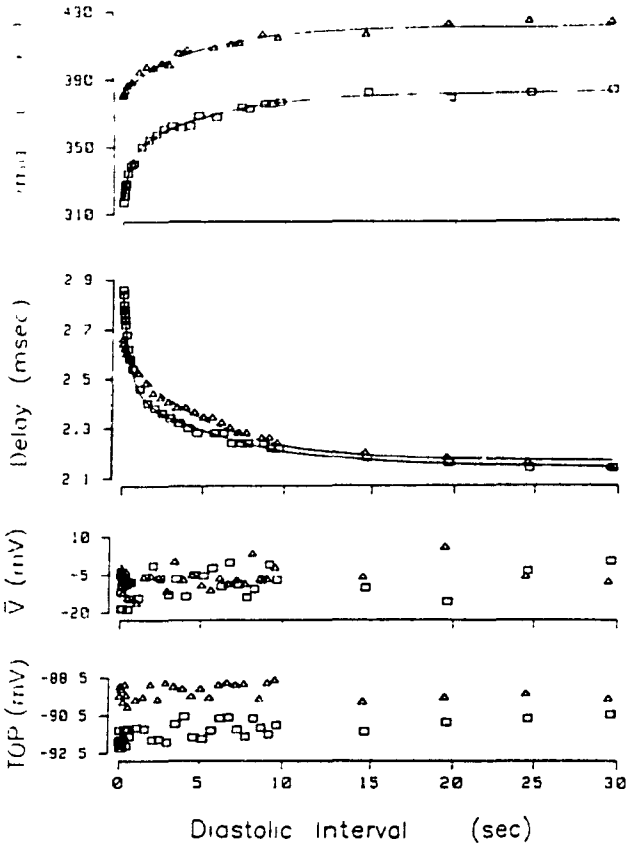

Figure 1.8. The time course of recovery of $\dot{V}_{max}$ and activation time in the presence of 5 μM quinidine and 30 minutes after switching to a combination of 5 μM quinidine plus 50 μM tocainide. $\dot{V}_{max}$ (uppermost panel), activation time (second panel), potential at which $\dot{V}_{max}$ occurs (third panel) and take-off potential (bottom panel) are plotted against diastolic interval. The curves represent nonlinear least squares fits of the data. Recovery in the presence of quinidine followed a single exponential with time constants of 4.96 and 5.03 seconds for $\dot{V}_{max}$ and activation time respectively. Recovery in the presence of the combination followed a double exponential with time constants of 0.37 and 5.39 sec for $\dot{V}_{max}$ and 0.44 and 6.20 sec for activation time respectively. Early diastolic block, defined as the magnitude of $\dot{V}_{max}$ depression at zero diastolic interval, was 9.14% in the presence of quinidine and 16.80% under the combination. Of the latter, 10.18% was attributable to quinidine suggesting that the two drugs had an additive effect. Both $\bar{V}$ and TOP remained essentially constant throughout the diastolic interval.

tocainide while the second time constant presumably corresponds to that of quinidine. The amount of block present at the beginning of diastole (early diastolic block) was significantly greater in the presence of the combination (22.57 ± 9.23%) than in the presence of quinidine alone (16.37 ± 5.72%; $P < 0.05$). The large depression of $\dot{V}_{max}$ of early premature responses was consistently accompanied by an increase in activation time suggesting that the depression of $\dot{V}_{max}$ of early responses reflects a slowing of conduction.

In two experiments, the concentration of tocainide in the combination was increased to 100 and 200 μM. There was no change in the time constants of recovery of $\dot{V}_{max}$ but there did occur a progressive increase in the amount of block present at the beginning of diastole. The greater magnitude of block was due to more tocainide block. The quinidine component of the block remained the same suggesting that greater early diastolic block was due to the additive effects of both drugs.

The membrane potential dependence of the time constant for recovery of $\dot{V}_{max}$ could not be determined in the presence of quinidine because of the long recovery intervals required and the likelihood of diastolic depolarization at these long intervals. At normal extracellular potassium concentration and membrane potentials greater than -85 mV, a few mV of depolarization did not prevent recovery of $\dot{V}_{max}$ at long diastolic intervals. However, when the preparations were depolarized by increasing the extracellular potassium concentration, even a small amount of depolarization (1-2 mV) prevented full $\dot{V}_{max}$ recovery and thus recovery time constants could not be accurately determined.

$\overline{V}$, the potential at which $\dot{V}_{max}$ occurs, remained essentially constant both

during onset and recovery from block. $\bar{V}$ only changed if $\dot{V}_{max}$ decreased as a result of membrane depolarization. Since membrane potential remained fairly constant (varying by no more than a few mV) during either onset or recovery from block, $\bar{V}$ would be expected to remain constant. Thus, reduction of $\dot{V}_{max}$ cannot be attributed to changes in sodium driving force ($V_m - V_{Na}$) at the time of $\dot{V}_{max}$.

Voltage Dependent Effects

In 9 experiments we assessed the effects of tocainide, quinidine and their combination on the relationship between $\dot{V}_{max}$ or activation time and membrane potential. Tocainide consistently shifted the $\dot{V}_{max}$ curves to more negative potentials suggesting an interference with sodium inactivation. This effect developed at stimulation rates at which tocainide shows no frequency dependent effects. *Figure 1.9A* shows that under control conditions, half inactivation, Vh_{50}, occurred at -71 mV. Tocainide, 50 μM, shifted the steady state inactivation curve along the voltage axis to more hyperpolarized potentials, half inactivation now occurring at -75 mV. The peak $\dot{V}_{max}$ obtainable was diminished slightly by tocainide suggesting that there was a small degree of resting block (less than 3%) at membrane potentials more negative than -90 mV. Tocainide shifted the curve an average of 3.25 mV (n = 2).

In contrast to tocainide, 5 μM of quinidine produced little or no shift in the $\dot{V}_{max}$-membrane potential curve (*Figure 1.9B*). Addition of tocainide to fibers equilibrated with quinidine produced a shift similar to that produced by tocainide alone in control fibers as seen in *Figure 1.9A*. In the presence of the combination, there was a greater depression of $\dot{V}_{max}$, particularly at more depolarized membrane potentials. The combination shifted the curve an average of 3.15 $\pm$ 0.11 mV (n = 4).

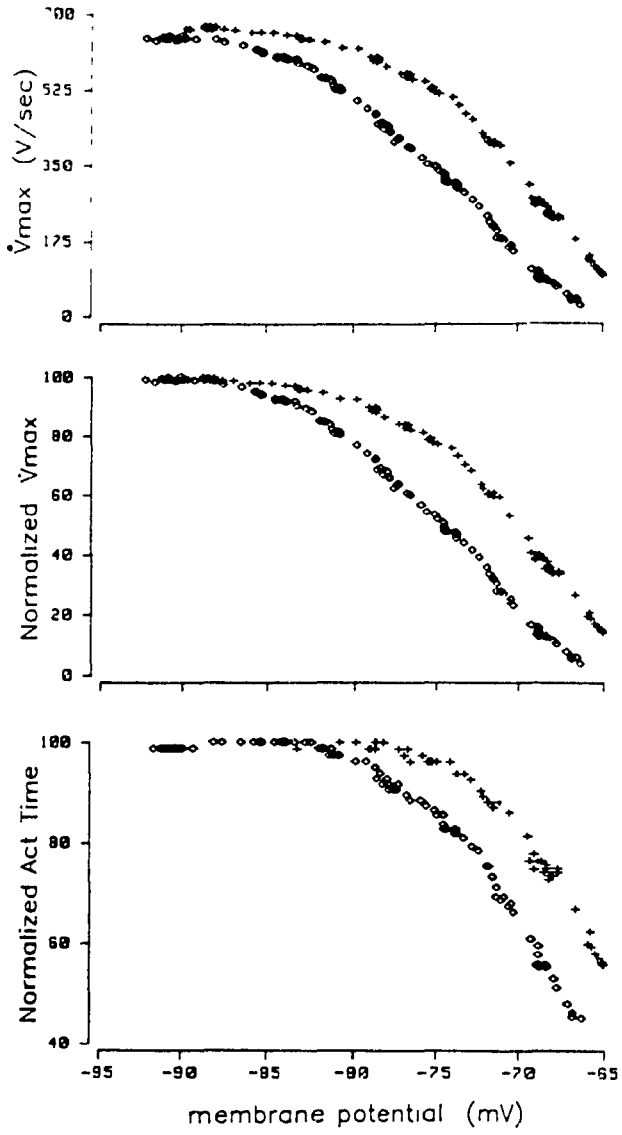

Figure 1.9A. Relationship between $\dot{V}_{max}$, activation time and membrane potential under control conditions (+) and in the presence of 50 μM tocainide (◇). Uppermost panel shows actual $\dot{V}_{max}$ values. In the middle and bottom panels, $\dot{V}_{max}$ and activation time have been normalized to their respective values at the most negative membrane potential. Membrane potential was decreased by increasing the K^+ concentration in the superfusate from 2.5 to 12 mM in 1-2 mM steps until the preparation stopped responding. Equilibration with each concentration was attained before switching to the next higher concentration. Stimulus cycle length was 10 seconds. Tocainide shifted the curves 4 mV in the hyperpolarizing direction.

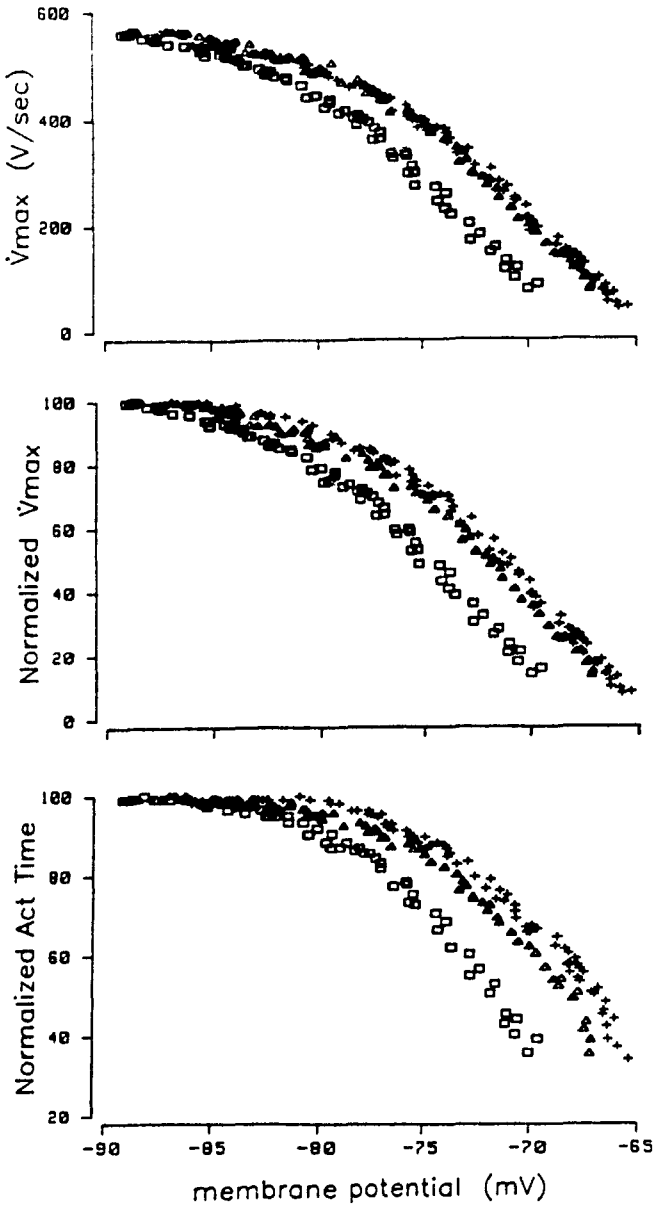

Figure 1.9B. Same relationship as in A under control conditions (+) and after exposure to 5 μM quinidine ($\triangle$) and a combination of 5 μM quinidine and 50 μM tocainide ($\square$), but in a different preparation than A. While quinidine shifted the curves minimally, the combination produced almost a 4 mV shift. This shift was similar to that produced by tocainide alone. Stimulus cycle length was 10 seconds.

DISCUSSION

In a beating heart, interaction of local anesthetic type drugs with the sodium channel receptor changes dynamically throughout the cardiac cycle. During an action potential, sodium channels cycle through three different states, viz., rested, open and inactivated, as described by Hodgkin & Huxley (1952). Hondeghem & Katzung (1977) proposed that each one of these three states can interact with antiarrhythmic drugs and that drug associated channels also rotate between the three states but they behave as if their voltage dependence is shifted to more hyperpolarized potentials.

Most class I antiarrhythmic drugs have a very low affinity for the receptor site when the channel is in the rested state, but have a high affinity for the receptor when the channel is open or inactivated (Hondeghem & Katzung, 1984). Thus, block develops during each action potential and then dissipates when the channels return to the rested state at the beginning of diastole. The rate of drug dissociation during diastole varies for different clinically effective class I drugs (Campbell, 1983b). Thus, sodium channel block evoked by these drugs is characterized by a pronounced dependence on stimulation rate. Our results suggest that for quinidine, the time constant of drug dissociation at normal diastolic potentials is slow enough (4.0 to 6.1 sec) so that block does not fully dissipate between beats throughout the clinical range of heart rates. Similar time constants of dissociation for quinidine have been obtained by others (Grant *et al*, 1982; Weld *et al*, 1982; Clarkson & Hondeghem, 1985). For tocainide, the time constant of drug dissociation in fully polarized cells is much faster (0.5 to 1.7 sec). Thus, in the presence of tocainide, a substantial amount of block can dissipate between beats at

slow heart rates. Similar time constants of dissociation for tocainide were obtained by Gintant *et al* (1983) in Purkinje fibers, and by Courtney (1980b) in guinea pig papillary muscles. Oshita *et al* (1980) found two components of recovery for tocainide in guinea pig papillary muscle using two different experimental protocols. It is unlikely that their first component is recovery from drug effect since they conducted their study at a basic cycle length of 4000 msec which is unlikely to produce any degree of block in the presence of tocainide. Thus, there could not have been any block from which to recover. The first component is more likely recovery from slow inactivation as described by Clarkson *et al* (1984). Their second component which is longer than our values was obtained at higher extracellular potassium concentrations.

In normal fully polarized cells, tocainide was more effective in depressing early premature impulses or those elicited at rapid heart rates but produced much less block at slow heart rates. Quinidine, on the other hand, produced less depression of early beats and a more uniform block throughout the clinical range of heart rates.

When administered in combination at clinically effective concentrations, the magnitude of the interaction between tocainide and quinidine was highly dependent upon the heart rate and the membrane potential. Tocainide depresses $\dot{V}_{max}$ and delays conduction at rapid heart rates and at more depolarized membrane potentials. At depolarized potentials, depression of $\dot{V}_{max}$ is due to a prolongation of the recovery time constant and possibly a greater magnitude of tonic block. At membrane potentials in the range of -75 mV, the rate of tocainide dissociation slows to the rates observed with quinidine at normal diastolic potentials. Unlike tocainide, quinidine in therapeutic concentrations

produces little or no shift of the curve relating $\dot{V}_{max}$ to activation voltage. Both Colatsky (1982) and Weld *et al* (1982) found a minimal shift of this curve in voltage clamped Purkinje fibers at much higher concentrations of quinidine (5 to 10 μg/ml). Thus, much higher concentrations than those effective clinically are required to produce a voltage shift of inactivation. We were unable to determine time constants of recovery for quinidine at depolarized potentials in the present study. Weld *et al* (1982) found that unbinding of quinidine from resting sodium channels is, in fact, strongly voltage dependent. However, in the -90 to -70 mV range of membrane potentials, the time constant for unbinding from resting channels for quinidine changes only from about 4 to 7 sec (their Figure 9). This contrasts with a 4-6 fold difference in the time constant for recovery of $\dot{V}_{max}$ for tocainide observed over a similar range of membrane potentials in the present study. In this respect tocainide resembles lidocaine and mexiletine, another lidocaine congener, both of which have been shown to have similar voltage dependence of the time constant for recovery of $\dot{V}_{max}$ (Chen *et al*, 1975; Oshita *et al*, 1980; Hohnloser *et al*, 1982). Thus, at clinical concentrations quinidine effect is dependent mostly upon heart rate while that of tocainide is dependent upon both heart rate and membrane potential.

Thus, when tocainide is combined with quinidine, the magnitude of the block produced by this combination at slow heart rates in normal fully polarized cells (greater than -90 mV) is not much greater than that produced by quinidine alone. However, at fast heart rates and particularly in depolarized cells, the magnitude of block produced by the combination is very much greater than that produced by quinidine alone. The greater amount of early diastolic block is due to an additive effect of both drugs. Similar interactions have been demonstrated in vitro with combinations of lidocaine and quinidine (Hondeghem & Katzung, 1980; Moyer & Hondeghem, 1980) and lidocaine and propafenone (Kohlhardt & Seifert,

1985).

The state dependence of drug-channel interaction can influence the interaction between two class I drugs. Steady state block caused by a drug with a high affinity for the inactivated site will be dependent upon the ratio of the action potential duration to diastolic interval. Block caused by a drug with a high affinity for the open channel will be influenced by rate, but not by action potential duration. Several lines of evidence support the view that quinidine binds preferentially to open channels (Weld *et al*, 1982; Hondeghem & Matsubara, 1984). There is no data available on the relative affinities for activated and inactivated channels for tocainide. If this drug resembles lidocaine, it may have a high affinity for inactivated channels. If there is substantial inactivated channel block, shortening of action potential duration by tocainide will decrease the relative degree of block produced by the combination. In addition to a prolonged diastolic interval, this may be an additional reason why we did not observe greater rate dependent block under combination versus quinidine alone at an interstimulus interval of 600 msec.

Clinical implications

The above described interactions occur at clinically relevant heart rates and at clinically relevant concentrations. The combination of tocainide and quinidine may be beneficial for several reasons. In the presence of quinidine, addition of tocainide can provide an extra depression of conduction of early extrasystoles by blocking those channels that are not occupied by quinidine while producing little further depression of basic beats since block of sodium channels by tocainide would be expected to dissipate rapidly. Such a combination may be beneficial in suppressing early extrasystoles that contribute to the generation of a

reentry mechanism.

The prolongation of the time constant of recovery of $\dot{V}_{max}$ at depolarized membrane potentials and a shift of the $\dot{V}_{max}$-membrane potential relationship to more hyperpolarized potentials by the tocainide component of the combination may have important implications in the treatment of ischemia induced arrhythmias. The combination would be expected to produce a greater depression of conduction in localized ischemic depolarized tissue without causing as much further depression in normal fully polarized tissue in the rest of the heart compared to that produced by quinidine alone. Thus, unidirectional block in partially depolarized ischemic tissue which is conducive to reentrant arrhythmias may be converted to bidirectional block resulting in abolition of the reentrant mechanism.

A drug such as tocainide which selectively blocks conduction in depolarized tissue and also shortens action potential duration can also facilitate induction of reentrant arrhythmias (Sasyniuk, 1984; Sasyniuk & McQuillan, 1985). The advantage of combining such a drug with quinidine is that selective depression of conduction in depolarized tissue is combined with prolongation of refractory period in fully polarized tissue.

The combination may also have beneficial effects in the treatment of arrhythmias due to enhanced automaticity. A combination of tocainide and quinidine suppresses Purkinje fiber automaticity much more than quinidine alone (Valois & Sasyniuk, unpublished observations). The escape intervals following a train of stimuli were very much longer in the presence of the combination compared to quinidine alone.

Tocainide may enhance the antiarrhythmic action of quinidine while

at the same time preventing quinidine induced toxicity. Quinidine induced increase in the QTc interval particularly in the presence of slow heart rates and low potassium concentration has been associated with Torsade de Pointes type of arrhythmias. Roden & Hoffman (1985) have shown that at cycle lengths greater than 4000 msec in low extracellular potassium, quinidine produced early after-depolarizations in canine Purkinje fibers. Early after-depolarizations were eliminated by interventions which abbreviated the action potential duration. They suggested that this form of triggered activity may play a role in quinidine induced Torsade de Pointes. We also found that tocainide, by preventing the marked prolongation of action potential duration by quinidine, particularly at slow heart rates and low potassium concentrations, abolished early after-depolarizations produced by quinidine (*Figure 1.10*). Hence, a combination of tocainide and quinidine might be expected to prevent the development of quinidine-induced Torsade de Pointes arrhythmias.

Thus, the present study provides a rationale for a possible enhanced antiarrhythmic efficacy of a combination of two Class I agents which have different kinetics of interaction with the sodium channel. Clinical instances often occur in which drugs with rapid kinetics are not quite effective and maximum tolerated doses of drugs with slow kinetics do not achieve the required suppression of extrasystoles. Thus, a combination of Class I drugs having slow and rapid recovery kinetics may provide a therapeutic efficacy not obtainable with either drug alone.

The beneficial effects of such a combination for suppression of ventricular extrasystoles has already been demonstrated in two clinical studies (Breithardt *et al*, 1981; Duff *et al*, 1983). In the study of Duff *et al* (1983), quinidine was combined with another lidocaine congener, mexiletine. Although

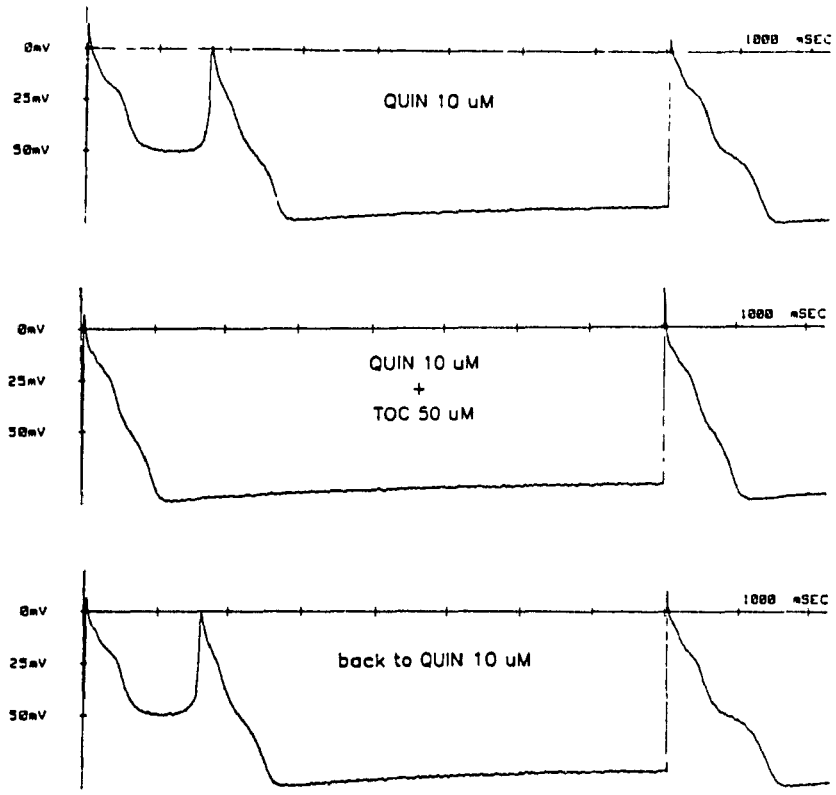

Figure 1.10. Abolition of early after-depolarizations (EADs) induced by 10 μM of quinidine in a preparation superfused with a modified Tyrode's solution (2.7 mM potassium, 12 mM $NaHCO_3$) and driven at a basic cycle length of 8 sec. Upper panel: EADs were obtained within 5 min of switching to a low rate of stimulation and continued in a stable alternating fashion. Middle panel: EADs were abolished after switching to a combination of 10 μM of quinidine and 50 μM of tocainide. Abolition was accompanied by shortening of action potential duration. Bottom panel: EADs reestablished after switching back to quinidine alone. All potentials shown are digitized traces. Similar results were obtained in 3 other experiments.

tocainide has rapid recovery kinetics compared to quinidine its kinetics are slower than those of lidocaine or mexiletine. This is probably related to tocainide's poor lipid solubility and thus more restricted access to the receptor in the chan nel (Courtney, 1980a).

ACKNOWLEDGEMENTS

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CHAPTER II

CHARACTERIZATION OF QUINIDINE-INDUCED TRIGGERED ACTIVITY AND ITS MODULATION BY CLASS IB DRUGS

Current Status: Valois M, Sasyniuk BI. In Review with *J Cardiovasc Pharmacol*

ABSTRACT

The present study examines the conditions under which quinidine induces early afterdepolarizations (EADs) that trigger action potentials and characterizes the cycle length dependence of the triggered activity. In bar-bell preparations of canine Purkinje fibers exposed to quinidine (5 to 10 μM) and hypokalemia ($[K^+]_o$ = 2.7 mM), acidosis (pH = 7.06 ± .08) was found to contribute to bradycardia-dependent action potential prolongation through its direct membrane effect. The degree of action potential prolongation seemed more important in the generation of triggered activity than the degree of bradycardia itself. Quinidine induced two types of triggered activity, namely arising from low membrane potentials (LMP) or from high membrane potentials (HMP). LMP triggered activity was more likely seen at normal pH whereas HMP triggered activity was seen both at normal and low pH. The characteristics of LMP triggered action potentials (amplitude, coupling interval, and activation voltage) were much less sensitive to changes in rate of stimulation that those of HMP triggered action potentials. As the rate of stimulation was decreased, HMP triggered action potentials occurred at more positive potentials and shorter coupling intervals. They were elicited over a narrow range of activation voltages (-45 to -60 mV), but occurred over a rather wide range of coupling intervals (600 to 2300 msec). Once HMP triggered responses were initiated, there was a positive relationship between their activation voltage and their amplitude. Class Ib antiarrhythmic drugs were not equally effective at abolishing LMP and HMP triggered activity. Therapeutic range of concentrations of mexiletine or tocainide abolished HMP triggered responses within minutes by progressively increasing their coupling intervals while shifting their activation voltages towards more negative potentials. Higher

concentrations of mexiletine and longer time periods were needed for the reversal of LMP responses. We conclude that the window and/or the steady state components of the sodium current may be important for the generation of triggered activity induced by HMP EADs and that our *in vitro* model of quinidine-induced triggered activity has several parallels with the arrhythmia torsade de pointes. However, the very slow rates of stimulation needed for the emergence of HMP triggered activity (mean minimum cycle length = 5.83 sec) and the prolonged coupling interval of HMP triggered responses (mean = 1042 msec) constitute features not compatible with clinical observations pertaining to prolonged QT related arrhythmias.

INTRODUCTION

Torsade de pointes arrhythmias are commonly associated with QT prolongation and have been reported to occur after the administration of several antiarrhythmic drugs including quinidine (Bauman *et al*, 1984; Roden *et al*, 1986a; Roden *et al*, 1986b), disopyramide (Wald *et al*, 1981; Schweitzer & Mark, 1982), N-acetylprocainamide (Olshansky *et al*, 1982), and sotalol (Kuck *et al*, 1984; Bennett *et al*, 1985). The most frequently reported drug associated with this arrhythmia is quinidine (Stratmann & Kennedy, 1987). Quinidine induced arrhythmias usually occur in the presence of normal or low plasma quinidine concentration and in the absence of marked QRS prolongation. These arrhythmias are usually treated by pacing or the administration of catecholamines (Keren *et al*, 1981). However, recent clinical reports indicate successful suppression of drug-induced torsade de pointes arrhythmias by administration of Class Ib drugs (Kellerman *et al*, 1982;

Shah & Schwartz, 1984; Thomas & Giles 1985; Bansal *et al*, 1986).

The mechanism underlying quinidine induced torsade de pointes arrhythmias is not well defined. Several laboratories have implicated bradycardia dependent triggered activity induced by early after depolarizations (EADs) as the initiating event (Brachman *et al*, 1983; Coulombe *et al*, 1985; Roden & Hoffman, 1985; Levine *et al*, 1985; El-Sherif *et al*, 1988; Kaseda *et al*, 1989). EAD induced triggered activity has been observed in isolated canine Purkinje fibers exposed to quinidine at slow stimulation rates and in the presence of hypokalemia (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Davidenko *et al*, 1989).

Previous studies do not differentiate between the early afterdepolarizations induced by quinidine and the rhythmic activity which they may generate. Furthermore, with the exception of the recent study by Davidenko *et al* (1989), they do not quantify the role of heart rate in their generation.

The purpose of the present investigation was to examine the conditions under which quinidine induces EADs and triggered activity consistently and reproducibly, to examine their characteristics, to determine the precise role of frequency in their initiation and to define more precisely the mechanism by which Class Ib antiarrhythmic drugs may abolish or prevent their initiation. We have already shown previously that tocainide abolishes quinidine-induced triggered responses (Valois & Sasyniuk, 1987).

Preliminary results have been published previously in abstract form (Valois & Sasyniuk, 1988; Valois & Sasyniuk, 1989).

METHODS

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg). The heart was quickly removed through a left thoracotomy and thoroughly flushed with cold oxygenated Tyrode's solution. Free running Purkinje fiber bundles with small pieces of attached ventricular muscle were dissected predominantly from the left ventricle and placed in a tissue bath. The fibers were superfused initially with control Tyrode's solution of the following composition (in mM): NaCl, 119.0; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHPO_4$, 0.9; dextrose, 5.5; and $NaHCO_3$, 25. pH of the solution was 7.33 ± 0.03. The solution was bubbled with a 95%O_2- 5% CO_2 mixture and the temperature was maintained at 37 ± 0.2° C.

In hypokalemic solutions, the KCl concentration was reduced from 4 mM to 2.7 mM. In acidotic solutions, the $NaHCO_3$ concentration was decreased from 25 to 12 mM and the NaCl concentration was raised to 131 mM to keep the extracellular sodium concentration constant. The pH of this solution was 7.06 ± 0.08 (mean ± S.D.).

CsCl (0.5 to 1 mM) was added to the superfusate in those preparations in which slow spontaneous rates could not be maintained. Although these concentrations of cesium produced some minor additional lengthening of action potential duration at longer cycle lengths of stimulation, neither EADs nor triggered activity were seen under normal, hypokalemic or hypokalemic-acidotic conditions (*Figure 2.1*). Cesium caused little or no change in action potential duration at the cycle lengths and conditions under which quinidine produced a

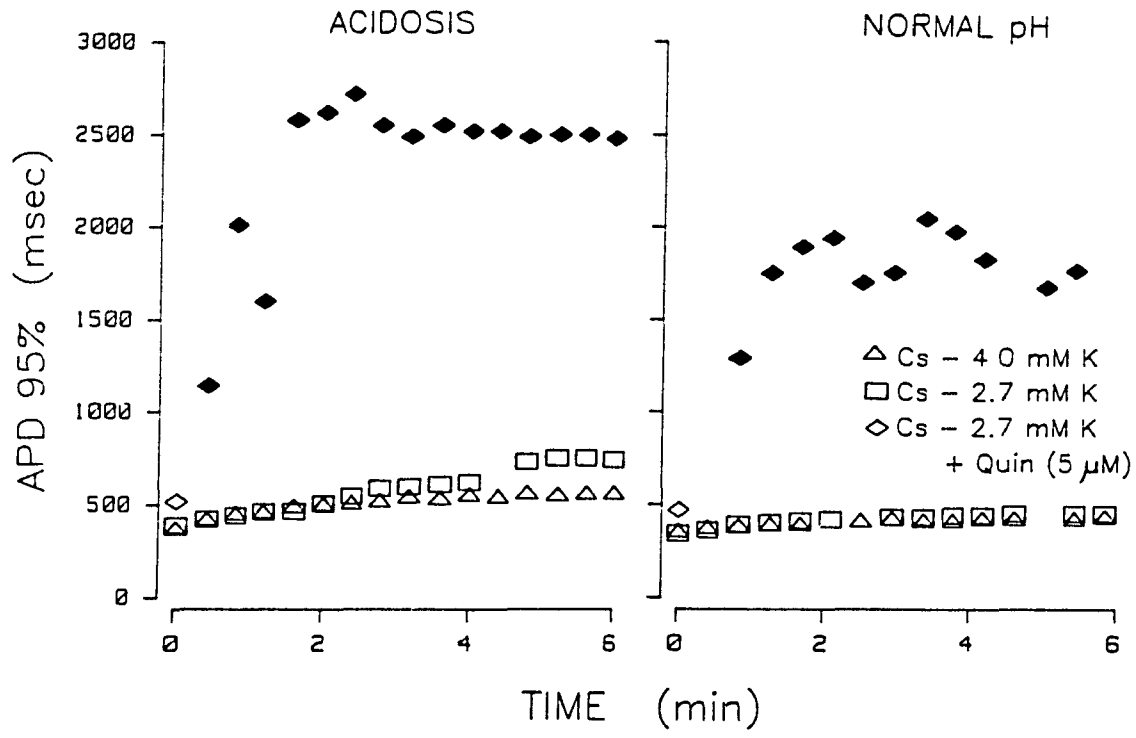

Figure 2.1. Lack of effect of cesium chloride, 1 mM, on action potential duration under conditions in which quinidine produces triggered activity. Data were obtained from two preparations, one with normal pH and one with a pH of 7.1. Each preparation was driven at a basic cycle length of 1 sec which was then abruptly increased to a longer cycle length, 8 sec (left panel) or 5 sec (right panel). Action potential duration is plotted as a function of time after switching to the longer cycle length. The first point of each graph is the last value recorded at a cycle length of 1 second prior to the abrupt increase in cycle length. Triggered activity was never observed under cesium alone under any condition. In the presence of quinidine, triggered activity occurred when action potential duration had exceeded 1 sec. Solid symbols indicate presence of triggered activity.

profound increase. These cesium concentrations have been shown to specifically block the pacemaker current, i_f, in Purkinje fibers (DiFranscesco, 1981). The preparations were exposed to quinidine for at least 60 minutes while being stimulated at a cycle length of 1 sec before any experimental protocol was begun.

Transmembrane potentials were recorded with glass microelectrodes filled with 3 mM KCl and coupled to the input of a high impedance, capacitance neutralized amplifier (Model KS-700, WPI instruments). The output of the KS-700 was displayed on a Tektronix 5113 dual beam storage oscilloscope and simultaneously displayed in digital form on a Data 6000 waveform analyzer (Data Precision, Inc.) for on-line analysis, as described previously (Sasyniuk & Jhamandas, 1984; Valois & Sasyniuk, 1987,. Action potentials were sometimes displayed on a Gould strip chart recorder (Model 2200).

The preparations were stimulated with rectangular pulses delivered through bipolar tungsten electrodes. Electrical stimulation was provided by a Model RS-660 Timing Simulator/Word Generator controlled by an HP 9816 computer. After a stabilization period of at least 1 hour, control transmembrane potential characteristics were measured at a drive cycle length of 1 sec. The preparations were stimulated over a wide range of basic cycle lengths (600 - 15,000 msec) beginning at the longest basic cycle length permitted by the intrinsic automaticity of the preparations. Each cycle length was maintained until either the action potential duration reached a steady state or triggered activity, if present, reached a stable state. After the effect had stabilized at each longer cycle length, stimulation was switched back to a cycle length of 1 sec. When a range of cycle lengths was found which was associated with EADs and/or triggered activity, this range was repeated to test for reproducibility of response.

The variables measured for all driven action potentials included action potential duration (APD) to various levels of repolarization (50%, 75% and 95%), action potential amplitude (AMP), $\dot{V}_{max}$, maximum diastolic potential (MDP) and take-off potential (TOP), i.e. the potential at which the driven action potential was initiated. The difference between TOP and MDP indicated the degree of phase 4 depolarization occurring during long cycle lengths.

EADs and triggered activity were defined according to Cranefield (1975) and Damiano & Rosen (1984). An EAD was considered an afterpotential that interrupts or delays the normal repolarization of the action potential. EADs and the triggered action potentials they induced were classified into two categories based on the membrane potential of the inflection point at which the repolarization process was interrupted (activation voltage). High membrane potential (HMP) EADs were defined as depolarizing afterpotentials that delayed the terminal repolarization phase at a membrane potential more negative than -40 mV. Low membrane potential (LMP) EADs were defined as depolarizing afterpotentials with activation voltages less negative than -30 mV. Occasionally, EADs arose at membrane potentials in between -40 and -30 mV and it was difficult to categorize them. LMP EADs often gave rise to small amplitude early second upstrokes. HMP EADs either caused a delay of repolarization or gave rise to a second nondriven large amplitude upstroke. Characteristics of these triggered action potentials and their preceding diastolic intervals were measured off line from stored digitized traces. Activation voltage (AV) was defined as the membrane potential just prior to initiation of the depolarization phase of the triggered action potential. The amplitude of the triggered action potential was defined as the difference between the activation voltage and the peak of the triggered response.

The coupling interval (CI) of the triggered response was defined as the time from the phase 0 upstroke of the driven action potential to the phase 0 of the upstroke of the triggered action potential. For multiple triggered responses, the coupling intervals of the second and subsequent triggered action potentials were calculated from the upstroke of the preceding triggered response to the upstroke of the subsequent one.

Five to ten μM of quinidine was used. This concentration of drug reflects the therapeutic plasma levels of free drug after correction for protein binding in patients with quinidine induced torsade de pointes arrhythmias (Bauman *et al*, 1984; Roden *et al*, 1986b; Thompson *et al*, 1988). Quinidine used was quinidine sulfate dihydrate obtained from J.T. Baker Chemicals. Mexiletine used was mexiletine hydrochloride generously supplied by Boehringer-Ingelheim. Tocainide used was tocainide hydrochloride generously supplied by Astra Pharmaceuticals. Concentrations ranging from 0.5 to 8 μg/ml - 2 to 36 μM for mexiletine and 9.6 μg/ml - 50 μM for tocainide were used.

All values are indicated as mean ± S.D. Statistical comparisons were made using students t-test for paired and unpaired data. Statistical analysis was performed using one way ANOVA followed by Scheffe's test as indicated. $p < 0.05$ was considered significant.

RESULTS

In initial experiments, we used a superfusion solution in which the extracellular potassium concentration had been decreased. Under conditions of hypokalemia and bradycardia, therapeutic concentrations of quinidine induced

EADs in 7 of 9 (77 %) of preparations and triggered activity in 5 (55%) of them. However, triggered action potentials occurred only episodically in 3 of the 5 preparations even when cesium chloride, 0.5 to 1.0 mM was added to the superfusate to slow the spontaneous rate. Thus, stable triggered activity which was appropriate for the study of its rate dependent characteristics and for the evaluation of drug interventions could be obtained in only 22%.

However, when we also decreased the concentration of bicarbonate in the superfusate to produce a mild acidosis (pH 7.06 ± .08), quinidine induced EADs in 100% of preparations and stable triggered activity in 86% (provided that a certain degree of bradycardia was present). Under these conditions, triggered activity was consistent within a preparation and reproducible from one preparation to another.

The degree of prolongation of APD by quinidine in the presence of hypokalemia versus the combination of hypokalemia and acidosis appeared to determine whether triggered activity would occur. *Figure 2.2* compares the degree of prolongation of APD in the presence of quinidine under normokalemic, hypokalemic and hypokalemic-acido.ic conditions when heart rate was either normal or extremely slow. The degree of bradycardia refers either to the slowest cycle length at which the preparations could be driven in the absence of triggered activity or to the shortest cycle length at which triggered activity appeared in those preparations in which such activity was present. In the presence of each condition, cesium chloride was added if the spontaneous cycle length was less than 3 sec to exclude the absence of a profound bradycardia as a factor. Thus, there was no significant difference in the degree of bradycardia achieved under each condition. However, in any single preparation the automatic rate was invariably slower in the

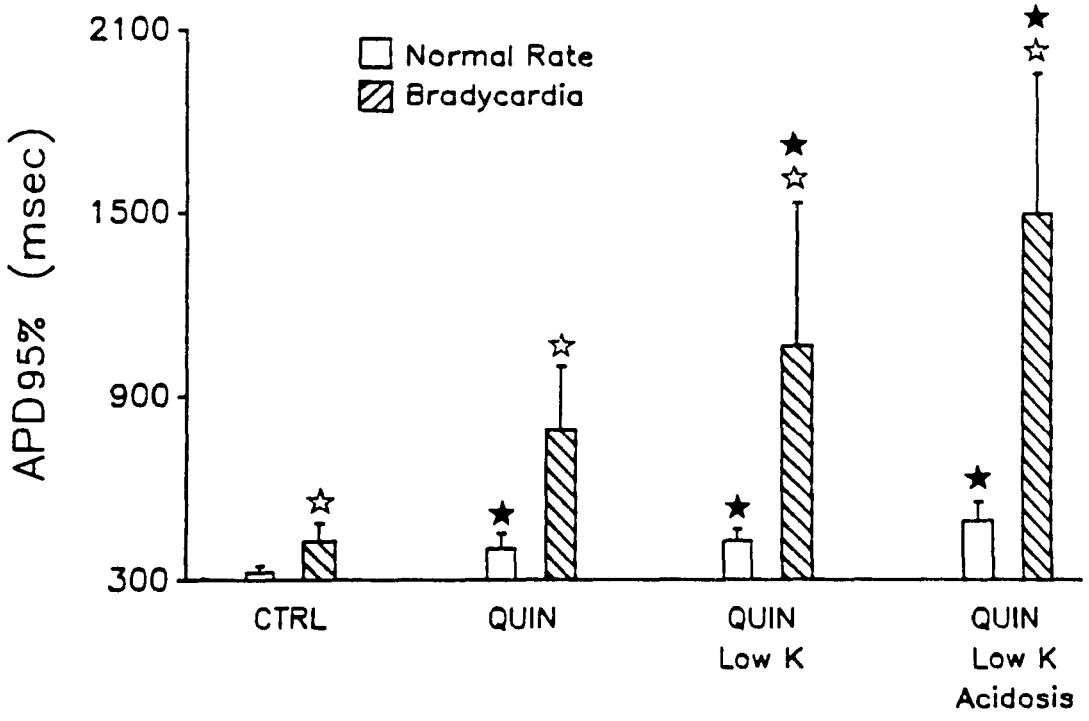

Figure 2.2. Effects of quinidine on action potential duration (APD 95%) during a normal rate (cycle length of 1 sec) versus a bradycardia under conditions of normokalemia, hypokalemia and a combination of hypokalemia and acidosis. The mean basic cycle length during bradycardia was 5.36 ± 1.43 sec (n = 11) under control conditions and 4.50 ± 1.00 (n = 4), 5.89 ± 1.27 (n = 9), and 6.0 ± 2.10 (n = 15) sec, respectively, in the presence of quinidine under the above three conditions. There was no statistically significant difference between bradycardic rates in the four groups. Open stars indicate a statistically significant difference between a bradycardic rate and a normal rate under each condition. Solid stars indicate a significant difference when compared to control.

presence of combined acidosis and hypokalemia than under hypokalemia alone. Cesium was required to slow the spontaneous rate in two-thirds of preparations exposed to hypokalemia alone, but in only 50% of preparations exposed to hypokalemia plus acidosis.

There was a significant difference in the degree of prolongation of APD under each condition in the presence of bradycardia versus a normal heart rate. But the most profound increase in APD during bradycardia was obtained under hypokalemic-acidotic conditions. This correlated with the highest incidence of triggered activity.

In order to further examine the role of acidosis, we evaluated its effect in 5 preparations in which the same bradycardic rate could be maintained under hypokalemic conditions versus hypokalemia plus acidosis in the absence of cesium in order to eliminate both cesium and bradycardia as a factor. *Figure 2.3* shows a typical example. Quinidine caused a further delay in the terminal phase of repolarization when acidosis was present resulting in triggered activity at the appropriate cycle length. Triggered activity was never observed under hypokalemic-acidotic conditions in the absence of quinidine. Clearly, mild acidosis facilitated the generation of triggered activity by quinidine by a mechanism other than the slowing of the spontaneous rate.

Quinidine induced two distinct types of EAD and triggered activity which either occurred independently or in the same action potential. Triggered activity appeared either at the level of the action potential plateau, usually at membrane potentials positive to -30 mV, or at more negative membrane potentials during phase 3 of the action potential.

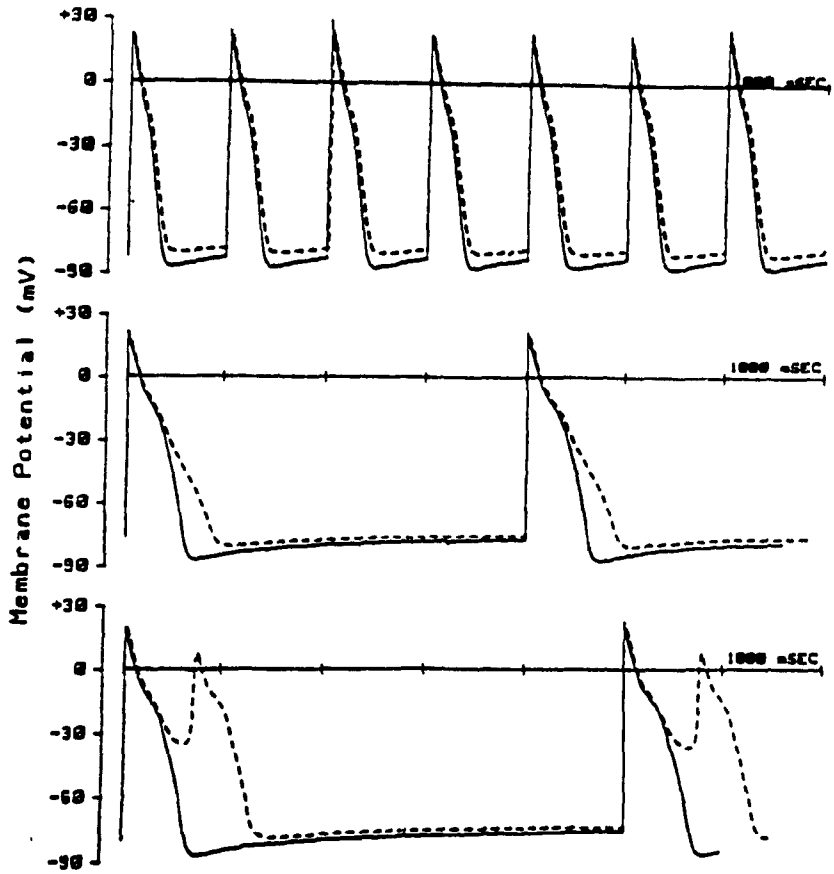

Figure 2.3. **Comparison of quinidine-induced changes in Purkinje fiber repolarization in the presence of hypokalemia versus a combination of hypokalemia and mild acidosis. In each panel, the solid line potentials were obtained in the presence of hypokalemia alone; the dashed line potentials when the concentration of bicarbonate in the superfusate was decreased. At a basic cycle length of 1 sec (upper panel), action potential durations were very similar under both conditions except that phase 4 diastolic depolarization was depressed under acidosis. When basic cycle length was increased to 4 sec (middle panel), a prominent hump (EAD) appeared on the terminal phase of repolarization in the presence of acidosis from which triggered activity arose when the cycle length was decreased further to 5 sec (bottom panel). Concentration of quinidine was 5 μM.**

The two types of triggered activity had different frequency dependence. Triggered activity due to LMP EADs first appeared at cycle lengths as short as 800 msec (mean cycle length 2.8 ± 1.5 sec) and at coupling intervals as short as 195 msec (mean coupling interval 365 ± 211 msec). Triggered activity due to HMP EADs was first manifest at much longer cycle lengths (mean shortest cycle length 5.83 ± 2.42 sec), and occurred at much longer coupling intervals, 1042 ± 320 msec.

We studied a total of 40 preparations; some form of triggered activity was seen in 36 of them. Triggered activity due only to LMP EADs occurred in 30% of preparations; that due to only HMP EADs occurred in 45%, while both types occurred in the same action potential in 25%. LMP EADs were more likely to occur in preparations not exposed to acidosis.

Figure 2.4 shows a typical example of the frequency dependence of activity arising at the level of the plateau in a preparation in which only this type of activity was observed. The major effect of bradycardia was to increase the number of oscillations. With plateau level oscillations, it was often difficult to distinguish an EAD from a triggered action potential. However, there was no correlation between frequency and either the amplitude of oscillations or the coupling interval at which they first appeared. Only the number of oscillations was altered. Thus, LMP EADs were not characterized any further.

The characteristics of triggered activity arising from HMP EADs were also very sensitive to changes in the stimulation rate, but throughout a much lower range of frequencies. *Figure 2.5* illustrates the frequency dependent changes in the steady state pattern of triggered activity arising from HMP EADs. At a basic

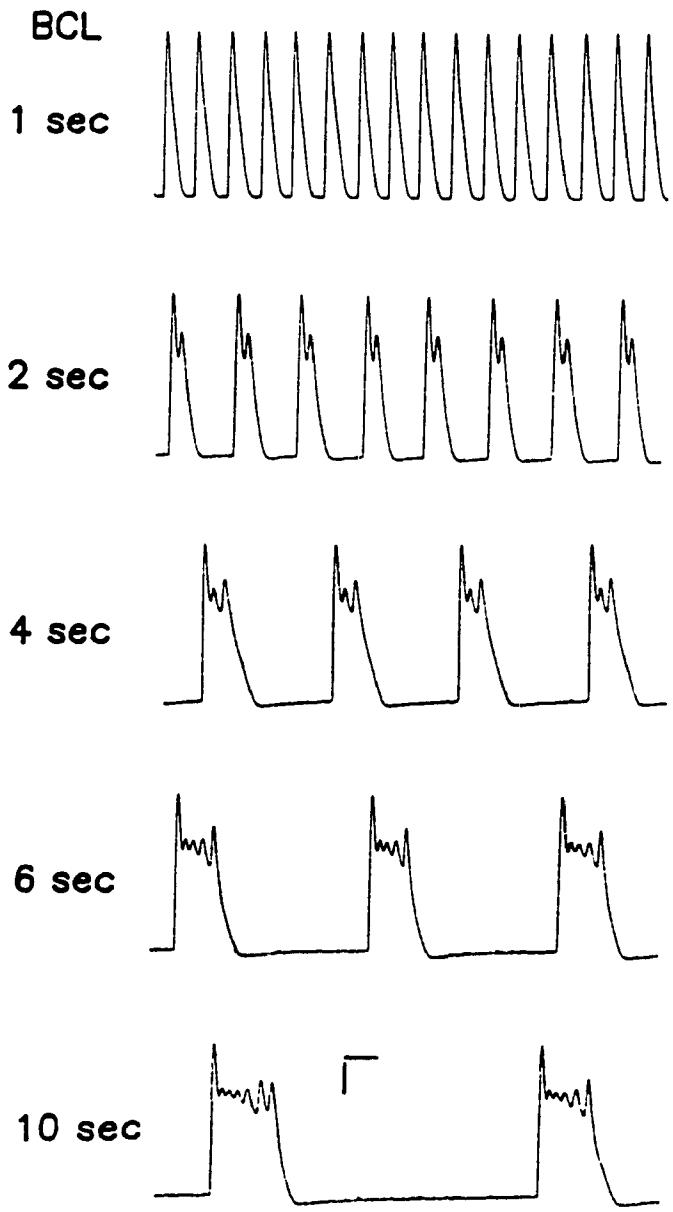

Figure 2.4. Frequency dependence of quinidine induced triggered activity occurring at the level of the plateau. Each panel shows recordings obtained from a Purkinje fiber superfused with a hypokalemic solution containing 5 μM of quinidine. Triggered action potentials first appeared at a basic cycle length of 2 sec. At longer basic cycle lengths progressively longer periods of oscillations followed each action potential. Vertical calibration, 1 second; horizontal calibration, 20 mV.

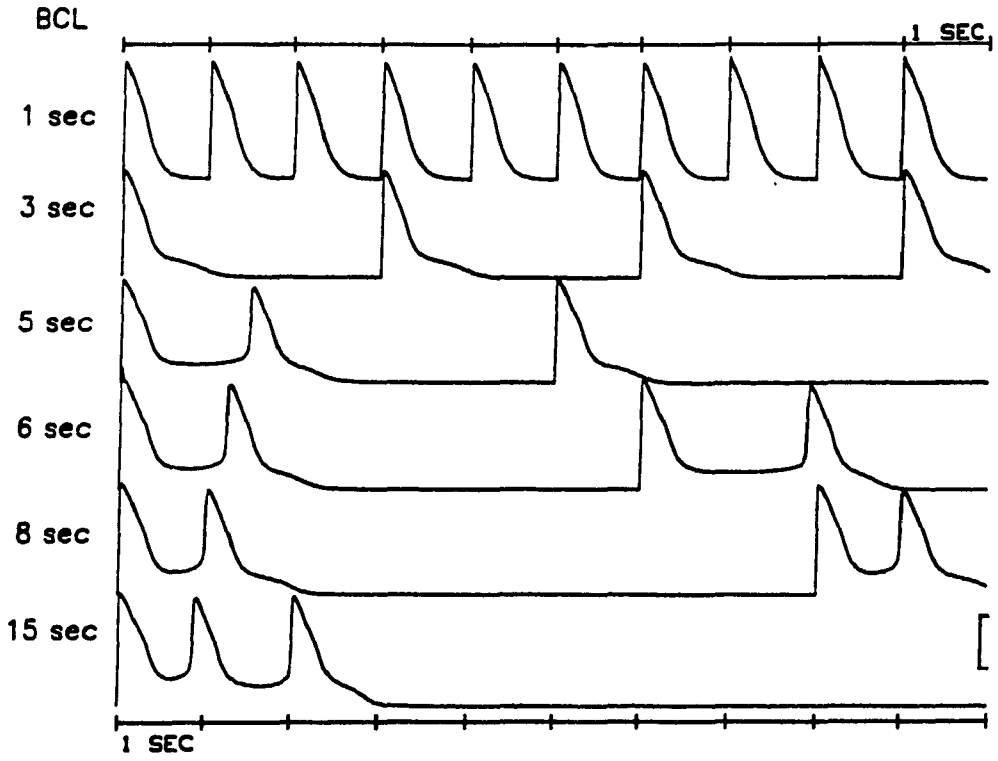

Figure 2.5. Frequency dependence of the pattern of triggered activity arising from HMP EADs. Each panel shows recordings obtained at steady state cycle lengths ranging from 1 to 15 sec following exposure to 10 μM of quinidine under conditions of hypokalemia and acidosis. BCL, basic cycle length. Vertical calibration is 50 mV.

cycle length of 2 sec or less triggered activity was absent. Prominent HMP EADs first appeared at a cycle length of 3 sec. When cycle length was increased to 5 sec, every other action potential was followed by a triggered response arising from a HMP EAD. At a cycle length of 6 sec, three quarters of the action potentials had triggered responses at variable coupling intervals. A consistent bigeminal pattern occurred at a basic cycle length of 8 sec. Finally, at an even longer cycle length of 15 sec, two triggered responses occurred. All triggered responses terminated in a prominent HMP EAD which returned the membrane potential to the resting level. A similar frequency dependent pattern was seen in all preparations in which triggered activity arose from HMP EADs.

Whether HMP EADs resulted in triggered activity depended upon a time and frequency dependent increase in their magnitude until a certain threshold activation voltage was reached. *Figure 2.6* shows a typical example. Following abrupt increases in cycle length to some critical value from a pacing rate of 1 sec, there was a gradual beat to beat increase in the size and duration of the HMP EAD (without a marked change in the plateau phase of the action potential) until during the longest action potential duration a second upstroke (triggered action potential) arose from the EAD. At progressively longer cycle lengths, the beat to beat increase in the magnitude of the EAD occurred progressively earlier and the coupling interval of the resultant triggered action potential was shorter. Similar beat to beat changes in the magnitude of the EAD following the first triggered response occurred until a triggered action potential generated (at very long cycle lengths).

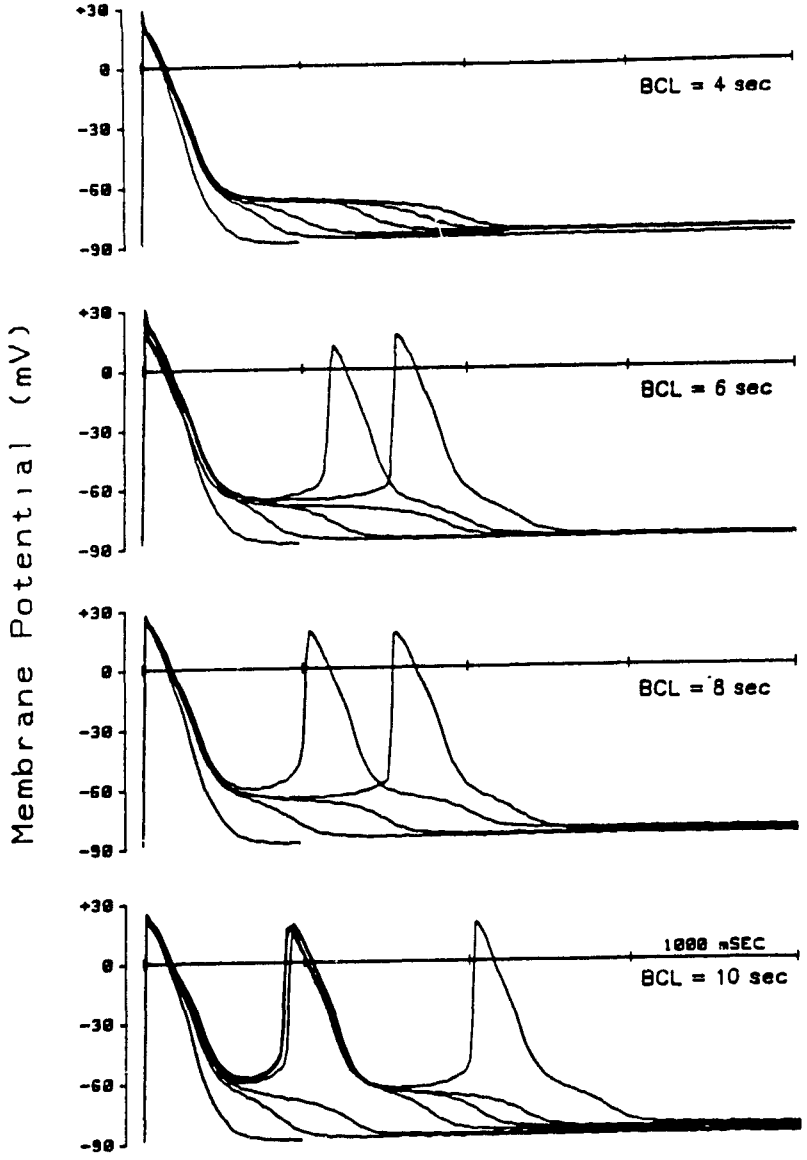

Figure 2.6. Time and frequency dependent changes in the magnitude of quinidine-induced HMP EADs following abrupt increases in basic cycle length (BCL) from a pacing rate of 1 sec. Each panel shows superimposed traces obtained at various times following the increase in cycle length. The first action potential is the last recorded potential at the pacing rate of 1 sec. At a BCL of 4 sec there was a progressive increase in the size and duration of EAD but even after 9.5 min at the slow rate triggered activity never occurred. At steady state, the membrane potential following each action potential remained stable at a depolarized level for over a second before repolarizing back to the diastolic level. At a cycle length of 6 sec, there was a more rapid and greater increase in the magnitude of the EAD which resulted in a triggered response in 2 min 42 sec following the rate change. The activation voltage of the EAD shifted toward more positive values compared to that at 4 sec. At 8 and 10 sec, triggered activity occurred in less than 2 min following the abrupt increase in cycle length. The activation voltage of the EADs was even more positive. At a BCL of 10 sec, there was a progressive increase in the size of the EAD following the first triggered action potential until a second triggered action potential occurred. 10 μM of quinidine was present under hypokalemic-acidotic conditions.

Generation of EADs required several minutes of a low drive rate. EADs were seldom generated after only a single pause following a normal driven rate. Furthermore, EADs generated after a single pause (even after a minute) were never of sufficient magnitude to generate triggered action potentials.

When triggered action potentials occurred over a wide range of cycle lengths, they occurred more closely coupled and at more positive activation voltages as cycle length was increased. At the critical minimum basic cycle length at which triggering occurred, the coupling intervals and activation voltages were variable due to variability in the preceding diastolic intervals as a result of a variability in the size of the EAD. At such a threshold cycle length, there was often a Wenckebach like periodicity in the size of the EAD until a triggered action potential occurred which shortened the immediately preceding diastolic interval. Alternation was also very common at cycle lengths at which triggered activity first appeared.

The variability in the activation voltages at which triggered responses occurred made it possible to plot the relation between the peak amplitude of the triggered response and its activation voltage. Comparison of results obtained in 5 preparations (*Figure 2.7*) showed a steep inverse relation between the peak amplitude of the triggered action potential and its activation voltage. This relation was not identical among preparations. Each preparation differed in the range of activation voltages over which triggered responses occurred and in the maximum peak amplitudes. However, in general, triggered responses occurred over a more positive range of activation voltages when the basic cycle length was very long. At cycle lengths in which two triggered responses occurred, the second response

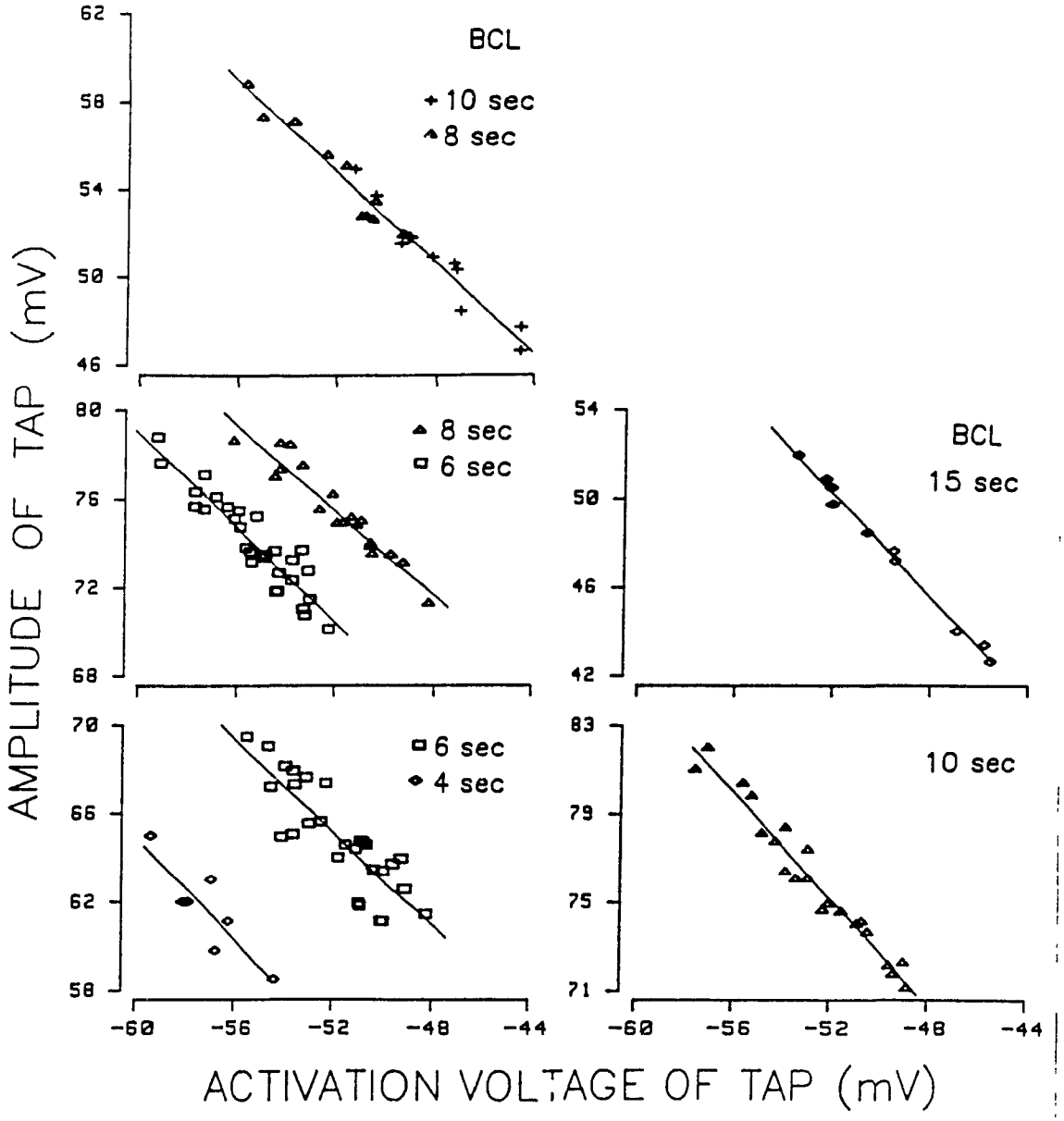

Figure 2.7. Relationship between the amplitude of triggered action potentials (TAP) due to HMP EADs and their activation voltage. Each panel shows this relationship in a different preparation. All were exposed to 10 μM of quinidine and a hypokalemic-acidotic superfusate. In the right hand panels the solid symbols indicate this relationship for a second triggered response. Triggered action potentials occurred at basic cycle lengths (BCL) ranging from 4 to 15 sec.

occurred at more negative voltages and was of larger amplitude than the first triggered response, but followed the same relation as the first response. All responses developed in a voltage range in which the fast sodium current is capable of being activated.

ABOLITION OF TRIGGERED ACTIVITY BY COMBINATION OF QUINIDINE WITH EITHER MEXILETINE OR TOCAINIDE

In 9 preparations triggered activity due to HMP EADs was sustained for a sufficiently long time to allow an evaluation of the effects of therapeutic concentrations of Class Ib drugs. Mexiletine, 0.5 to 2.0 μg/ml (2-8 μM) was evaluated in 6 preparations and, tocainide, 9.6 μg/ml (50 μM) in 3 preparations. These drugs eliminated both HMP EADs and triggered activity after an average of 4.5 $\pm$ 2 minutes of superfusion for cycle lengths ranging from 4 to 10 sec.

A characteristic series of events occurred upon exposure to the Class Ib drug. *Figure 2.8* shows a typical example in a preparation in which each action potential was followed by a HMP EAD which alternately triggered one or two action potentials. Abolition of the triggered response was always preceded by a progressive beat to beat lengthening of its coupling interval and a progressive shift to more negative values of its activation voltage. The upstroke was abolished at the point the activation voltage reached a membrane potential of -60 mV. Eventually EADs were also abolished (thus decreasing the duration of the terminal phase of repolarization, APD 95%) but the duration of the plateau phase of the driven action potential did not change. Triggered responses were eliminated before there were any changes in the prominent EADs, suggesting that the drug

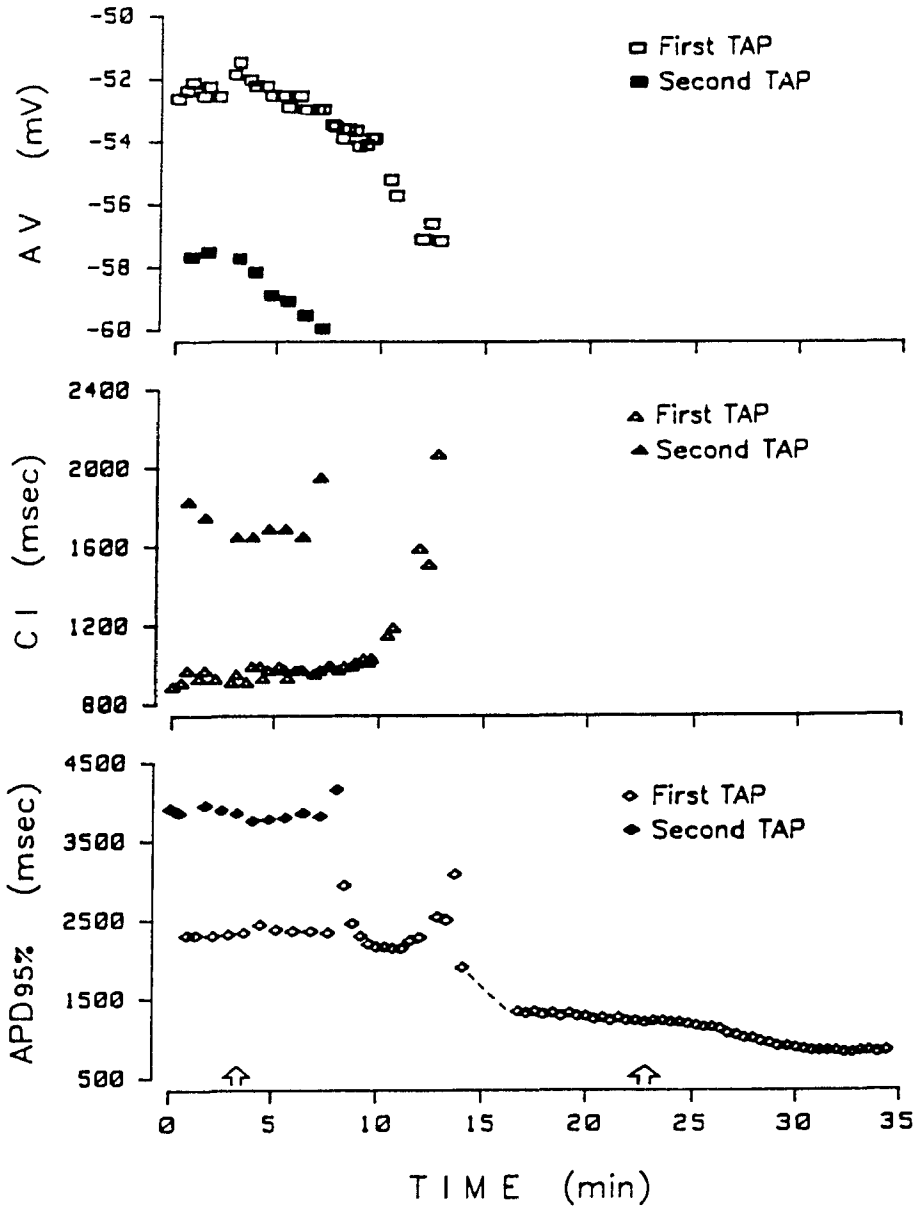

Figure 2.8A. Characteristic sequence of events during abolition of quinidine induced EADs and triggered activity by mexiletine. Action potential duration (APD 95%), coupling interval (CI) of the triggered action potentials (TAP) and their activation voltages (AV) are plotted versus time. The left arrow indicates time of exposure to mexiletine (1 μg/mL). In this preparation, each action potential was followed by EADs which alternately resulted in one or two triggered responses. Mexiletine abolished the second TAP first and then eliminated the first TAP. Abolition was accompanied by a progressive increase in the coupling interval and in the negativity of the activation voltage of the TAP. It was only after abolition of both triggered responses that the APD shortened. At the second arrow, the concentration of mexiletine was increased to 2 μg/mL. This was accompanied by further shortening of APD as the EAD was also eliminated. Dashed line on APD 95% curve indicates time during which records were not obtained.

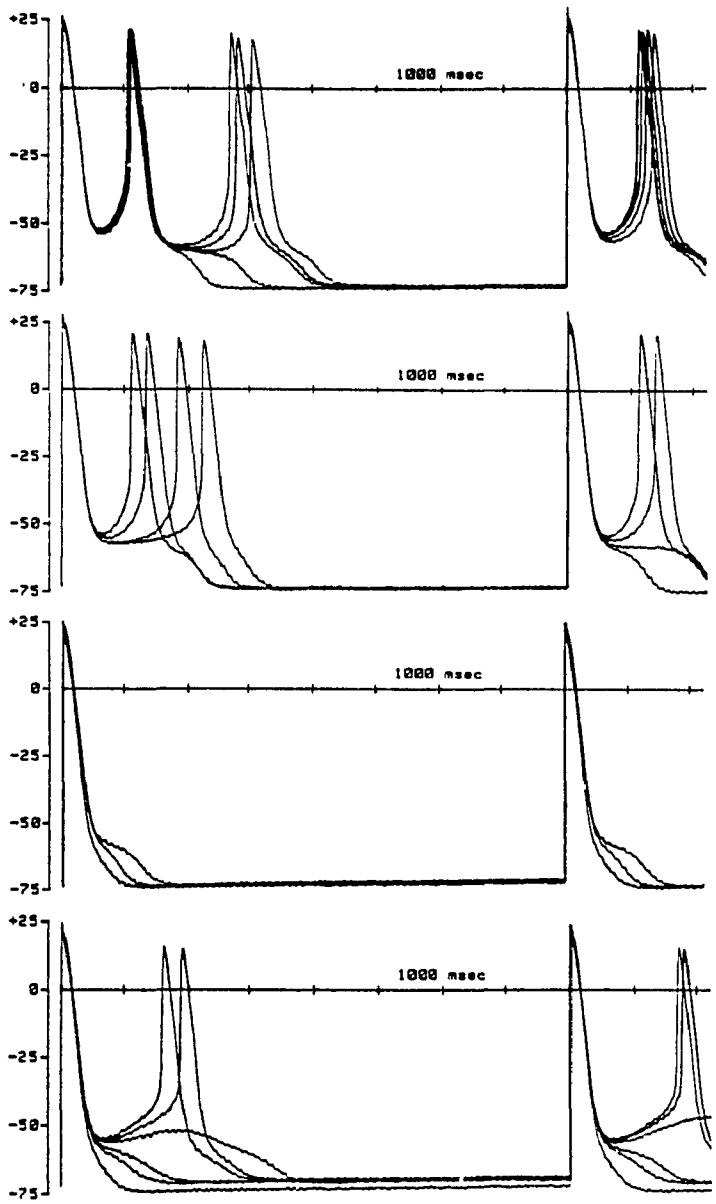

Figure 2.8B. Superimposed action potentials showing characteristic sequence of events plotted in *Figure 8A*. *First panel*: abolition of second triggered action potential following progressive increase in coupling interval. *Second panel*: abolition of the first triggered action potential after exposure to 1 μg/ml of mexiletine with prominent EAD still present. *Third panel*: elimination of the EAD following exposure to 2 μg/ml of drug. *Fourth panel*: reinduction of EADs and triggered action potentials following washout of mexiletine.

had altered an inward current necessary for initiation of the triggered response. Progressive lengthening of the terminal phase of repolarization followed by triggered activity always occurred upon washing out the Class Ib drug.

Mexiletine was less effective in abolishing triggered activity associated with LMP EADs. Higher concentrations of drug were necessary and it took a longer time to be effective. *Figure 2.9* shows a typical example. In the presence of 5 μM of quinidine and hypokalemia triggered activity occurred at low membrane potentials accompanied by a lengthening of the terminal phase of repolarization (HMP EAD). Addition of mexiletine, 4 μg/mL, lengthened the coupling interval of the triggered response and increased the membrane potential from which it was initiated but did not abolish triggered activity. 8 μg/mL of mexiletine was necessary to abolish triggered activity. This was accompanied by a shortening of total action potential duration. Upon washout of mexiletine, triggered activity returned. Similar effects were observed in 3 other preparations.

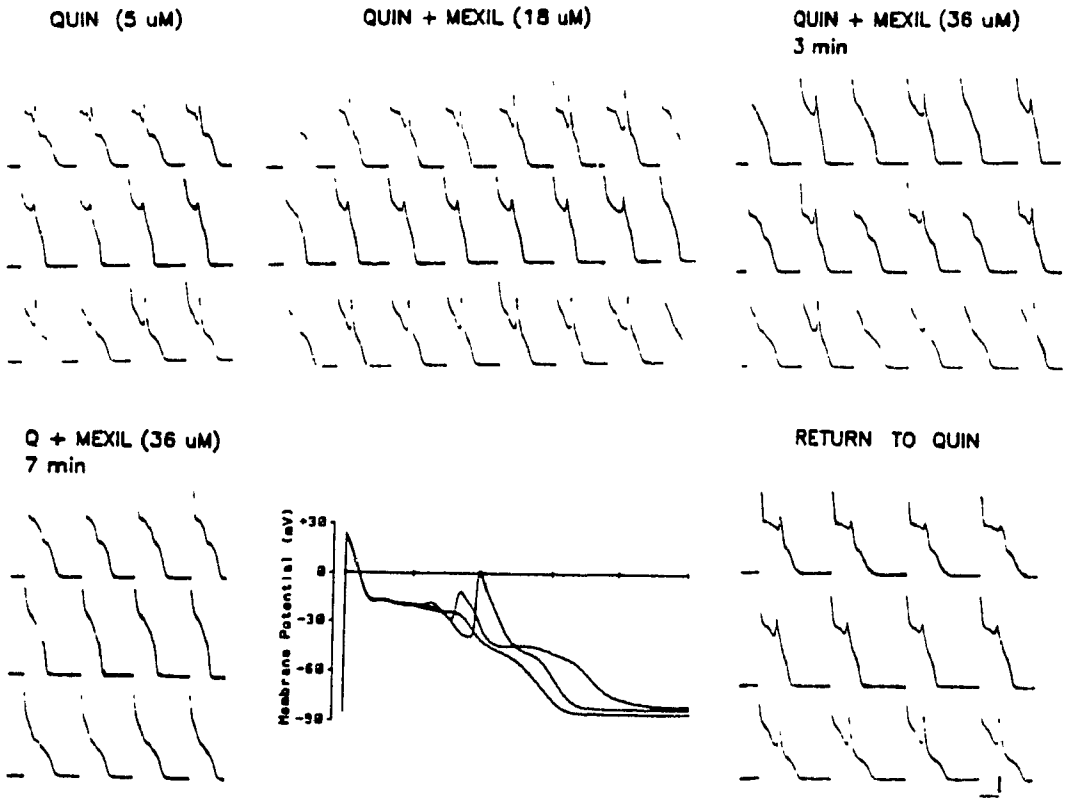

Figure 2.9. Abolition of triggered action potentials arising during the plateau phase by high doses of mexiletine. Each panel shows three simultaneous records obtained from the septal insertion of the free running strand, the papillary muscle insertion of the free running strand and from a subendocardial Purkinje fiber at the tip of the papillary muscle. In the bottom middle panel are superimposed waveforms obtained from the septal end of the free running strand before and after exposure to mexiletine showing abolition of triggered activity after exposure to mexiletine, 8 μg/ml. Basic cycle length was 3 sec. Horinzontal calibration is 500 msec. Abolition was accompanied by a shortening of action potential duration and an increase in negativity of the activation voltage of the triggered response to -42 mv. Triggered activity returned after washing out the mexiletine, but only at a basic cycle length of 4 sec. Horizontal calibration 1 sec; vertical calibration 20 mV.

DISCUSSION

In the present study, we describe the characteristics of quinidine induced triggered activity in terms of its dependence on pH of the superfusate and frequency of stimulation in preparations of false tendons attached to ventricular muscle. In the presence of hypokalemia and mild acidosis, triggered activity could be obtained consistently and reproducibly provided that extreme bradycardia was also present. Failure to obtain triggered activity was usually due to an increase in automaticity in the presence of hypokalemia and the failure to attain a sufficiently long cycle length to allow triggered activity to be manifest.

We observed two types of EADs, those arising at the level of the action potential plateau (which we call low membrane potential - LMP - EADs) and those arising during the terminal phase 3 of the action potential (which we call high membrane potential - HMP - EADs). LMP EADs were more likely to occur under conditions of normal pH and hypokalemia and were usually less consistent. Acidosis favored the induction of HMP EADs which occurred either alone or together with LMP EADs in the majority of preparations.

Both types of triggered activity were sensitive to changes in the stimulation frequency, but these effects were less well defined for LMP EADs. LMP EADs occurred in those fibers in which an increase in the cycle length was accompanied by a particular prolongation of duration at the level of the action potential plateau. This was not usually the major effect of quinidine. Whenever both types of EADs occurred in the same preparation, LMP EADs always occurred

earlier and at shorter cycle lengths than those at which HMP EADs were first manifest. Increases in the cycle length led to an increase in the incidence of such activity (also see Sasyniuk *et al*, 1989). However, in any one preparation, there did not appear to be any correlation between frequency and the amplitude of EADs nor the coupling interval at which they occurred. Because LMP EADs occurred less consistently, their characteristics were not studied further.

Davidenko *et al* (1989) also distinguished two types of EAD and triggered activity in preparations exposed to quinidine under normo- and hypokalemic conditions. However, the two types occurred only under normokalemic conditions. Phase 2 triggered activity was never observed under hypokalemic conditions. The incidence of both types of triggered activity also depended on the rate of stimulation, but throughout a much faster range of frequencies than in the present study. This difference may be related in part to anatomic differences (eg. the absence of ventricular muscle in their preparations). Ventricular muscle would normally act as a current sink tending to repolarize Purkinje fibers adjacent to them via electrotonic interactions. Whenever triggered activity could not be obtained in our preparations, severing the Purkinje strand from its connections to ventricular muscle invariably induced them (unpublished observations).

In those experiments in which HMP EADs occurred, they could be readily induced or eliminated merely by changes in cycle length. In each preparation, there was a well defined relationship between frequency of stimulation and incidence of triggered activity at steady state. HMP EAD-induced triggered action potentials were sensitive to changes in stimulation frequency throughout a much lower range of frequencies (mean shortest BCL = 5.83 sec). However, the

degree of prolongation of the terminal phase of the action potential was more important in determining the emergence of triggered activity than the degree of bradycardia *per se*. We showed that under similar conditions of bradycardia, the prolongation of the terminal phase of repolarization by quinidine was much more marked in the presence of hypokalemia and acidosis than under hypokalemia alone and thus, the incidence of triggered activity was higher. This result suggests that it was the direct effect of acidosis on membrane currents which was important in facilitating the manifestation of triggered activity rather than the indirect effects in slowing the spontaneous rate.

In the presence of quinidine, hypokalemia and acidosis, there was usually minimal prolongation of the plateau phase of the action potential following an abrupt deceleration of rate (i.e. beyond that already present at a cycle length of one second). Only the terminal phase of repolarization (at potentials more negative than - 40 mV) was seen to significantly prolong. This was particularly striking when mild acidosis was also present. Thus, prolongation of the plateau phase of the action potential was not critical to the development of HMP EADs and triggered action potentials. Rather a cycle length dependent lengthening of action potential duration at membrane potentials more negative than -40 mV and exceeding 1000 msec was a prerequisite for initiation of triggered activity.

Quinidine's effect on generation of HMP EADs was markedly dependent not only upon stimulation frequency *per se*, but also upon the time after the rate change. After an abrupt increase in cycle length, the magnitude of the EAD increases progressively with time until a triggered action potential occurs. The activation voltage of the EAD becomes progressively more positive until a membrane potential is reached at which a second upstroke takes off. This process

must involve a progressive decline in an outward current until a point is reached when the net current becomes inward. A sufficient degree of depolarization then brings the membrane to the level at which there is a regenerative increase in an inward current. Thus, initiation of triggered activity must involve both a time and voltage dependent mechanism.

Once triggered action potentials were initiated, a steep relation existed between their amplitudes and activation voltages. There was an inverse relationship between cycle length and the activation voltage, the longer the former, the less negative the latter. In all experiments, triggered action potentials were elicited over a fairly narrow range of activation voltages (-40 to -60 mV), but occurred over a rather wide range of coupling intervals.

The development of conditions needed to initiate triggered activity reflects the sum of the currents that regulate the terminal phase of repolarization in Purkinje fibers. These include the steady state "window" (Attwell *et al*, 1979) or the slowly inactivating (Gintant *et al*, 1984; Saikawa & Carmeliet, 1982) components of the sodium current, the potassium background current i_{k1} (Carmeliet, 1982), the delayed outward rectifying current (Gintant *et al*, 1985), and the sodium-potassium pump current (Gadsby & Cranefield, 1979). Thus, conditions that diminish repolarizing current relative to depolarizing current at voltages more negative than -40 mV would favor induction of triggered activity.

Since quinidine would be expected to produce lesser block of sodium channels at the slow rates at which triggered activity is manifest (Valois & Sasyniuk, 1987), the progressive decline of an outward current with increase in cycle length would allow the inward currents flowing during repolarization to be

unmasked. These inward currents may be the sodium "window" current and/or its slowly inactivating component. The voltage dependence of the "window" current is such that it peaks at the potentials at which HMP EADs become manifest.

The progressive decline in an outward current may be attributed to quinidine's effect in blocking the delayed rectifier which has clearly been shown to be bradycardia and disuse dependent. Roden and coworkers (1988) reported that quinidine's effect on the delayed rectifier, I_K, is to preferentially block the rested state of the channel at the negative membrane potentials present during diastole and to unblock during the action potential. Hypokalemia would facilitate the blocking of the delayed rectifier by keeping the membrane potential at a more negative level (Roden & Iansmith, 1987). In fact, we found that if the fibers depolarized to levels more positive than -70 mV in the presence of acidosis, quinidine, and a slow rate, triggered activity was less likely to occur, if at all.

Part of the reduction in the outward current may be attributed to a reduction in electrogenic Na^+/K^+ exchange which would be expected to occur during a period of low stimulation frequency, thereby diminishing the intensity of the outward current generated by the electrogenic pump (Gadsby, 1985). In fact, the longer the cycle length during deceleration, the earlier was the appearance of triggered activity. Rate alone, however, was usually insufficient to induce EADs in the presence of quinidine. Lowering the extracellular potassium concentration would further contribute to a reduction of Na^+/K^+ exchange. Low extracellular potassium also lowers the permeability of the membrane to potassium. Acidosis has been shown to depress the time-dependent potassium conductance and to shift the steady state current-voltage relationship in the inward direction in Purkinje fibers at membrane potentials negative to -10 mV (Brown *et al*, 1978). Thus,

reduction of stimulation frequency, hypokalemia and acidosis may all act in a synergistic way with quinidine to induce early afterdepolarizations.

It seems clear that triggered activity occurring during the terminal phase of repolarization is very sensitive to abolition by Class Ib drugs. The mechanism of abolition appears to result from a change in the balance between the inward and outward currents so that the cell must repolarize to more negative values before it can generate enough inward current.

Class Ib drugs can prevent triggered activity by a number of different mechanisms. These drugs decrease the fast inward sodium current in a voltage dependent manner (Sánchez-Chapula *et al*, 1983; Valois & Sasyniuk, 1987) and have their greatest effect at those membrane potentials at which triggered activity occurs. Thus, these drugs likely decrease the excitability of the membrane, thereby increasing the threshold for initiation of triggered activity by the EADs. EADs, while still present, become ineffective in initiating triggered responses. The voltages at which HMP triggered activity occurs are those in which there are the greatest number of reopenings of Na^+ channels. It is the reopenings of these channels which are most sensitive to lidocaine (Hondeghem & Bennett, 1989). If other Class Ib drugs act similarly, inhibition of reopening of sodium channels may be the mechanism by which mexiletine and tocainide abolish triggered activity. Class Ib drugs eventually eliminated the HMP EAD and shortened the entire action potential. However, this does not seem to be the mechanism of the abolition, since the upstroke of triggered action potentials was abolished prior to elimination of the EAD. This disagrees with the conclusions of Nattel & Quantz (1988) that termination of quinidine induced triggered activity by tetrodotoxin and sodium channel blocking drugs is due to abbreviation of action potential duration.

The effectiveness of Class Ib supports the suggestion that sodium window current or the slowly inactivating component of the sodium current may be important for the generation of triggered activity induced by HMP EADs. Further support for the involvement of the sodium "window" current is the computer simulation of EADs and triggered activity by Coulombe *et al* (1980, 1985) under conditions of hypokalemia and profound acidosis.

The mechanism for quinidine induced triggered activity and EADs arising at the level of the action potential plateau is less clear. January *et al* (1988) examined the mechanism of EADs induced near plateau voltages with the calcium current agonist Bay K 8644. They concluded that the induction of EADs at plateau voltages required a lengthening and flattening of the plateau within a voltage range where recovery from inactivation and reactivation of L-type calcium channels can occur. Calcium overload with the concomitant induction of an I_{Ti}-like current was not required to elicit EADs. A similar conclusion was reached by Marban *et al* (1986) for EADs induced by cesium. January & Riddle (1989) showed that drugs (both calcium and sodium channel blocking drugs) and ionic interventions that suppress EADs and shorten action potential duration shift the steady state current voltage relation outwardly near action potential plateau voltages. Nattel & Quantz (1988) showed that calcium channel blockers abolish quinidine induced triggered activity without changing the magnitude of EADs. Their results, based on measurements of action potential duration, are difficult to interpret because heart rate was not kept constant before and after addition of drug and also because of the known property of these drugs to indirectly affect potassium permeability (i.e. the background outward current (Bassingwaighte *et al*, 1976) and the delayed rectifier (Colatsky & Hogan, 1980)) by decreasing i_{si}.

Class Ib drugs were also effective in abolishing LMP EADs in the present study. However, high concentrations of drug were required and it took 20 - 30 minutes to eliminate the triggered activity versus only a few minutes for elimination of HMP triggered activity. Elimination of LMP EADs was accompanied by shortening of action potential duration. If the mechanism of quinidine induced LMP EADs is similar to that proposed by January *et al* (1988), then shortening of the action potential by a drug which decreases the sodium "window" current should abolish this activity. However, the high concentrations required make it unlikely that such a mechanism would be effective clinically.

Quinidine-induced polymorphous ventricular tachycardia known as torsade de pointes is frequently associated with hypokalemia, therapeutic plasma levels of quinidine and a slow heart rate or long pauses (Bauman *et al*, 1984; Roden *et al*, 1986b; Jackman *et al*, 1988). Furthermore, such arrhythmias have been shown to be effectively controlled by Class Ib drugs (Kaplinsky *et al*, 1972; Kellerman *et al*, 1982; Shah & Schwartz, 1984; Thomas & Giles 1985; Bansal *et al*, 1986). A number of investigators have suggested that these arrhythmias may be precipitated by triggered responses arising from EADs that develop in Purkinje fibers (Brachman *et al*, 1983; Cranefield & Aronson, 1988; Jackman *et al*, 1988). Although our data and that of others (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Davidenko *et al*, 1989) show a clear congruity between the conditions that predispose to torsade de pointes and those under which quinidine induces triggered activity, there are several inconsistencies between the two which will have to be addressed before observations in the tissue bath can be extrapolated to the clinic.

In the present study, single pauses up to 15 sec failed to induce triggered activity. Progressive prolongation of action potential duration over a

number of beats was necessary for induction of triggered activity. Furthermore, the cycle lengths required to induce triggered activity *in vitro* in preparations in which Purkinje fibers are not isolated, but connected to muscle were very much longer than those which occur clinically. The average coupling interval at which triggered activity occurs is very much longer than the coupling intervals of the premature beats which trigger episodes of torsade de pointes arrhythmias.

The discrepancy between frequency dependent characteristics of triggered activity *in vitro* and the behavior of torsade de pointes arrhythmias poses a number of possibilities. Perhaps, triggered activity can play a role in the clinical arrhythmia when it is restricted to a protected parasystolic focus with entrance block and exit conduction. The conditions *in vivo* may be such that the phenomenon can occur at shorter cycle lengths. Likewise, conditions of altered sympathetic tone or conditions of altered calcium loading may allow the phenomenon to be induced more readily *in vivo* and to enhance the frequency of its generation.

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CHAPTER III

MODULATION OF QUINIDINE-INDUCED TRIGGERED ACTIVITY BY ADRENALINE

Current Status: Valois M, Sasyniuk BI. Submitted to *J Pharmacol Exp Ther*

ABSTRACT

The present study examines the modulation of quinidine-induced triggered activity by adrenaline in preparations of false tendons attached to ventricular muscle with multiple recording sites from the free-running Purkinje strand and the subendocardial portion (Purkinje or muscle) of the preparation. In preparations in which quinidine (5 μM) induced triggered activity in the presence of low $[K^+]_o$ (2.7 mM), adrenaline was found to facilitate its manifestation by significantly decreasing the minimum cycle length for triggered activity from 6.0 ± 2.16 to 3.25 ± 1.5 sec (means ± S.D.) ($p < 0.05$), shortening the coupling interval of high membrane potential (HMP) triggered responses, and transforming single triggered responses into multiple. In preparations in which triggered activity was not seen in the presence of quinidine and low $[K^+]_o$, adrenaline induced triggered responses arising from the plateau that were associated with extra ventricular muscle responses with an average coupling interval of 517 ± 87 msec (mean ± S.D.). The induction of triggered activity occurred despite increased rate of automaticity seen in preparations beating spontaneously. Whenever the acceleration of automaticity was too pronounced, adrenaline eventually abolished triggered activity after a transient potentiation. Adrenaline also induced activity resembling sustained triggered activity as well as triggered responses that were associated with multiple extra-systoles. We conclude that the presence of adrenaline makes quinidine-induced triggered activity a stronger model for bradycardia-dependent arrhythmias by allowing it to occur at rates of activity closer to a physiologically relevant range and by facilitating the transmission of triggered action potentials to ventricular muscle.

INTRODUCTION

Torsade de pointes, as a complication of antiarrhythmic therapy with agents such as quinidine that prolong cardiac repolarization, is a distinct clinical and electrophysiologic entity. However, the precise mechanism that underlies this abnormality is still not well defined. Arrhythmias related to QT prolongation have traditionally been ascribed to heterogeneity of repolarization in the ventricles (Surawicz & Knoebel, 1984; Surawicz, 1989). Such heterogeneity would be expected to increase in the presence of hypokalemia and abrupt changes in cycle length. However, no existing experimental model dependent on increased dispersion resembles conditions encountered clinically.

Data from several laboratories, including our own, have shown that concentrations of quinidine associated with torsade de pointes cause early afterdepolarizations (EADs) in canine Purkinje fibers in the presence of hypokalemia and slow stimulation rates (Roden & Hoffman, 1985; Valois & Sasyniuk, 1987; Nattel & Quantz, 1988; Davidenko *et al*, 1989; Sasyniuk *et al*, 1989; Valois & Sasyniuk, submitted). EADs arose either during the plateau phase or during the terminal phase of repolarization and were readily reversed by raising extracellular potassium, increasing stimulation rate, raising the magnesium concentration or administering class Ib antiarrhythmic drugs. Hence, the hypothesis was advanced that EADs play a role in the genesis of quinidine induced torsade de pointes arrhythmias.

While the existing data show a clear congruity between the characteristics of quinidine-induced triggered activity in Purkinje fibers and quinidine-induced torsade de pointes, there are several problems with the

acceptance of this hypothesis. Quinidine-induced triggered activity in Purkinje fibers attached to muscle was observed at extremely low frequencies (seldom greater than 20 per minute. While bradycardia favours quinidine-induced torsade de pointes arrhythmias, the latter generally occur at heart rates of 50 to 70 per minute. Furthermore, the intrinsic frequency of triggered activity generated *in vitro* is far less than a typical tachycardia rate (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Valois & Sasyniuk, submitted). We speculated that either triggered activity was restricted to a protected parasystolic focus, as originally suggested by Mendez & Delmar (1985) and only became manifest at certain well defined intervals, or that conditions of altered sympathetic tone or the presence of circulating catecholamines may allow the phenomenon to be induced more readily *in vivo* and to enhance its intrinsic frequency of generation. Thus, catecholamine administration in the setting of abnormally prolonged repolarization may facilitate the induction of triggered activity under conditions more similar to those which occur clinically.

Triggered activity due to a variety of experimental conditions has only been demonstrated to occur in Purkinje fibers (Coraboeuf *et al*, 1980; El-Sherif *et al*, 1988; Nattel & Quantz, 1988). If such activity is to have relevance as a mechanism of arrhythmia generation *in vivo*, then one has to demonstrate that either triggered activity is capable of occurring in ventricular myocardium or that activity generated in Purkinje fibers is transmitted to ventricular muscle. Mendez & Delmar (1985) showed that triggered activity induced in Purkinje fibers by hypoxia can be propagated into apparently normal adjacent Purkinje tissue or muscle only when the amplitude of the triggered responses exceeded 40 mV and their activation voltages were more negative than -55 mV, suggesting that only phase 3 triggered activity can propagate. Any role for EADs elicited by quinidine

must take into consideration not only propagation into muscle, but also the effect of electrotonic interactions between Purkinje and muscle.

Thus, the present study was designed to determine what effect catecholamines might have on both the behaviour and the rate of quinidine-induced triggered activity in an attempt to reconcile the *in vitro* data with clinical observations. The second aim of this study was to determine more precisely the site of origin of triggered activity and the characteristics of its propagation into surrounding myocardial tissue.

The results suggest that the presence of adrenaline can facilitate the appearance of quinidine-induced triggered activity, cause it to occur at cycle lengths more likely to be present clinically, and increase the intrinsic rate of a salvo of such rhythms. Furthermore, the site of origin of triggered activity was established to be solely within the Purkinje system. Adrenaline facilitates its transmission to adjacent ventricular muscle.

METHODS

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg). Their hearts were removed via a left thoracotomy. Both ventricles were thoroughly flushed with chilled, oxygenated Tyrode's solution. Long free-running Purkinje fiber bundles (between 6 and 16 mm) connecting two pieces of ventricular muscle were quickly excised from either ventricle and pinned to a Sylgard block at the bottom of a tissue bath (4 mL capacity). Fibers were continuously superfused with modified Tyrode's solution aerated with 95% 0_2 - 5%

$C0_2$ at a rate of 10 mL/min. Our standard Tyrode's solution contained (in mM): NaCl, 119.0; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHPO_4$, 0.9; dextrose, 5.5; and $NaHCO_3$, 25.

The preparations were stimulated with rectangular pulses through bipolar tungsten electrodes. Electrical stimulation was provided by a Model RS-660 Timing Simulator/Word Generator controlled by a HP9816 computer in combination with a Digitimer stimulus isolation unit (model DS2).

Transmembrane potentials were recorded with glass microelectrodes filled with 3 mM KCl and coupled to the inputs of high-impedance, capacitance-neutralized amplifiers (Model KS-700 and/or model 705 dual microprobe (WPI instruments). The outputs of the amplifiers were displayed on a Tektronix 5113 dual-beam storage oscilloscope and simultaneously displayed in digital form on a Data 6000 waveform analyzer (Data Precision. Inc). Data was simultaneously stored on videocassettes using a Sony model PCM-501ES digitizing unit and a JVC model HR-D225U video cassette recorder. A strip chart recorder (Mingograph model EEG-16) was used to obtain a hard copy of the data.

The preparations were allowed to stabilize for one hour under control conditions and at least one to two hours under quinidine, 5 μM. CsCl, 0.5 to 1.0 mM, was added to the superfusing solution to limit spontaneous automaticity upon exposure to hypokalemic conditions. We established previously that hypokalemic cesium containing solutions did not produce EADs but that they appeared in a consistent, stable fashion only after the addition of quinidine (Valois & Sasyniuk, submitted). Furthermore, comparable concentrations of cesium were found by Brown *et al* (1981) and DiFrancesco

(1981) to have a highly selective inhibitory effect on the pacemaker current.

During control measurements and equilibration with quinidine, the preparations were stimulated at a basic cycle length of 1000 msec. Action potentials were monitored on line and the following variables were measured: action potential duration (APD) to various levels of repolarization (50%, 75%, 95%), maximum diastolic potential (MDP), action potential amplitude, $\dot{V}_{max}$, and take-off potential.

In most preparations, action potentials were monitored from three different sites. At least one of the sites was from a subendocardial Purkinje or muscle fiber whereas the other two sites were usually located on the free running strand. Action potentials were observed over a wide range of pacing cycle lengths and during spontaneous rhythm in the presence of quinidine and hypokalemia (K_o^+ - 2.7 mM). If EADs and triggered activity occurred, their characteristics and the range of cycle lengths over which they were observed were determined. If triggered activity did not occur, then the cycle length dependent characteristics of the action potentials were determined.

The effects of adrenaline were tested in two ways. The preparations were either superfused with a constant concentration of drug added to the quinidine superfusate or adrenaline was added directly to the tissue bath in the form of a bolus. EDTA, 1 μM, was added to the adrenaline containing solutions. The solutions were covered with aluminum foil to inhibit photolytic oxidation. Whenever constant superfusion was used, the preparations were stimulated at a cycle length of 1000 msec during equilibration with adrenaline. Action potentials were then observed over the same range of pacing cycle lengths

as in the presence of quinidine and hypokalemia alone. Usually, it was not possible to observe the entire range of pacing cycle lengths because of enhancement of automaticity. Whenever adrenaline was added directly to the tissue bath, the preparations were stimulated at a particular cycle length until a stable response was obtained and 5 μL of a commercial stock solution (adrenaline chloride, Parke-Davis) was added. The concentration of the stock solution was 4.54 mM. It was estimated that the peak concentration achieved transiently in the tissue bath was no greater than 1 μM. The adrenaline effect was observed to occur within 30 to 60 seconds after addition to the bath. The effect lasted from 30 seconds to as long as 8 minutes.

Measurements of the characteristics of triggered activity were similar to those described previously (Valois & Sasyniuk, submitted). Characteristics of triggered action potentials were measured off line from stored digitized traces. Activation voltage was defined as the membrane potential just prior to initiation of the depolarization phase of the triggered action potential. The amplitude of the triggered action potential was defined as the difference between the activation voltage and the peak of the triggered response. The coupling interval of the triggered response was defined as the time from the phase 0 upstroke of the driven action potential to the phase 0 of the upstroke of the triggered action potential. For multiple triggered responses, the coupling intervals of the second and subsequent triggered action potentials were calculated from the upstroke of the preceding triggered response to the upstroke of the subsequent one.

EADs and the triggered action potentials they induced were classified into two categories based on their activation voltage. High membrane

potential (HMP) EADs were defined as depolarizing after potentials that delayed the terminal repolarization phase at a membrane potential more negative than -40 mV. Low membrane potential (LMP) EADs were defined as depolarizing after potentials with activation voltages less negative than -30 mV. EADs arising from activation voltages ranging between -40 and -30 mV were difficult to categorize.

Quinidine (5 μM) used was quinidine sulfate dihydrate obtained from J.T. Baker Chemicals; noradrenaline used was noradrenaline hypochloride obtained from Sigma.

Data are expressed as means ± S.D. Statistical analysis was done using the student t-test.

RESULTS

The effects of adrenaline were studied in a total of 17 preparations (13 from the left ventricle and 4 from the right ventricle) exposed for at least one to two hours to 5 μM of quinidine under hypokalemic conditions. Ten of the 13 left ventricular preparations displayed some form of EADs and/or triggered activity when driven at an average cycle length of 6 sec. Two of the three preparations from the left ventricle which did not display triggered activity had high spontaneous rates. Neither EADs nor triggered activity were observed in any of the preparations obtained from the right ventricle despite the fact that

these preparations could be driven at cycle lengths as long as 15 sec.

Adrenaline had two major effects. It induced EADs and triggered activity in all right ventricular preparations in which such activity was not present after exposure to quinidine alone. It shortened the minimum basic cycle length at which triggered activity could be obtained and increased the number of triggered responses in those preparations in which it was present before addition of adrenaline.

In the 4 preparations in which similar ranges of stimulation rates were tested before and after adrenaline, the average minimum cycle length for triggered activity decreased by one-half from 6.0 to 3.25 sec. *Figure 3.1* shows a typical example of the effects of adrenaline on the relationship between steady state cycle length and pattern of triggered activity in a preparation from the left ventricle. Under quinidine alone, consistent single triggered responses occurred only after the preparations were driven at cycle length of six seconds. In the presence of adrenaline, triggered responses occurred at cycle lengths as short as two sec. The number of triggered responses increased as the cycle length was increased. The preparations could not be stimulated at cycle lengths greater than three sec because of enhanced automaticity.

Figure 3.1 also shows the marked shortening of the coupling intervals of the triggered action potentials in the presence of adrenaline. When adrenaline was added to the superfusing solution, the first and subsequent triggered responses occurred at coupling intervals less than 300 msec versus 1500 msec under quinidine alone. At least part of the shortening of the coupling

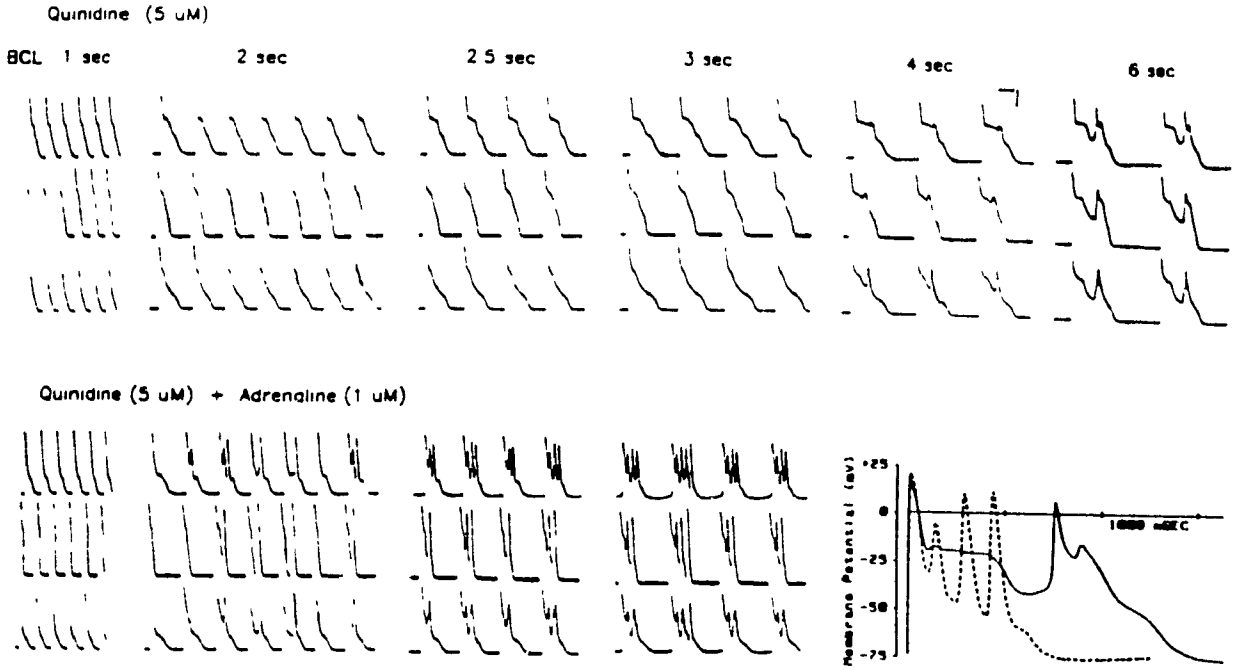

Figure 3.1. Typical example of the effects of superfusion with adrenaline, 1 μM, on the relationship between steady state cycle length and pattern of triggered activity in the presence of quinidine and hypokalemia. Each panel from top to bottom shows action potentials recorded from the septal and papillary muscle insertions of the free running Purkinje strand and from a subendocardial Purkinje fiber at the tip of the papillary muscle in a preparation from the left ventricle. The bottom right panel shows superimposed digitized traces of action potentials recorded from the septal insertion of the free running strand under quinidine alone and a cycle length of 6 sec (solid line) and in the presence of quinidine plus adrenaline at a cycle length of 3 sec (dashed line). Vertical calibration: 20 mV; horizontal calibration: 1 sec.

intervals may be due to a difference in the basic cycle length at which triggered responses occurred. However, in 5 preparations, the same long cycle lengths at which triggered action potentials occurred under quinidine alone could be maintained after the addition of adrenaline to the bath so that the effects of cycle length could be eliminated. *Figure 3.2* shows a typical example. Prior to the addition of adrenaline, each action potential displays a LMP EAD followed by a triggered response induced by a HMP EAD. Single HMP triggered action potentials occurred at an average coupling interval of 1245 ± 197 msec (mean ± S.D.). In the presence of adrenaline, each action potential was followed by two triggered action potentials. The average coupling interval of the first HMP triggered response decreased to 716 ± 28 msec while that of the second was 390 ± 63 msec. Despite the increased number of triggered responses, the duration of the entire response decreased.

Thus, in preparations from the left ventricle in which triggered activity could be obtained in the presence of quinidine under hypokalemic conditions but only if extreme bradycardia was also present, addition of adrenaline markedly reduced the degree of bradycardia necessary for induction of triggered activity. Furthermore, single triggered action potentials were converted to multiple triggered responses with markedly reduced coupling intervals.

In several preparations from the left ventricle, action potentials were recorded from subendocardial muscle fibers at the tip of the posterior papillary muscle to determine if triggered responses were also generated in ventricular muscle and/or if triggered responses generated in Purkinje fibers were propagated to muscle. We found that propagation to ventricular muscle could not be studied because action potentials in ventricular muscle could only be obtained

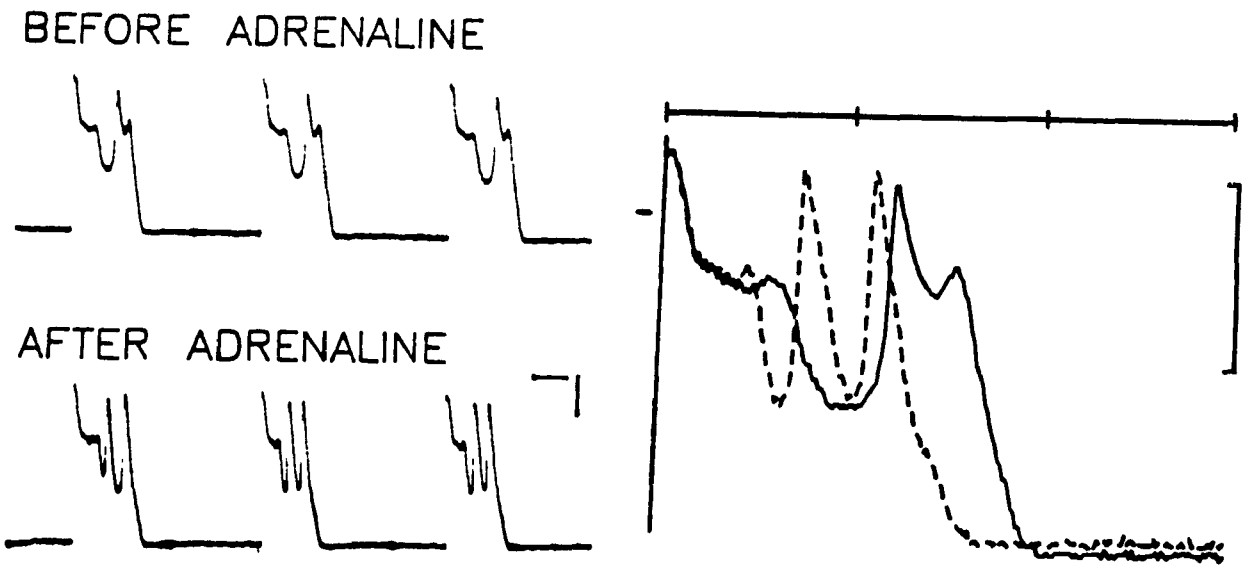

Figure 3.2. Shortening of the coupling interval of triggered responses by adrenaline in a left ventricular preparation in which the same cycle length (5 sec) was maintained before and after the addition of adrenaline. Only the recording from the septal insertion of the free running strand is included for simplicity. The lower trace shows the response 100 sec after adrenaline was added directly to the tissue bath. In the panel on the right, the responses before (solid line) and after (dashed line) addition of adrenaline have been superimposed. Vertical and horizontal calibrations: 50 mV and 1 sec.

when the stimulating electrodes were located in muscle. There was no propagation from Purkinje to muscle even for regular potentials. We speculated that the reason for this lack of propagation was related to the type of preparation which was used. According to the studies of Veenstra *et al* (1984) and Myerburg *et al* (1972), the Purkinje system as it enters the posterior papillary muscle at its tip is electrotonically isolated from muscle and instead makes contact with muscle about the midpoint of the papillary muscle. Conduction to the tip of the papillary muscle where the bundle enters it thus occurs after activation of muscle fibers at the base. When only the tip of the papillary muscle is excised, the normal connections from Purkinje to muscle are cut off.

Figure 3.3 shows an example of multiple recordings from the free running Purkinje strand and from subendocardial Purkinje and muscle fibers in a left ventricular preparation during a stable triggered activity. The stimulating site was near the muscle recording site. Activity occurred in muscle in response to the basic stimulus. However, the triggered action potential failed to produce activity in muscle even though it occurred at an activation voltage greater than -40 mV and at a time when repolarization in muscle was complete. When the stimulating electrodes were placed on the septal insertion of the free running strand, no activity at all was recorded in muscle (not shown).

Therefore, we studied 4 preparations from the right ventricle in which the free running Purkinje strand was attached to the base of the papillary muscle at its proximal end and the free wall at its distal end. In these preparations, quinidine did not induce EADs and triggered activity, even when they were driven at cycle lengths ranging from 8 to 15 sec. However, triggered activity could be induced in all of them upon addition of adrenaline. *Figure 3.4*

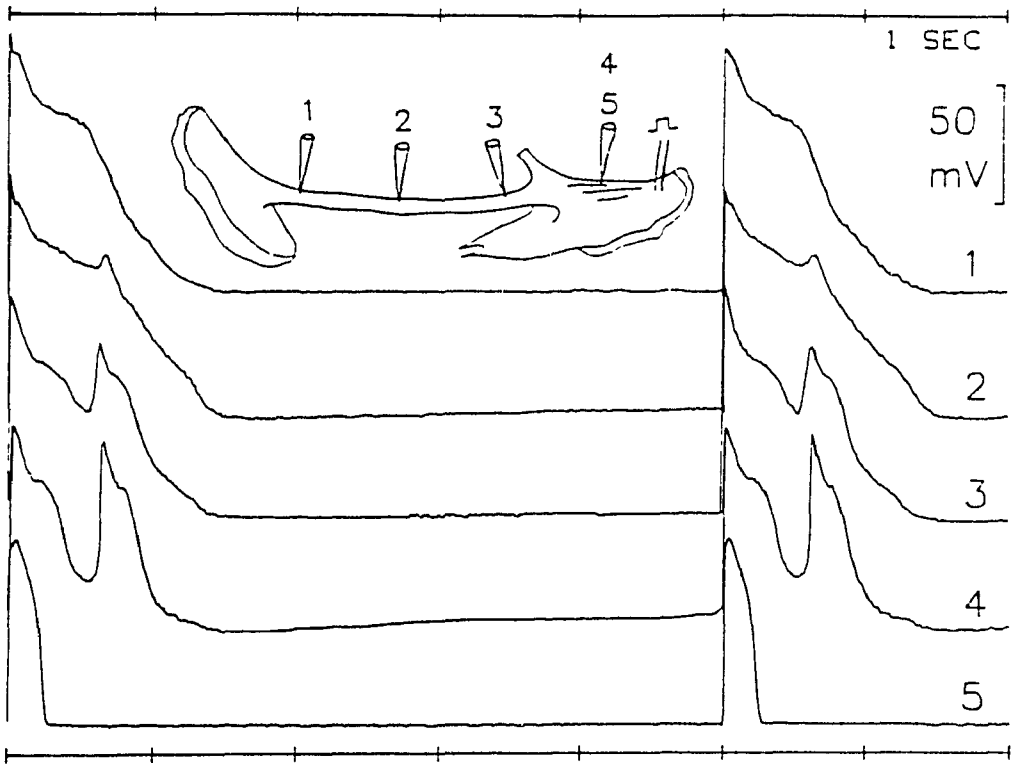

Figure 3.3. Mapping of stable triggered activity in a left ventricular preparation. Triggered activity remained stable for 8 min allowing multiple impalements of the preparation. 200 nM of noradrenaline was present in the superfusing solution. One microelectrode was maintained in a constant position (recording site # 3). A second microelectrode was used to record action potentials from sites 1, 2, 4, and 5. The electrode at site 4 recorded activity in a subendocardial Purkinje fiber. The electrode was then advanced one cell down to record activity in muscle. Thus, the muscle recording site was immediately adjacent to the Purkinje site. The preparation was stimulated at a basic cycle length of 5 sec. The coupling interval of the triggered action potentials ranged from 750 to 800 msec. Triggered activity was clearly not transmitted uniformly to all recording sites.

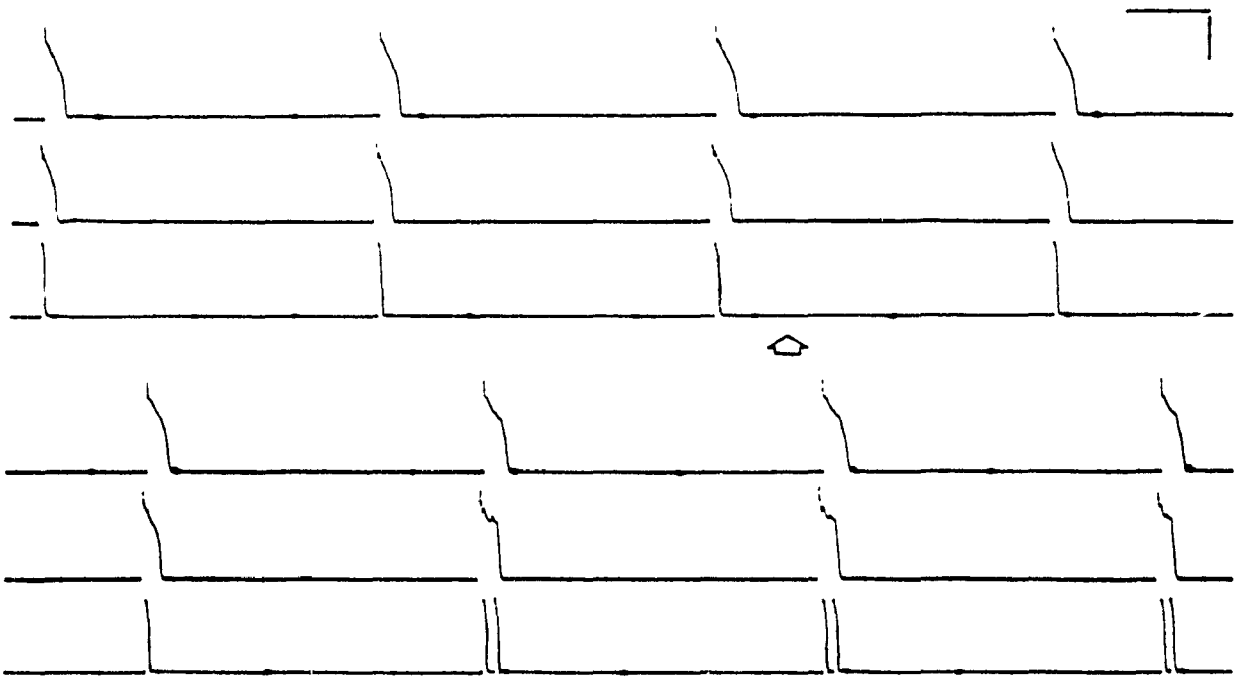

Figure 3.4. Induction of coupled ventricular muscle responses by adrenaline in a right ventricular preparation stimulated at a cycle length of 15 sec. Adrenaline was added directly to the tissue bath at the time indicated by the arrow. The two panels are continuous. Each panel shows action potentials recorded from a fiber on the free wall (top panel) and papillary muscle insertions (middle trace) of the free running Purkinje strand, and a ventricular muscle at the base of the papillary muscle (bottom trace). Vertical calibration: 40 mV; horizontal calibrations: 5 sec.

shows a typical example. In this preparation, quinidine failed to induce triggered activity even though action potentials recorded from the Purkinje strand were prolonged beyond 1 sec. Upon addition of adrenaline, there was further prolongation (by approximately 100 msec) and flattening and elevation of the plateau phase of the action potential, particularly at the recording site at the papillary muscle insertion with no change in the ventricular muscle action potential duration. This was accompanied by coupled ventricular muscle responses with coupling intervals ranging from 405 to 495 msec.

Adrenaline also induced multiple triggered activity in Purkinje fibers which was accompanied by multiple ventricular muscle responses. *Figure 3.5* shows a typical example. In the absence of adrenaline, the terminal phase of repolarization was markedly prolonged at the recording sites from the free running Purkinje strand accompanied by increasing disparity between durations in Purkinje fibers and ventricular muscle. When the preparation was allowed to beat spontaneously, even long periods of quiescence ranging from 45 to 120 seconds did not result in triggered activity which was propagated to muscle. After addition of adrenaline to the bath, each action potential was followed by multiple plateau type triggered activity in Purkinje fibers which was associated with coupled responses in ventricular muscle as shown in the middle panel. The third panel was preceded by a quiescent period of 50 sec. The first action potentials generated after the pause displayed multiple triggered activity in Purkinje fibers and were accompanied by multiple responses in ventricular muscle. Such activity was never seen in the absence of adrenaline and it was always preceded by long pauses. In a total of 14 trials in 3 experiments in which multiple LMP triggered activity were seen, the coupling interval of the first extra ventricular response averaged 639 ± 110 msec (mean ± S.D.). It was

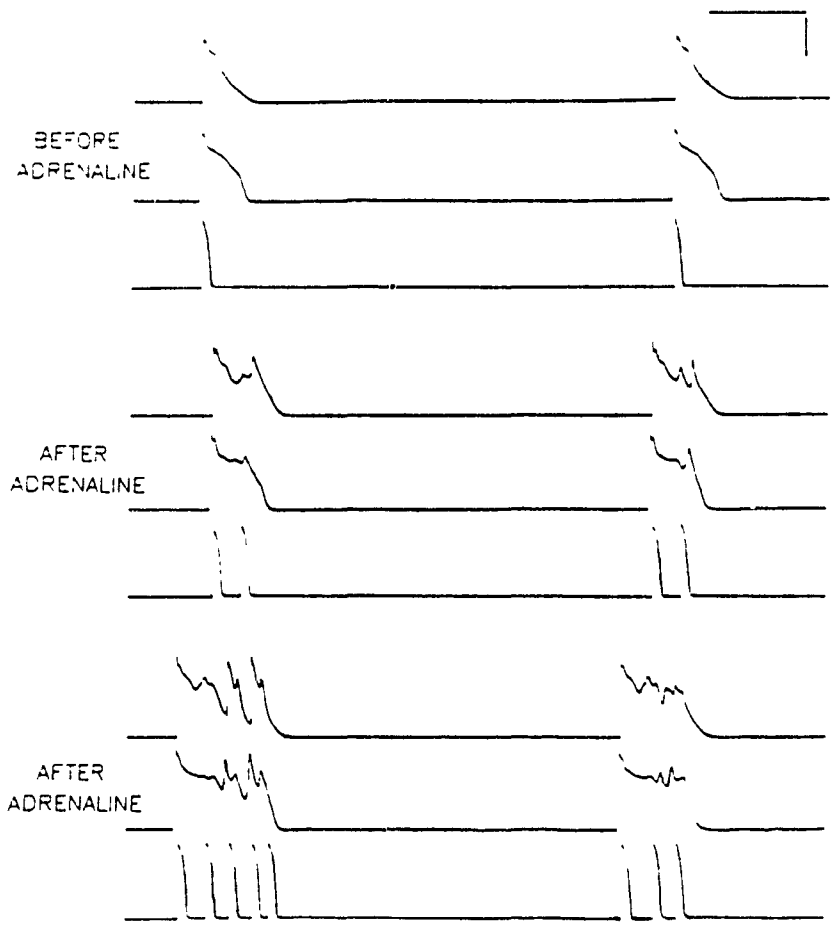

Figure 3.5. Induction of multiple phase 2 triggered activity in Purkinje fibers and multiple ventricular muscle responses in a preparation from the right ventricle following addition of adrenaline. Recording sites were similar to those in the preparation described in *Figure 3.4.* Stimulation rate was 10 sec before adrenaline. Before addition of adrenaline, stimulation was stopped and the preparation was allowed to beat spontaneously. The spontaneous rate was very irregular with long pauses between potentials. The middle panel was obtained 10 sec after a second addition of adrenaline to the bath. A previous bolus had been administered 4.5 min earlier. Following the two spontaneous action potentials, there was a long quiescent period of 50 sec. The first action potentials on the bottom panel are the first responses recorded after the pause. Vertical calibration: 40 mV; horizontal calibration: 1 sec.

significantly longer than the coupling interval of all subsequent ventricular responses (398 ± 94 msec). Triggered activity was never observed in venticular muscle.

The ultimate effect of adrenaline was to enhance automaticity. However, as *Figure 3.6* shows, adrenaline still caused transient induction of triggered activity despite the simultaneous enhancement of automaticity. Within seconds of the addition of adrenaline, the plateau phase of the Purkinje fiber action potentials lengthened to twice their values before adrenaline. There was a flattening of the plateau at a more positive potential level with the emergence of LMP EADs. Such potentials were accompanied by coupled responses in ventricular muscle. In contrast, the action potential duration of the ventricular muscle responses was slightly shorter than that prior to adrenaline, probably related to an enhancement of automaticity. The adrenaline effect lasted 2 min after which only enhancement of automaticity was evident.

We measured the coupling intervals of ventricular muscle responses of 88 potentials in the 4 preparations which were associated with LMP triggered activity in Purkinje fibers. The mean coupling interval of these responses was 517 ± 87 msec (mean ± S.D.).

In some instances, the predominant effect of adrenaline was to enhance automaticity and abolish triggered activity as shown in *Figure 3.7*. Multiple triggered activity was present prior to the addition of adrenaline. The automatic rate accelerated soon after the addition of adrenaline. As the rate increased, multiple triggered responses were converted into single triggered

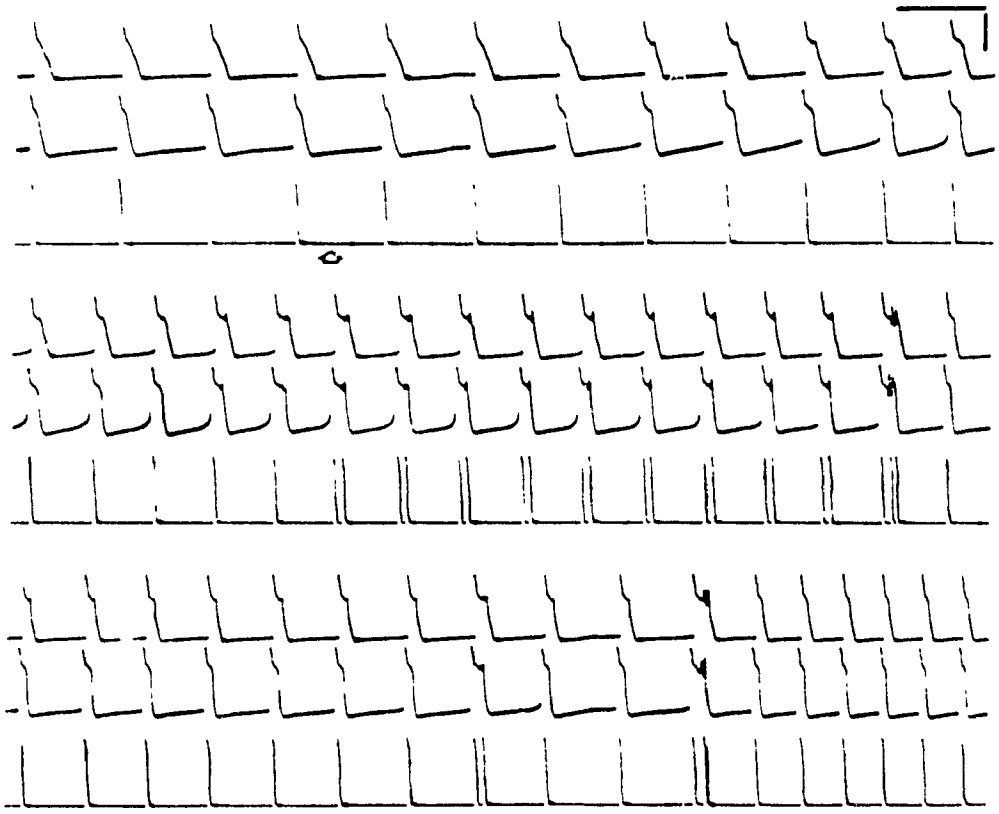

Figure 3.6. Induction of LMP triggered activity by adrenaline in a right ventricular preparation despite a simultaneous increase in automaticity. Adrenaline was added directly to the tissue bath at the time indicated by the arrow. The three panels are continuous. The preparation was beating spontaneously. Each panel shows action potentials obtained from the middle of the free running strand (top trace), the papillary muscle insertion of the free running strand (middle trace) and a ventricular muscle fiber on the papillary muscle (bottom trace). Vertical calibration: 40 mV; horizontal calibration: 5 sec.

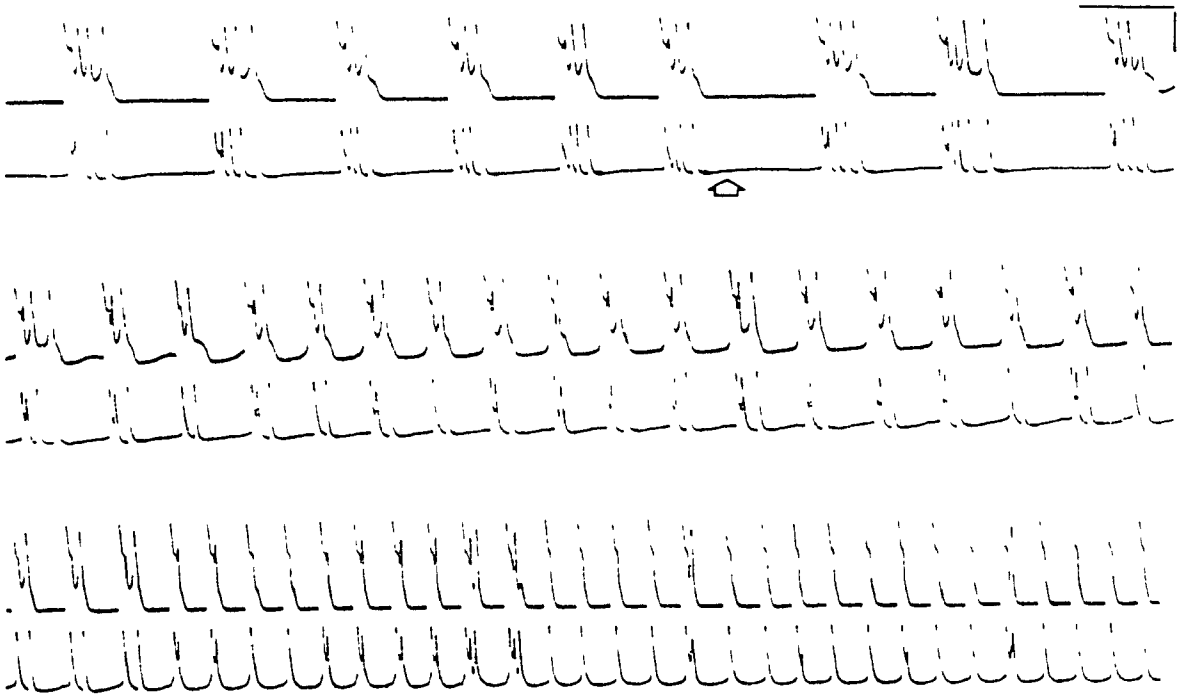

Figure 3.7. Abolition of quinidine induced triggered activity by adrenaline through an increase in automaticity. Each action potential was followed by multiple triggered activity in a left ventricular preparation beating spontaneously. Each panel shows action potentials obtained from the middle of the false tendon (top trace) and from a subendocardial Purkinje fiber at the tip of the papillary muscle (bottom trace). The panels are continuous. Adrenaline was added directly to the tissue bath at the time indicated by the arrow. Vertical calibration: 40 mV; horizontal calibration: 5 sec.

responses which eventually disappeared as the rate accelerated further.

It was not possible to predict the effect of adrenaline in those preparations in which triggered activity was already present prior to the addition of adrenaline. Abolition was seen in 5 instances in 5 preparations. In 3 of them, a transient facilitation of triggered activity occurred prior to abolition. On 7 occasions, activity resembling a rapid, sustained triggering was seen after the addition of adrenaline. Such sustained activity lasted for several seconds to as long as 6 minutes in different preparations. *Figure 3.8* shows an example of such activity. After addition of adrenaline, there was an increase in the duration and flattening of the plateau phase at all recording sites as described previously. When the durations had prolonged to almost 1300 msec at the septal site, multiple activity occurred after which sustained activity followed and continued for 77 sec. The intrinsic cycle length of the sustained activity ranged from 615 to 660 msec. Since the action potentials at the septal recording site were extremely prolonged, this site failed to respond with full blown potentials until the end of the period of sustained activity when presumably the action potential duration had decreased. Termination of the period of sustained rapid activity was followed by coupled responses manifest at the papillary muscle recording sites with an average coupling interval of 754 ± 54 msec (n = 20; mean ± S.D.). Termination of coupled triggered potentials was accompanied by a progressive lengthening of the coupling interval ending in potentials with prolonged plateaus at the septal recording site, HMP EADs at the papillary muscle insertion and only brief potentials at the subendocardial site.

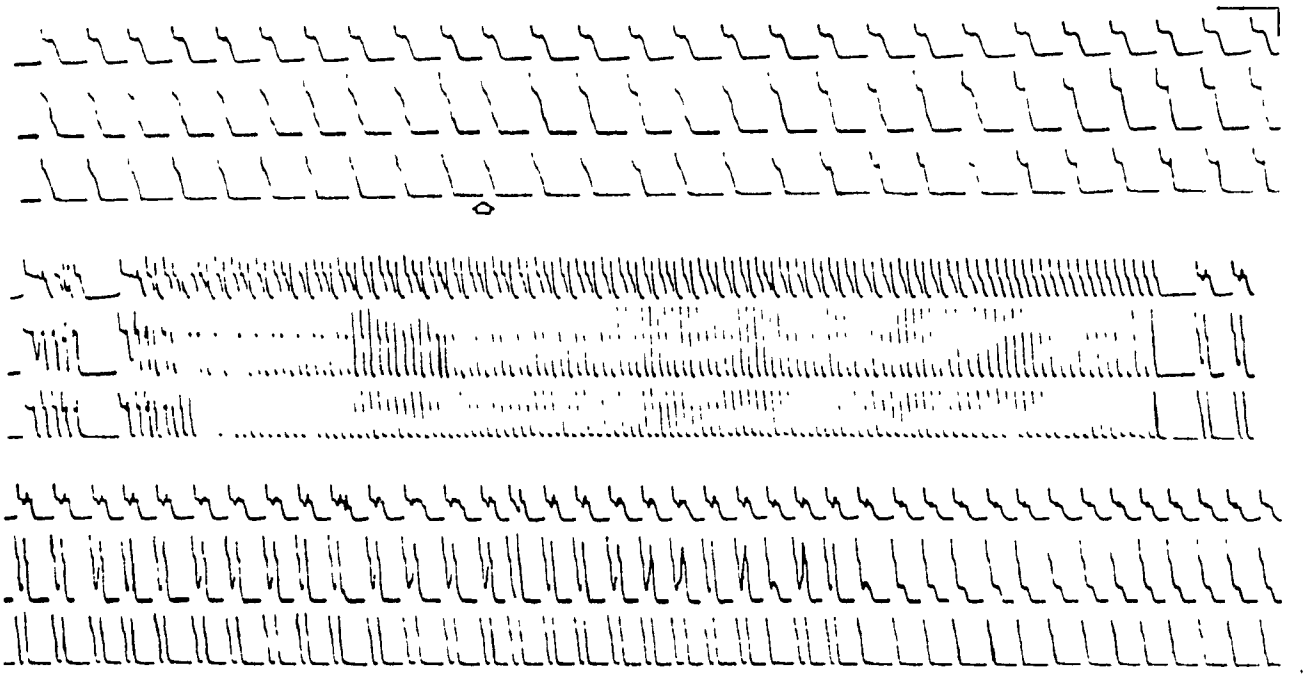

Figure 3.8. Induction of sustained rapid activity by adrenaline in a left ventricular preparation during spontaneous activity. Adrenaline was added directly to the bath (at arrow). The three panels are continuous. Each panel shows action potentials recorded from the septal (top trace) and papillary muscle (middle trace) insertions of the free running strand and from a subendocardial Purkinje fiber on the papillary muscle (bottom trace). Vertical calibration: 40 mV; horizontal calibration: 5 sec.

DISCUSSION

It has been suggested by a number of investigators that triggered activity initiated by afterdepolarizations may be the cause of quinidine-induced extrasystoles or polymorphous ventricular tachycardia, especially those seen in the presence of QT interval prolongation (Cranefield & Aronson, 1988a; Jackman *et al*, 1988; El-Sherif *et al*, 1989). The appearance of early afterdepolarizations (EADs) in preparations studied *in vitro* are favored by the same conditions as those which are associated with torsade de pointes in the whole heart. EADs have been recorded from the endocardial surface using monophasic action potential recordings in dog hearts injected with either CsCl (Levine *et al*, 1985) or anthopleurin-A (El-Sherif *et al*, 1988). No one has succeeded in developing an *in vivo* model of quinidine induced torsade de pointes arrhythmia which mimics the well described clinical features of the syndrome. Therefore, the role of EADs in this arrhythmia must be inferred from *in vitro* data or from monophasic action potential recordings *in vivo* (which only indirectly record activity in subendocardial Purkinje fibers).

We and others showed that quinidine produces two distinct types of EADs (Davidenko *et al*, 1989; Sasyniuk *et al*, 1989; Valois & Sasyniuk, submitted). Brugada & Wellens (1985) hesitated to attribute torsade de pointes arrhythmias to EADs occu[illegible]igh membrane potentials because of the previous data of Damiano & Rose[illegible]984) showing that cesium induced triggered activity was rather slow. They suggested instead that this arrhythmia might be caused by a mechanism called "prolonged repolarization dependent re-excitation". According to this mechanism, cells arrested at or near the plateau level of the action potential due to LMP EADs can reexcite neighboring cells because of

current flow between these cells and repolarized normal cells. Cranefield & Aronson (1988a), on the other hand, argued that triggered activity arising from HMP EADs can explain all of the features of torsade de pointes. They could account for the feature of extreme bradycardia by postulating the occurrence of EADs in a small focus with rate dependent entry block. Thus, the rate of the focus could be one-half of the sinus rate or much less.

The present results suggest that either mechanism may be possible and neither mechanism may be excluded. In a previous study, we showed that quinidine-induced triggered activity due to HMP EADs also occurred only in the presence of extreme bradycardia and its intrinsic activity was too low to account for a typical tachycardia (Valois & Sasyniuk, submitted). Furthermore, triggered activity occurred at extremely prolonged coupling intervals (> 1000 msec) unlikely to occur clinically and a salvo of triggered activity was extremely rare.

The present study shows, however, that the presence of catecholamines clearly facilitates the generation of triggered activity due to HMP EADs. In preparations in which quinidine induced triggered activity but only if extreme bradycardia was present, adrenaline decreased the minimal cycle length at which triggering first occurs to values more clinically relevant. Moreover, triggered action potentials with long coupling intervals to the basic beat occurred at considerably reduced coupling intervals.

Adrenaline never induced triggered activity due to HMP EADs in preparations in which no such triggered activity could be obtained in its absence. Its major effect was to increase the trains of triggered activity and markedly increase its rate (up to 200 beats/min as shown in *Figure 3.1*). Thus, in the

presence of adrenaline, triggered activity generated by HMP EADs can account for coupled ventricular beats and a mild sustained ventricular tachycardia even without postulating the presence of rate dependent entrance block, provided that some "minimal" conditions (in particular, marked lengthening of action potential duration) for its initiation are already present. However, the presence of rate dependent entry block should greatly facilitate its occurrence, provided that adrenaline also enhances exit conduction. Otherwise, the incidence of such activity must be low under clinical conditions, even in the presence of adrenaline because a moderately slow rate is still mandatory for its initiation in intact preparations in which Purkinje fibers are not isolated from muscle.

Under conditions of bradycardia and hypokalemia in which triggered activity still failed to occur under quinidine alone, adrenaline readily induced LMP triggered activity. The role of such activity in the generation of the clinical arrhythmia is less clear. Generation of LMP EADs in Purkinje fibers created marked differences in repolarization between Purkinje cells on the free running strand and those on the subendocardial surface which have durations similar to those in adjacent ventricular muscle. These differences only became significant in the presence of adrenaline. While minimal further lengthening of action potential duration preceded the initiation of EADs in free running Purkinje strands in the presence of adrenaline, a much more striking feature was an increase in the action potential plateau voltages to more positive values. A flattening and increase in duration of the action potential plateau at voltages more positive than -20 mV was associated with coupled responses in ventricular muscle which occurred at much shorter coupling intervals than those associated with HMP EADs in the absence of adrenaline.

How LMP EADs originating in Purkinje fibers give rise to coupled responses in muscle is not clear. One possibility is via the mechanism of "prolonged repolarization-dependent reexcitation" described above. The capacity for re-excitation by this mechanism will depend not only on the intensity of the current created by differences in the membrane voltage of contiguous cells, but also on the excitability of the already repolarized cells. Thus, the current generated must bring to threshold subendocardial cells that are connected to a large ventricular muscle mass and have high excitability thresholds. The possibility of this mechanism resulting in excitation of ventricular muscle must indeed be very low. Adrenaline appears to increase that possibility. If this is the mechanism of torsade de pointes, its incidence must be greatly influenced by the presence of a surge of catecholamines.

Only Purkinje fibers developed marked bradycardia dependent prolongation of action potential duration. In fact, the APD of ventricular muscle sometimes shortened in the presence of adrenaline. Thus, triggered activity appears to originate only in Purkinje fibers and is accompanied by coupled or multiple activity in ventricular muscle. Whenever multiple activity occurred, the coupling interval of the first coupled beat was always significantly more prolonged than that of subsequent beats, resembling the initiating sequence of a drug-induced torsade de pointes arrhythmia (Roden *et al*, 1986; Cranefield & Aronson, 1988a, 1988b; Jackman *et al*, 1988). Coumel *et al* (1985) and El-Sherif *et al* (1989) reported that ventricular ectopic beats associated with the U wave which preceded quinidine-induced torsade de pointes arose at coupling intervals (between 600 and 700 msec) which were within the range reported in the present study for coupled ventricular beats following the administration of adrenaline (between 400 and 700

msec).

It seems clear from the present study that triggered activity is not as uniform a phenomenon as previously believed from studies of short segments of Purkinje fibers and that its interpretation may require multiple recordings from many sites. In the presence of adrenaline, different effects occurred at different recording sites within the Purkinje system which may confound interpretation of the data and suggests that the mechanism may be more complicated than originally assumed. In our experience, LMP triggered activity was more likely to occur in fibers on the free running strand most remote from muscle. HMP triggered activity was more likely to occur in more peripheral fibers with shorter plateaus. Our data show that significant differences in repolarization of Purkinje fibers can occur at very close distances under the conditions of our experiments. An electrotonic gradient can be set up in the Purkinje network because of the electrotonic influences of ventricular muscle on Purkinje tissue. Thus, two possibilities can be considered for the site of origin of triggered activity. 1) A potential difference exists between the prolonged action potentials (particularly prolonged plateaus) in the free running Purkinje strand and Purkinje fibers on the subendocardial surface under conditions which induce triggered activity. HMP EADs originating in subendocardial fibers can reach threshold sooner and trigger action potentials earlier because they repolarize earlier. These triggered potentials are reflected back to the free running strand and may appear as humps on the repolarization phase ("LMP triggered activity"). Thus, in some cases LMP EADs occurring in free running Purkinje fibers may in fact be electrotonic manifestations of HMP activity arising on the subendocardial surface; or 2) LMP EADs originating in free running fibers may induce potential changes in subendocardial fibers which appear as HMP EADs. On reaching threshold, the cells with shorter action potentials initiate a

triggered action potential which appears as a HMP triggered response. In both cases, triggered activity would be associated with closely coupled responses in ventricular muscle. If one were to record from the free running strand, one would conclude that coupled responses in muscle were due to the development of focal reexcitation as a result of LMP triggered activity. If one were to record only from the subendocardial Purkinje fibers, one might conclude that coupled ventricular responses were due to the propagation of HMP triggered responses. Yet, both are probably correct and inextricably linked and may account for the inability of El-Sherif *et al* (1989) to correlate the presence of EADs on MAP recordings with the occurrence of ectopic beats.

Adrenaline has multiple electrophysiological actions, most notable of which is an enhancement of automaticity. Clearly, if adrenaline is to facilitate the occurrence of triggered activity, its facilitatory effect on triggered activity must supersede its effect on automaticity. In most of our preparations, we used cesium to prevent this action of adrenaline, presumably via its effects on the i_f current. However, we did find that, despite an enhancement of automaticity, adrenaline could still facilitate triggered activity, albeit transiently. Eventually, the rate accelerated and triggered activity was abolished.

Two previous studies have evaluated the influence of sympathetic stimulation on triggered activity induced by increasing doses of cesium (Ben-David & Zipes, 1988; Hanich *et al*, 1988). In the study of Hanich *et al* (1988), β-blockade prevented cesium induced sustained ventricular arrhythmia without eliminating EADs observed on MAP recordings. On the other hand, left stellate stimulation or infusion of noradrenaline was associated with larger EAD amplitudes and areas and higher incidence of tachycardias in the study of Ben-David & Zipes (1988).

Our results suggest that multiple recordings from a number of different sites on the endocardial surface may be needed to establish causality between EADs and incidence of ventricular tachycardia *in vivo*.

Adrenaline has several effects on membrane currents involved in repolarization which may account for its effects in facilitating triggered activity. The major ionic effects of catecholamines are to increase Ca^{2+} inward current (Boyett, 1978), to increase the hyperolarization activated pacemaker current, i_f (DiFranscesco *et al*, 1986), to stimulate the Na^+/K^+ pump (JoeBlo *et al*, 1990), and to increase K^+ outward currents (Bennett *et al*, 1986; Giles *et al*, 1989). In the presence of adrenaline, the emergence of LMP EADs was preceded by plateau elevation and prolongation suggesting the involvement of an L-type calcium channel in a manner postulated by January *et al* (1989) to be the mechanism of Bay K 8644-induced triggered potentials. A decrease in the recovery time of the Ca^{2+} current by adrenaline (Shimoni *et al*, 1984) would facilitate the occurrence of EADs.

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GENERAL DISCUSSION

The main objective of this thesis was to examine the concept that the combined use of quinidine, a Class Ia drug, with a Class Ib drug could improve its antiarrhythmic effect and prevent its proarrhythmic effect. For this purpose, we studied two aspects of the electrophysiological actions of quinidine in canine Purkinje fibers, namely, its antiarrhythmic effect on the depolarizing phase of the action potential and its arrhythmogenic effect on the repolarization phase, and the modulation of these effects by Class Ib drugs. Tocainide and mexiletine, two lidocaine-like agents, were used as representatives of Class Ib drugs, mainly because they present the advantage over lidocaine of being orally active. Our results suggest that the concomitant administration of a Class Ib drug improves the therapeutic effect of quinidine.

Data presented in the first chapter of the results section provided a basis for the enhanced antiarrhythmic efficacy of a combination of two Class I agents which exhibit different kinetics of interaction with the sodium channel in accordance with the prediction of the Hondeghem & Katzung model (1980). In this series of experiments, the maximum upstroke velocity of the action potential ($\dot{V}_{max}$) was used as an index of peak sodium conductance.

The time constant of recovery from quinidine-induced frequency-dependent block averaged 4.81 sec. It follows that quinidine-induced block of $\dot{V}_{max}$ would tend to accumulate at heart rates encountered physiologically. On the other hand, the mean time constant of recovery from tocainide induced frequency-dependent block was 1.04 sec. Thus, in the presence of tocainide, a substantial degree of block can dissipate between beats at slow heart rates. The recovery from $\dot{V}_{max}$ depression induced by the combination of the two drugs followed a bi-

exponential time course, each component (respective means of 0.43 and 5.94 sec) presumably contributed by one drug. The combination significantly increased the degree of "early diastolic block" as compared with quinidine alone, confirming a greater depression of $\dot{V}_{max}$ of closely coupled extra-systoles. The clinical study of Barbey *et al* (1988) also showed additive effects of quinidine and tocainide on closely coupled ectopic beats; the combination increased the coupling interval of such beats to a greater extent than either drug alone.

At therapeutic concentrations, the effect of quinidine was mostly dependent on the rate of stimulation while that of tocainide was dependent both on the rate of stimulation and the membrane potential. First, quinidine produced little or no shift in the curve relating membrane potential to $\dot{V}_{max}$ which suggests that tonic block is not likely to contribute significantly to quinidine-induced depression of $\dot{V}_{max}$ at depolarized potentials. On the other hand, tocainide-induced depression of $\dot{V}_{max}$ and delay in conduction increased at rapid rates of stimulation and at more depolarized membrane potentials. At depolarized potentials, i.e. approximately -75 mV, depression of $\dot{V}_{max}$ was due to a prolongation of the recovery time constant and to a greater magnitude of tonic block.

Ischemia tends to depolarize resting membrane potential through potassium leakage, and could make arrhythmias more likely to occur or more severe. At depressed resting membrane potentials, as likely to occur under ischemic conditions, the time constant of recovery from tocainide induced frequency dependent block slowed to similar values as those observed with quinidine at normal diastolic potentials.

The kinetics of recovery from tocainide-induced depression of $\dot{V}_{max}$

are rapid at normal, but slow at depolarized potentials. Therefore, a combination of quinidine with tocainide would be expected to produce a greater depression of conduction in localized ischemic depolarized tissue without causing as much further depression in fully polarized normal tissue in the rest of the heart compared with that produced by quinidine alone. This is an important aspect of the benefit of the combination of the two drugs since an additive depression of the conduction of normal cardiac rhythms may tend to favor reentrant arrhythmias and hence would be undesirable.

During the course, or after the completion of our study, electrophysiological interactions between Class Ia and Class Ib drugs have been studied in other laboratories.

Duff & Gault (1986) studied the combination of mexiletine and quinidine in isolated perfused rabbit hearts after circumflex occlusion-reperfusion and found its effect "supra-additive"; low concentrations of mexiletine and quinidine which, when given alone, had little or no electrophysiological or antiarrhythmic activity, had antiarrhythmic activity greater than that seen with high-concentration single treatment. This antiarrhythmic activity was measured as a prolongation of infarct zone conduction time and refractoriness. More recently, Duff (1989) confirmed in an *in vivo* model of dogs with inducible sustained ventricular tachyarrhythmias late after ischemic injury that enhanced antiarrhythmic activity of the combination of quinidine and the Class Ib agent mexiletine was paralleled by electrophysiologic changes occurring in the perinfarct zone.

Valenzuela & Sánchez-Chapula (1989) studied the frequency-

dependent effects of a combination of quinidine and mexiletine as compared to individual drugs in isolated guinea pig ventricular muscles using a sucrose gap technique. Using 5.4 mM K^+_o, and very high concentrations of both drugs (25 μM), they concluded that mexiletine and quinidine competed for the same receptor within the sodium channel because the addition of mexiletine reduced the early diastolic block provoked by quinidine alone. The reduction in early diastolic block, which contradicts our own results, is probably due to the high concentrations of drugs they used, which would be expected to interact in a competitive manner (Clarkson & Hondeghem, 1985).

Duff *et al* (1986) compared the combination of quinidine and mexiletine with each drug alone in the isolated perfused rabbit heart. They estimated sodium blockade with the prolongation of ventricular refractoriness, i.e. VERP/mAPD (the ratio of the ventricular effective refractory period over the action potential duration of monophasic action potentials) as assessed by the extra-stimulus technique. The preparations were always stimulated at the same cycle length of 400 msec. They concluded that the synergism on ventricular refractoriness seen with low concentrations of the two drugs could explain the prolongation of the coupling interval between sinus beats and ectopic depolarizations seen in their earlier clinical study (Duff *et al*, 1983). Similar results were reported by Costard-Jaeckle *et al* (1989).

Duff *et al* (1989) combined mexiletine and quinidine in the isolated rabbit heart before and after circumflex occlusion-reperfusion. Combinations of mexiletine and quinidine at concentrations which alone had little electrophysiological activity produced antiarrhythmic activity greater than that seen with high concentrations of mexiletine or quinidine alone. Antiarrhythmic activity

was assessed with the induction of ventricular fibrillation with extra-stimuli, prolongation of ventricular refractoriness and conduction. On the basis of a similar antiarrhythmic efficacy achieved with the combination of tetrodotoxin and quinidine, the authors concluded that enhanced antiarrhythmic activity of the combination of quinidine with mexiletine involved, at least in part, blockade of the cardiac sodium channel.

Bajaj *et al* (1987) studied the frequency dependence of the same combination (quinidine and mexiletine) *in vivo* on dogs with heart block, allowing a range of cycle lengths to be studied from 250 to 1500 msec. Sodium channel interactions by clinically relevant concentrations of the two drugs were assessed by the recovery of conduction time from frequency-dependent depression in both the transverse and the longitudinal orientations. They concluded that the effect of the combination represented additive frequency-dependent effects of the individual agents.

Burke *et al* (1986) studied the interaction of quinidine and mexiletine on $\dot{V}_{max}$ of the Purkinje action potential, but used only one cycle length (666 msec) and very high concentrations of both drugs (from 3.1 to 50 μM). They concluded that a combination of quinidine and mexiletine achieved the same level of $\dot{V}_{max}$ depression as did a solution containing twice the concentration of quinidine, supporting an additive interaction on sodium channel inhibition.

We found that quinidine and tocainide also interact on the repolarization phase of the action potential. This was expected as the two drugs are known to have opposite effects on the duration of action potential. Quinidine prolongs the action potential duration mainly through a block of the delayed

rectifier i_K (Colatsky, 1982; Hiraoka *et al*, 1986), inward rectifier i_{k1} (Hiraoka *et al*, 1986; Salata & Wasserstrom, 1988), and i_{to} (Imaizumi & Giles 1987) whereas tocainide shortens the action potential duration mainly through a block of the sodium "window" current (Carmeliet & Sairawa, 1982). Two types of interventions revealed the ability of tocainide to reverse the action potential prolongation by quinidine under bradycardic conditions, namely steady state changes in rates of stimulation (cycle length ranging from 300 to 2000 msec) and long single quiescent pauses (duration ranging between 20 and 30 sec). This suggests that the much documented proarrhythmic QT prolongation by quinidine (Bauman *et al*, 1984; Roden *et al*, 1986a; Levine *et al*, 1989) could be potentially controlled by concomitant administration of a Class Ib drug.

Proarrhythmic effects of quinidine are most commonly a consequence of its ability to prolong the QT interval. The likelihood of occurrence of these arrhythmias is enhanced by the concomitant presence of conditions that also prolong QT interval, such as bradycardia and electrolyte imbalance. Although polymorphic ventricular arrhythmias (torsade de pointes) due to the acquired long QT syndrome have been reported with almost every drug that prolongs the action potential duration (in particular Class Ia and III drugs), quinidine remains the most frequent culprit associated with these arrhythmias, with an incidence of up to 10% (Thompson *et al*, 1988). Despite the identification of several factors associated with its occurrence, torsade de pointes is still considered an idiosyncratic, and rather unpredictable, response to drugs that prolong repolarization.

The overall effect of the antagonistic activities of tocainide and quinidine on repolarization varied with the rate of stimulation. The steady-state shortening of the action potential by the addition of tocainide to quinidine-treated

fibers was minimal at faster rates of stimulation, but more prominent at slower rates of stimulation. Thus, the interaction of quinidine and tocainide on repolarization has opposite frequency dependence to their interaction on $\dot{V}_{max}$. Under conditions of slow rates of activity where quinidine-induced APD prolongation would be expected to be proarrhythmic, the addition of a Class Ib agent will abbreviate the action potential duration to a greater extent, thereby adding a safety factor to bradycardia-dependent arrhythmias.

Roden *et al* (1987) compared the effects of the combination of quinidine and mexiletine with that of individual drugs (in a therapeutic concentration range) on both $\dot{V}_{max}$ of the Purkinje action potential and the repolarization phase over a wide range of cycle lengths of stimulation. They measured the kinetics of onset of and recovery from rate-dependent block. They claimed their results were compatible with a single binding site to the sodium channel for Class I antiarrhythmic drugs and that the beneficial outcome of such a combination may reflect opposing effects on repolarization and at least additive actions on sodium current blockade.

In summary, a combination of quinidine with a Class Ib drug would be beneficial in antiarrhythmic therapy by preventing quinidine-induced excessive APD prolongation seen under bradycardic conditions as well as enhancing the depression of closely coupled premature ventricular contractions or rapid tachycardias without further depressing normal conduction beyond that induced by quinidine alone.

Chapter two of the results section deals with the characterization of quinidine-induced triggered activity. This served as an *in vitro* model for

bradycardia-dependent arrhythmias which are the most commonly encountered complication of proarrhythmia due to quinidine treatment. Experimental conditions include therapeutic quinidine concentrations (5 to 10 μM), low $[K^+]_o$ (2.7 mM), and slow rates of stimulation (cycle lengths between 3 and 15 seconds).

We first established the importance of acidosis in the generation of triggered activity. The contribution of acidosis (pH = 7.06 ± 0.08 - mean ± S.D.) was two fold: a direct increase in the magnitude of EADs and an indirect facilitatory role by slowing down automaticity, thereby allowing preparations to follow slower rates of stimulation for longer periods of time. This suggests that even a small decrease in pH may be an efficient potentiating factor in the genesis of torsade de pointes arrhythmias when both quinidine and hypokalemia are present. One previous study has shown that more profound acidosis (pH = 6.6) and low $[K^+]_o$ (2.7 mM) could induce triggered activity arising from HMP EADs (Coraboeuf *et al*, 1980).

In the presence of low $[K^+]_o$, quinidine induced two types of triggered activity, viz arising from the plateau or low membrane potential (LMP) and arising from phase 3 or high membrane potential (HMP). LMP triggered activity was found more frequently at normal pH, whereas HMP triggered activity occurred both at low and normal pH.

Prolongation of the action potential in response to a decrease in the rate of the stimulation appears more important in the emergence of triggered activity than bradycardia itself. Under our experimental conditions, the combination of mild acidosis, hypokalemia and cesium failed to induce on its own the preliminary APD prolongation necessary for triggered activity. The presence of

quinidine was essential for this purpose, possibly implicating a primary role for the blockade of i_K, and perhaps also the delay of its activation process (Roden *et al*, 1988), as well as for the blockade of i_{to} (Imaizumi & Giles, 1987).

The generation of HMP triggered action potentials was dependent on prolongation of the terminal phase of repolarization with the prolongation of the plateau playing a minimal role. These were elicited over a fairly narrow range of activation voltages (-40 to -60 mV), but occurred over a relatively wide range of coupling intervals. Once HMP triggered action potentials were initiated, a negative relationship existed between their amplitudes and activation voltages over the range of stimulation rates studied. The same relationship also existed for double triggered action potentials, but at a more negative range of activation voltages for the second triggered action potential than the first one.

As the rate of stimulation was decreased, steady state HMP triggered action potentials were elicited from more positive activation voltages and at shorter coupling intervals. This may be due to a greater inhibition of the delayed rectifier, i_K, by quinidine at longer than at shorter cycle lengths of stimulation (Roden *et al*, 1988), provided that the mechanism of blockade of i_K in Purkinje is similar to that in ventricular muscle where quinidine is though to promote occupancy of closed state of the potassium channel. This greater degree of depression would facilitate the generation of net inward current by allowing the membrane to depolarize earlier and at more positive potentials.

The range of activation voltage of HMP triggered action potentials and their abolition by Class Ib drugs tend to suggest an involvement of the sodium current, through the non-inactivating steady-state "window" (Attwell *et al*, 1979)

and/or the slowly inactivating component (Gintant *et al*, 1984). However, standard microelectrode techniques do not allow to discriminate between these two possible candidates. Torsade de pointes and triggered activity are seen only under conditions of low concentrations of quinidine and slow rates of activity, where the block of sodium current flowing during repolarization ("window" current) is expected to be minimal. The involvement of the sodium current in the upstroke of triggered responses is further supported by studies which reported the prevention of arrhythmias related to quinidine induced QT prolongation with Class Ib agents tocainide (Kim *et al*, 1987; Barbey *et al*, 1988) or mexiletine (Duff *et al*, 1983; Giardina & Wechsler, 1990) and by studies reporting control of torsade de pointes episodes with the same drugs (Kaplinsky *et al*, 1972; Kellerman *et al*, 1982; Shah & Schwartz, 1984; Thomas & Giles 1985; Bansal *et al*, 1986).

HMP triggered action potentials were abolished by clinically relevant concentrations of tocainide or mexiletine. The events preceding the abolition of the upstroke were similar with the two drugs and took place over a period of few minutes. At a constant rate of stimulation, a progressive prolongation of the coupling interval of the triggered action potential and a shift of its activation voltage towards more negative potentials were the first changes induced by mexiletine or tocainide. The elimination of the upstroke of the triggered action potential occurred at the point the activation voltage reached a membrane potential of -60 mV. This strongly suggests that Class Ib drugs abolish triggered action potentials through their sodium blocking properties. The abolition of triggered action potentials preceded any decrease in the magnitude of the EAD, again reinforcing the importance of the contribution of sodium in their upstroke. *Figure D.1* provides a continuous view of the sequence of elimination of multiple, then double, and finally single triggered action potentials by mexiletine. The

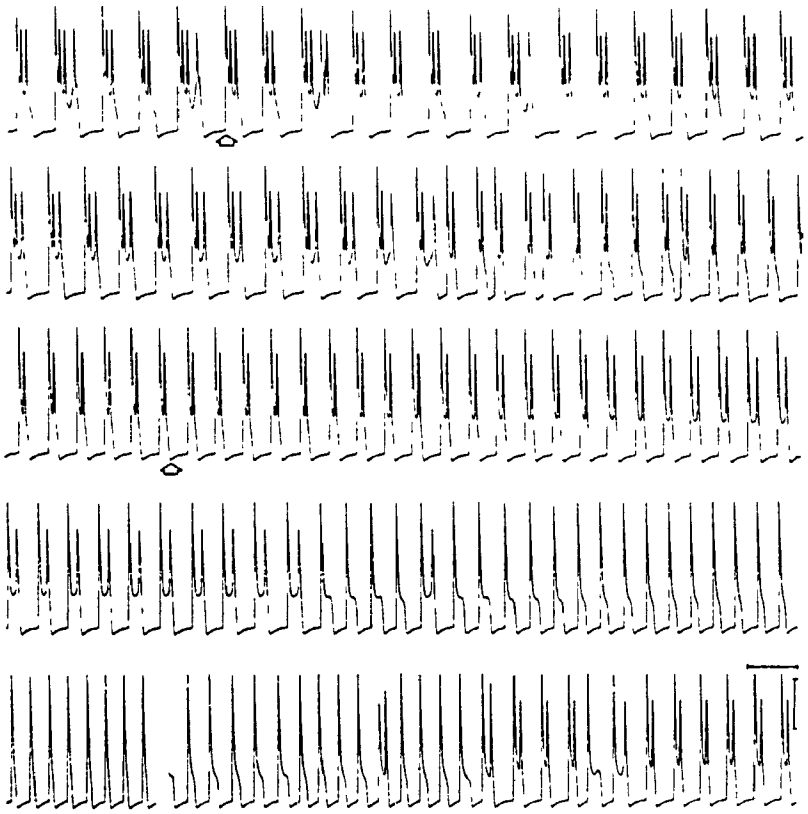

Figure D.1: Abolition of triggered action potentials which occurred during a spontaneous rhythm by a combination of quinidine and mexiletine. Multiple triggered action potentials occurred during exposure to quinidine alone (10 μM). Arrow in first panel indicates time of exposure to combination of quinidine plus 2 μM of mexiletine. First and second panels are continuous. First panel shows conversion of multiple triggered responses to double triggered responses. Second panel shows conversion of double triggered responses to single triggered responses. Three minutes of record were deleted between panels two and three. In panel three, the arrow indicates time of exposure to a combination of quinidine and 4 μM of mexiletine. Panels three and four are continuous. Panel four shows progressive prolongation of the coupling interval of single triggered responses followed by their abolition and eventual shortening of action potential duration 2 min later (left hand portion of fifth panel). Fifth panel shows recurrence of triggered action potentials 6 1/2 minutes after perfusion with quinidine alone. Vertical calibration is 40 mV; horizontal calibration is 10 sec.

progressive lengthening of the coupling interval of the triggered response preceding its abolition is obvious in the three instances. The bottom strip of *Figure D.1* shows re-exposure to quinidine alone after mexiletine was washed out. At the point triggered responses returned, their coupling interval was long with relatively negative activation voltages at first, and then stabilized later at shorter steady state values with less negative activation voltages.

It has been demonstrated that quinidine-induced triggered action potentials could also be abolished by the calcium antagonists nifedipine (0.1 μM) and verapamil (1 or 10 μM) (Nattel & Quantz, 1988). Before interpreting such data as direct evidence for the exclusive involvement of calcium as the inward current responsible for the depolarization phase of triggered action potentials, one should consider the study of January *et al* (1988) in which sodium-blocking (lidocaine, tetrodotoxin) as well as calcium-blocking (nitrendipine) pharmacological interventions abolished triggered activity induced by the calcium agonist Bay K 8644 in similar ways. To our knowledge, Bay K 8644 has no documented sodium channel blocking properties. Therefore, sodium should not necessarily be ruled out as taking part into Bay K 8644 induced triggered activity. On the contrary, the fact that lidocaine can abolish it tends to support at least some involvement of sodium. However, some possibility remains that lidocaine abolished Bay K 8644 induced triggered activity through a direct calcium blocking effect (Ono *et al*, 1986; Josephson, 1988; Kotake *et al*, 1988).

Nevertheless, the fact that quinidine-induced triggered action potentials could be abolished by sodium and calcium blocking drugs raises the possibility that both a calcium and a sodium components are necessary for their upstroke. This hypothesis, which is supported by Lazzara (1989) and Liu (1989),

may explain the inconsistency of success in the treatment of torsade de pointes with Class Ib drugs (Jackman *et al*, 1988).

Although standard microelectrode techniques cannot directly identify the ionic currents involved in the two types of triggered activity, several lines of evidence suggest that LMP and HMP triggered action potentials depend on different ionic mechanisms.

First, LMP and HMP triggered action potentials arise at different membrane potentials. LMP triggered potentials are of much lower amplitude than HMP triggered potentials, which probably reflects a lower net current through a lesser degree of outward current blockade at the plateau level. Second, they did not occur over the same range of cycle lengths. LMP triggered potentials manifest at much shorter cycle lengths (mean for the shortest cycle length 2.8 ± 1.5 sec) than HMP triggered potentials (mean for the shortest cycle length 5.83 ± 2.42 sec). A similar observation has been previously reported for quinidine-induced (Davidenko *et al*, 1989) as well as cesium-induced (Damiano & Rosen, 1984) triggered activity, although both studies reported that LMP triggered activity could only be observed at $[K^+]_o$ = 4.0 mM. Third, HMP and LMP triggered action potentials exhibited different frequency-dependence. The characteristics of LMP triggered action potentials (activation voltage, amplitude, and coupling interval) were little affected by changes in rate of stimulation whereas those of HMP triggered action potentials followed the pattern described above. Fourth, mexiletine was not equally effective in abolishing the two types of triggered activity. HMP triggered action potentials were abolished within minutes and at therapeutic concentrations whereas exposure to much higher drug concentrations for longer periods was needed to abolish LMP triggered action potentials. Further exploration of the question of ionic currents

involved in LMP versus HMP triggered activity would require voltage clamp techniques.

For a triggered response to hold an arrhythmogenic potential, its occurrence in the Purkinje network somehow has to be transmitted to ventricular muscle in order to re-excite the whole myocardium. At the present, the question of the possible arrhythmogenic meaningfulness of LMP and HMP triggered responses remains unresolved. Mendez & Delmar (1985) have shown that only triggered responses with activation voltages negative to approximately -55 mV could produce an extra muscle response. Moreover, Damiano & Rosen (1984) reported that cesium can induce both LMP and HMP EADs, but only HMP EADs were capable of inducing triggered activity. The present study does show plateau oscillation at very slow rates of stimulation; however, it is difficult to speculate at this point whether these could actually propagate to ventricular muscle or alternatively, generate extra-systoles by "prolonged repolarization-dependent reexcitation", as postulated by Brugada & Wellens (1985). El-Sherif *et al* (1988) have shown that anthopleurin-A induced triggered action potentials arising from EADs with activation voltages positive to -20 mV were associated with extra ventricular muscle responses while EADs themselves were not.

Our results have confirmed several parallels between quinidine-induced triggered activity and the ventricular arrhythmia torsade de pointes. The *in vitro* conditions permissive to triggered activity also predispose to arrhythmias secondary to excessive QT prolongation by quinidine. As previously observed by others (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Davidenko *et al*, 1989), EAD-induced triggered activity was produced by a therapeutic drug concentration. Likewise, a good number of clinical studies on torsade de pointes induced by

quinidine reported serum drug levels within or below therapeutic values (Jenzer & Hagemeijer, 1976; Bauman *et al*, 1984; Roden *et al*, 1986b; Stratmann & Kennedy, 1987; Jackman *et al*, 1988).

Electrolyte imbalance favors both quinidine-induced triggered activity and torsade de pointes. Low potassium (Roden & Hoffman, 1985; Nattel & Quantz, 1988; Davidenko *et al*, 1989) as well as low magnesium (Nattel & Quantz, 1988; Davidenko *et al*, 1989) concentrations have been used in several *in vitro* studies. Hypokalemia and hypomagnesemia are among the most frequently identified conditions associated with drug-induced torsade de pointes (Sclarovsky *et al*, 1979; Keren *et al*, 1981; Bauman *et al*, 1984; Stern *et al*, 1984; Jackman *et al*, 1988).

Acceleration of the cardiac rate, through pacing or administration of catecholamines, is the first line of treatment against recurrent drug-induced torsade de pointes attacks (Jackman *et al*, 1984 and 1988). The principle of abolition by acceleration in the rate of stimulation was confirmed in our *in vitro* model (see *Figure D.2*).

Electrophysiological observations of our *in vitro* model and of the arrhythmia torsade de pointes strongly suggest a similarity between the two. However, features of the *in vitro* model, such as the very slow rates of stimulation necessary for the manifestation of triggered action potentials and the slow intrinsic rate of firing of multiple triggered responses, still constitute clear discrepancies from the heart rates during torsade de pointes.

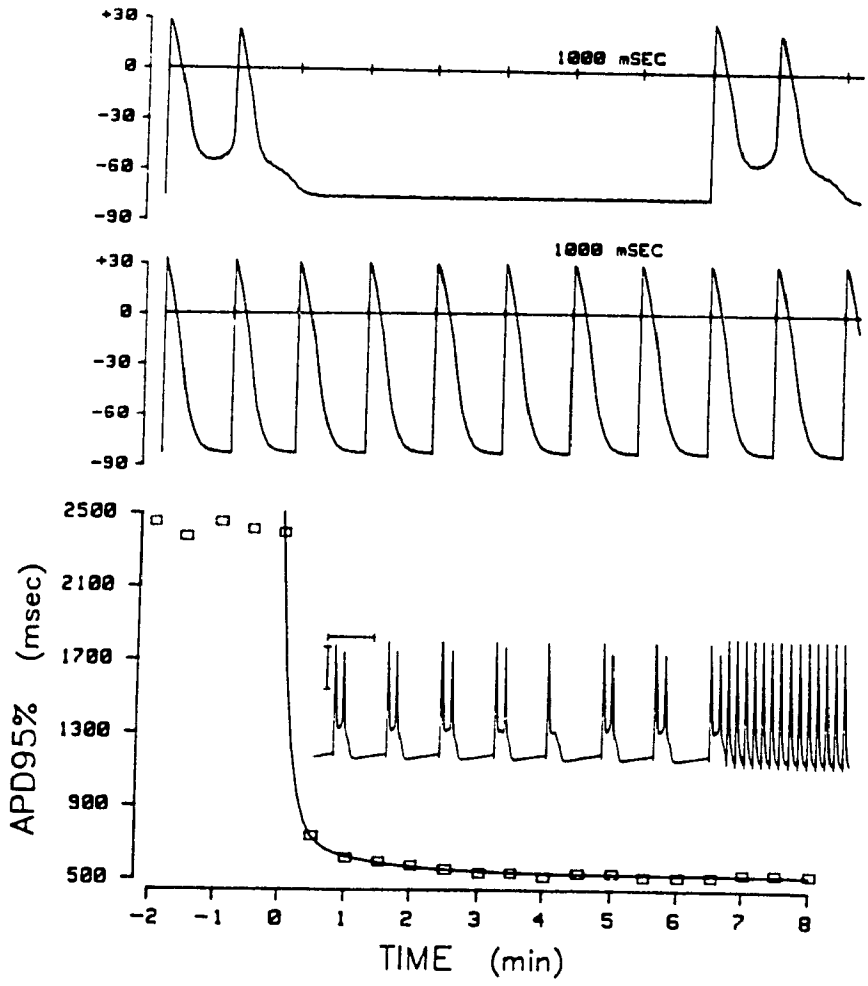

Figure D.2: Abolition of triggered activity by pacing at a cycle length of 1 sec. Upper panel shows single triggered action potentials which occurred at a cycle length of 8 seconds. Middle panel shows action potentials recorded during a pacing rate of 1 Hz. Bottom panel shows time dependent changes in $APD_{95\%}$ when cycle length was abruptly changed from 6 to 1 sec. Triggered responses followed most driven action potentials at this slow rate. APD was recorded every 30 seconds. Inset shows record from which graph was obtained. Vertical calibration is 20 mV; horizontal calibration is 5 sec. This data was obtained from a different preparation than the top panels.

Although bradycardia is a prerequisite for the induction of the *in vitro* phenomenon and the clinical arrhythmia, the cycle lengths required for the generation of EAD-induced triggered activity were generally longer than those which occur clinically (mean minimum cycle length of 5.83 sec). Jackman *et al* (1984) mentioned that marked sinus bradycardic rate below 50 beats/min were infrequently observed in their study. Moreover, whenever we observed more than one triggered action potential following a stimulated action potential, the intrinsic rate was too slow to account for the sustaining mechanism of torsade de pointes where heart rates often exceed 200 beats/sec. *Figure D.3* shows an example where quinidine induced sustained triggered activity with an intrinsic rate between 35 and 45 beats/min.

If such slow cycle lengths are necessary for the manifestation of HMP triggered activity in isolated cardiac tissue, it may be an indication that it could only emerge within a protected focus, as suggested by several workers (Damiano & Rosen, 1984; Mendez & Delmar, 1985; Cranefield & Aronson, 1988b), where profound bradycardia could be maintained through entry block. Alternatively, it could mean that certain features of the *in vivo* situation are not present in the *in vitro* model. A likely candidate is the presence of catecholamines *in vivo*; we tested this possibility with respect to quinidine-induced triggered activity.

The sympathetic and parasympathetic nervous systems can profoundly influence the electrophysiological properties of the heart and consequently the initiation and evolution of ventricular arrhythmias. Catecholamines have been used for the treatment of drug-induced torsade de pointes because of their ability to accelerate the heart rate. However, in the presence of abnormally prolonged repolarization, they may become

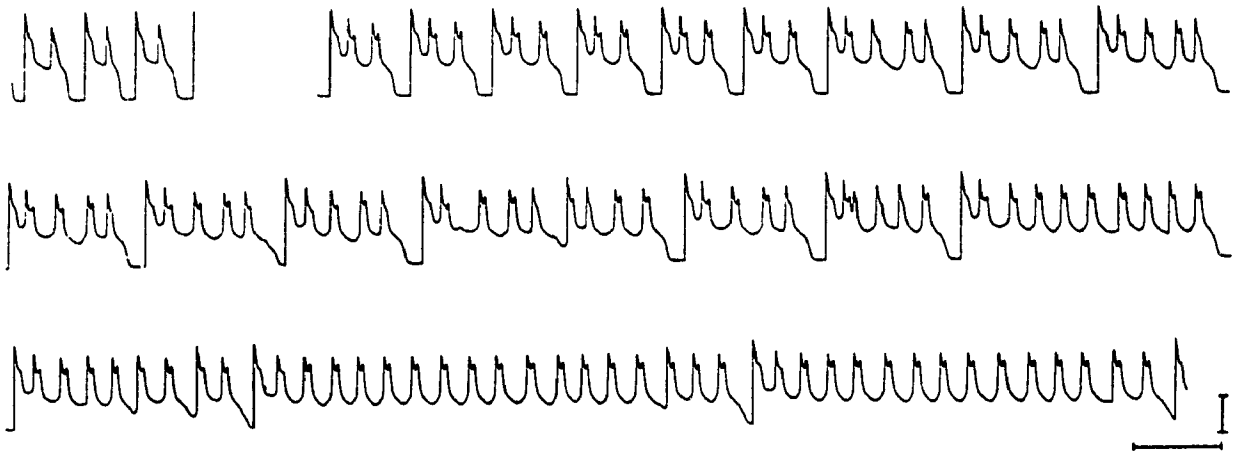

Figure D.3: Typical example of quinidine-induced sustained triggered activity. The preparation is superfused with quinidine (5 μM), 12 mM $NaHCO_3$, CsCl (1 mM), and KCl (2.7 mM). The short strip at the beginning was taken 5 min before the rest of the figure. The long strip is continuous. Vertical calibration is 40 mV and horizontal calibration is 5 sec.

arrhythmogenic by allowing triggered activity to occur more readily *in vivo* and enhancing its intrinsic rate of generation.

It was found that adrenaline can indeed modulate quinidine-induced triggered activity. A facilitation of quinidine-induced triggered activity by adrenaline was observed whether or not quinidine had previously induced triggered activity under hypokalemic conditions. First, in experiments in which HMP triggered activity was previously seen under quinidine alone, the facilitation was measured by the shortening of the minimum cycle length for the manifestation of triggered activity, by the conversion of single into multiple triggered action potentials, and by the shortening of the intrinsic coupling interval of sustained triggered activity. Second, in experiments in which triggered activity was not previously seen under quinidine alone, the addition of adrenaline permitted the generation of LMP triggered action potentials arising from the plateau associated with extra ventricular muscle responses, single, double or even multiple.

The facilitation of quinidine-induced triggered activity by adrenaline occurred even in the presence of acceleration of rate in automatic firing in spontaneously beating preparations. Whenever automaticity increased too much, triggered activity disappeared. Thus, the facilitation of quinidine-induced triggered activity by adrenaline depended upon the crucial balance between its two opposing effects on repolarization, namely, its direct membrane effects versus the APD shortening effect secondary to an acceleration of automaticity rate.

Our results clearly indicate that triggered activity occurs only in Purkinje fibers and not in ventricular muscle. LMP triggered action potentials seen after the addition of adrenaline gave rise to closely coupled extra-systoles in

ventricular muscle when associated with LMP triggered action potentials (517 ± 87 msec). Under our experimental conditions, the effects of adrenaline were mainly on the Purkinje action potential, with ventricular muscle exhibiting minimal change in the shape of its repolarization phase. Therefore, the main mechanism involved in the development of triggered activity must be specific to this tissue.

Hanich *et al* (1988) recently provided evidence that the autonomic nervous system exerts relatively small effects on the magnitude of cesium-induced EADs (recorded *in vivo* with monophasic action potentials), but nevertheless seems to markedly influence the outcome of EADs into ventricular tachycardia. Moreover, β-blockade can prevent sustained arrhythmias without eliminating EADs. Ben-David & Zipes (1988) have shown that the incidence of ventricular tachyarrhythmia secondary to cesium-induced EADs is higher with concomitant bilateral or left ansae subclaviae stimulation than controls with CsCl injections only.

In summary, the presence of adrenaline allows quinidine-induced triggered activity to manifest at cycle lengths closer to those observed during arrhythmias related to prolonged QT intervals. Thus, *in vitro* preparations with triggered activity occurring at physiologically relevant rates constitute an improved model of the arrhythmia torsade de pointes.

An important question in the study of triggered activity is the role of electrotonic interactions in its manifestation. The few recording sites used in the present thesis cannot address this question completely, but may provide some indication. *Figures D.4* and *D.5* show examples of an electrotonus.

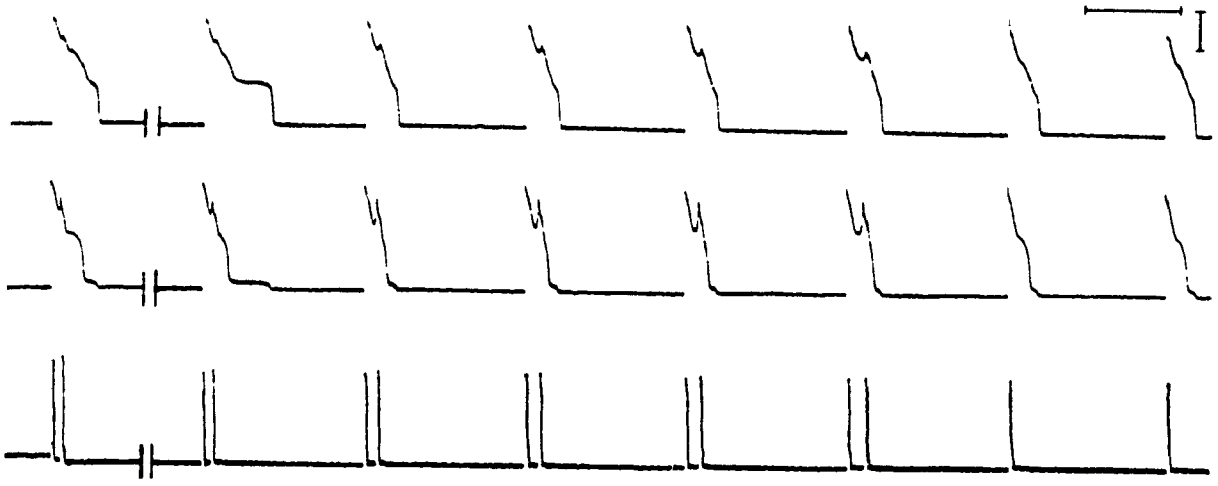

Figure D.4: Preparation from the right ventricle with 3 simultaneous recording sites exposed to quinidine (5 μM), CsCl (1 mM), and 2.7 K^+_o. The top trace is an impalement at the free wall insertion of the free-running Purkinje strand; the middle trace is an impalement at the papillary muscle insertion of the free-running Purkinje strand; the bottom trace is a ventricular muscle impalement in the papillary muscle. The interruption of the record is a time laps of 30 seconds. From the second action potentials to the end, the preparation is stimulated at a basic cycle length of 8 seconds. Vertical calibration is 10 mV and the horizontal calibration is 1 second.

In *Figure D.4*, EADs arising from the terminal portion of repolarization of the action potential are electrotonically transmitted at more negative membrane potentials across the Purkinje fiber, but fail to generate extra responses in muscle. On the other hand, extra-systoles seem to be associated with the elevated plateau. The strongest evidence for a direct relationship between the LMP triggered action potentials and the extra response in muscle is that the progressive prolongation of the coupling interval of the extra-response in muscle follows that of the triggered action potential. Moreover, the extra ventricular muscle response disappears with the elimination of the upstroke of the LMP triggered action potential.

Figure D.5 shows that triggered activity seems to originate from the papillary insertion of the free-running strand and propagate toward the middle of the fiber. At the recording site closest to the origin, triggered action potentials arise from a more positive activation voltage. EADs follow the same pattern, also occurring at a more positive membrane potential at the site closest to the site of origin and being electrotonically transmitted toward the opposite end of the Purkinje strand. However, EADs are not transmitted to the ventricular muscle impalement possibly because the recording site for the muscle is several space constants away from the insertion.

The non-uniformity of triggered activity in our experiments raises a certain ambiguity about LMP triggered activity. We have shown in *Figures 3.3* and *3.8* that triggered activity seemingly arising from the plateau at one recording site may be in fact partial electrotonic transmission of a HMP triggered action potential arising at some other recording site. Mapping of activity throughout the preparation is therefore important in determining whether or not LMP triggered

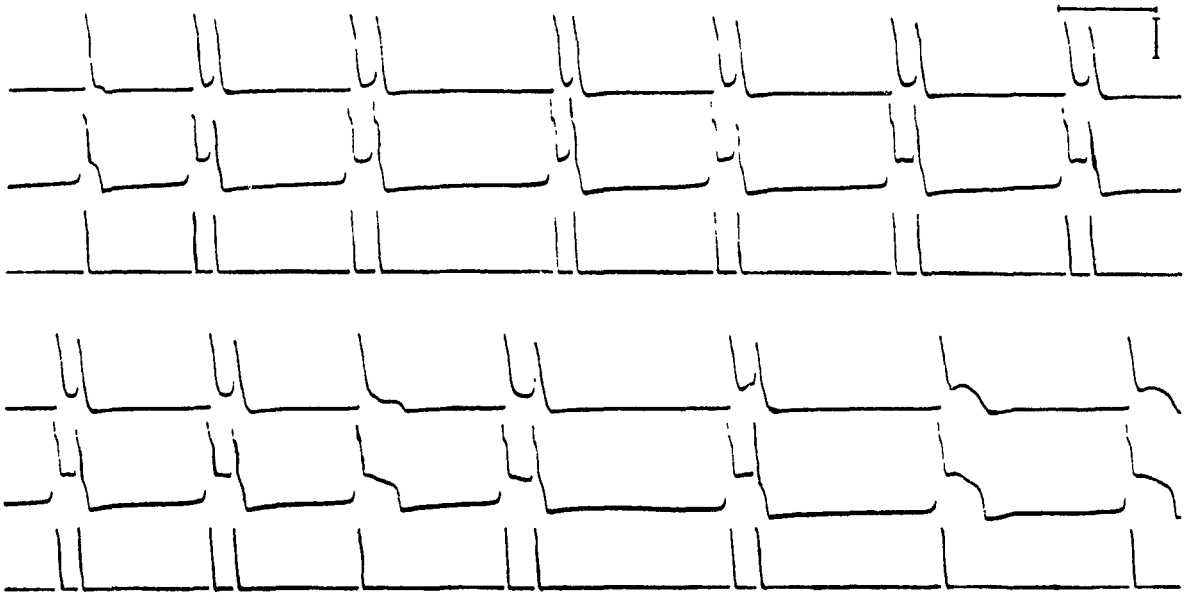

Figure D.5: Preparation from the right ventricle under quinidine (5 μM), CsCl (0.5 mM), and 2.7 mM K^+_o. The top trace is a recording site at the middle of the free-running Purkinje strand; the middle trace is a recording site at the papillary muscle insertion of the free-running Purkinje strand; the bottom trace is a ventricular muscle action potential from the papillary muscle. The horizontal calibration is 5 sec and the vertical calibration is 20 mV.

activity actually occurs at more than one recording site. The possibility of electrotonic transmission in multicellular preparations requires caution in the interpretation of such data and reinforces the importance of identifying the site of origin of triggered activity. The difficulty of distinguishing between "true" LMP triggered activity and mere incomplete transmission of HMP triggered activity or passive electrotonic influences from a distal site remains a drawback of our technique. This could be overcome by increasing the number of multiple recording sites to monitor activity through-out an entire bar-bell preparation.

Two main mechanisms for the ability of triggered action potentials to re-excite the ventricular mass have so far been proposed in the literature, one by Brugada *et al* (1985) and the other by Cranefield & Aronson (1988a, 1988b). Brugada & Wellens (1985) brought forward the concept of "prolonged repolarization-dependent reexcitation" based on the electrotonic interactions of LMP EADs occurring in the Purkinje system on the ventricular muscle mass. According to this hypothesis, HMP triggered responses do not have a primary role in initiating bradycardia-dependent arrhythmias because of the long coupling interval of their sustained activity and the long cycle lengths needed to record them *in vitro* (Damiano & Rosen, 1984). Our results suggest that when prolonged repolarization is to generate muscle extra-systoles, it occurs at a certain level of membrane potential, i.e. positive to -20 mV. Prolonged repolarization occurring at more negative membrane potential failed to induce extra ventricular muscle responses unless a depolarizing phase was present. Cranefield & Aronson (1988a, 1988b) proposed an alternative hypothesis according to which HMP triggered activity would play the relevant role in the genesis of arrhythmias. This mechanism of transmission of triggered activity to ventricular muscle involves propagation via exit conduction from a protected focus where entrance block would allow local

bradycardia.

Our results do not allow to exclude the possibility of either mechanism. This would require a depolarizing current in a compartment with Purkinje action potentials to stop repolarization at different levels of membrane potential and simultaneous recording in ventricular muscle in another chamber not subjected to these depolarizing currents. One could thereby determine whether prolonged repolarization fails to generate extra ventricular muscle responses as it progressively takes place at more negative membrane potentials.

Electrotonic influence from ventricular muscle was found to exert a strong effect on the APD prolongation of the Purkinje system in response to an increase in cycle length. That is to say, the very slow rates of stimulation needed for triggered activity under quinidine alone cannot be explained solely on the basis of the kinetics of the ionic currents involved in the repolarization phase. Additional factors influencing the manifestation of triggered activity *in vitro* include, among others, the electrotonic influence from neighboring cells, either favoring or opposing its emergence.

Figure D.6 illustrates the influence of ventricular muscle on Purkinje fiber. The same conditions that induced triggered activity at a BCL of 3 sec in a preparation without ventricular muscle activity were insufficient to induce it in a preparation with ventricular muscle activity (see inset), even at much longer cycle lengths. Ventricular muscle activity was tested by positioning the stimulating electrode on the septal side of the Purkinje strand of the bar-bell shaped preparation very close to the insertion of the free-running strand while obtaining a ventricular muscle impalement on the papillary muscle. If the ventricular muscle

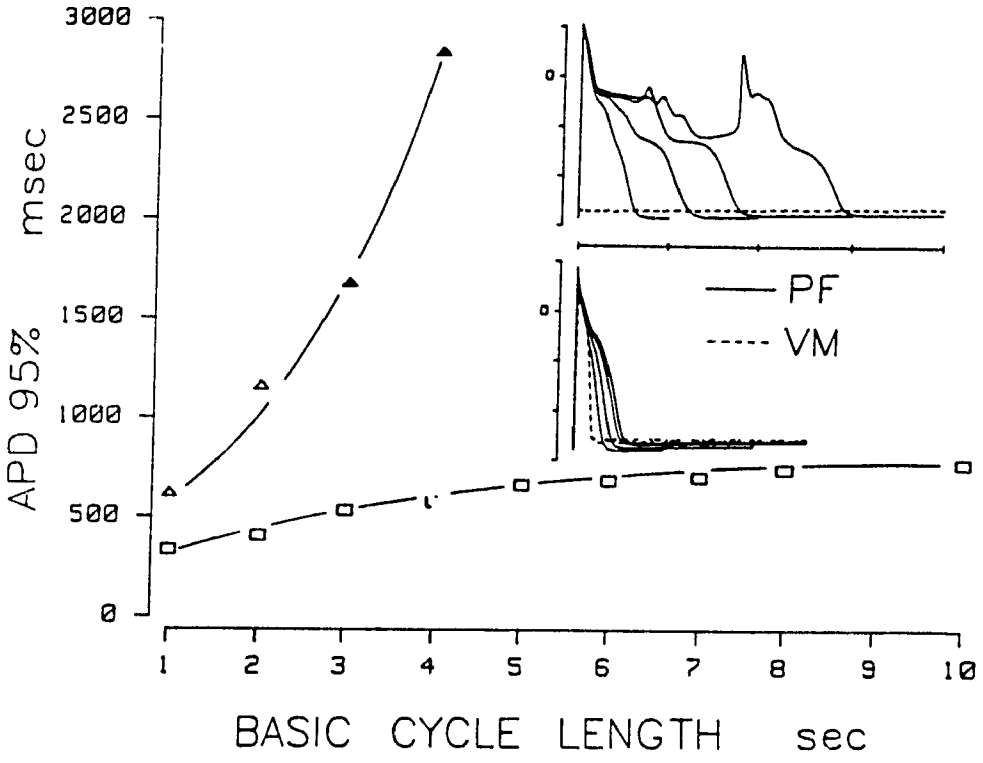

Figure D.6: Effect of the presence versus the absence of concomitant ventricular muscle activity on the cycle length dependent prolongation of the action potential duration under quinidine (5 μM), CsCl (1 mM) and 2.7 mM $[K^+]_o$. Each point represents steady-state reading of the total action potential duration. Cycle length was steadily increased from 1 sec to the longest that a preparation could follow, with sufficient time at each cycle length for the APD to reach equilibrium (between 2 and 4 minutes). The squares represent a typical preparation from the right ventricle with concomitant ventricular muscle activity; the triangles represent a typical preparation from the left ventricle in which there was no concomitant ventricular muscle activity. The closed symbols are action potentials followed by triggered activity. The inset is the superimposition of typical action potentials at basic cycle lengths of 1, 2, 3, and 4 sec respectively. The full lines represent the Purkinje action potentials and the dashed lines represent the ventricular muscle action potentials. The top panel is the waveforms series from the left ventricle preparation and the bottom panel from the right ventricle preparation. The vertical calibration is 50 mV and the horizontal calibration is 1 sec.

action potential could follow the stimulation from across the free-running strand, conduction was assumed to have taken place at the junction between Purkinje and ventricular muscle. When the ventricular muscle could not follow the stimulation from the septal side of the Purkinje free-running strand, we interpreted this as a lack of junction between Purkinje and muscle in that particular preparation.

Marked APD prolongation in Purkinje in response to steady increase in cycle length and emergence of triggered activity was correlated with the absence of Purkinje-ventricular muscle junction in 8 preparations. Minimal APD prolongation in Purkinje (and lack of EADs) in response to the same intervention was related to the presence of Purkinje-ventricular muscle junction in 7 preparations.

These results are supported by Balser & Roden (1987). They found that the presence of ventricular muscle reduces the incidence of EAD-induced triggered activity in preparations exposed to 10 μM quinidine-2.7 mM $[K^+]_o$ and blunts the APD prolongation seen at cycle lengths greater than 4 seconds.

In a recent study with preparations of short strips (between 2.5 and 6 mm long) of free-running Purkinje strands with no pieces of ventricular muscle attached at either insertion (Davidenko *et al*, 1989), the minimum basic cycle length was approximately 1 and 2.5 sec for LMP and HMP triggered responses, respectively. Since the difference between the study of Davidenko *et al* (1989) and our study is the presence of ventricular muscle pieces in the preparations, it is possible that the attachment of the pieces of muscle at the two insertions of the free-running Purkinje fibers in our experiments behaves as a current sink, thereby exerting electrotonic repolarizing influence, which would increase the need for very

low frequencies of stimulation.

Assuming that failure of ventricular muscle to follow stimulation from across the Purkinje fiber reflects the absence of junction between Purkinje and ventricular muscle, results from *Figure D.6* tend to suggest that uncoupling from ventricular muscle facilitates triggered activity. This indirectly supports the hypothesis of Cranefield & Aronson (1988a, 1988b). A certain degree of uncoupling may be necessary for the emergence of EAD-induced triggered activity in Purkinje fibers, possibly originating within a parasystolic focus. According to Cranefield & Aronson (1988a), this level of uncoupling should cause entry block without causing exit conduction block in order to favor the initiation of arrhythmias by triggered activity. The right balance has to exist between the inadequacy of the muscle to abbreviate the Purkinje action potential through electrotonic repolarizing influence versus the ability of the triggered potential to reexcite the muscle. Arrhythmia genesis would require the proper degree of uncoupling to allow triggered activity to develop and propagate throughout the heart would be one less that what would cause conduction delay or conduction block, but enough to isolate a segment from adjacent normal repolarizing regions. Entrance block would play a dual role by 1) allowing profound bradycardia could be attained locally; and 2) isolating this focus of Purkinje cells from the repolarizing electrotonic influence from the ventricular muscle, thereby permitting emergence of EADs giving rise to triggered upstrokes.

The need for triggered activity to originate within a isolated focus with such delicate conditions of conduction (i.e. entry block without exit block) in order to generate arrhythmias may well explain the rarity, and perhaps also the unpredictability, of torsade de pointes as a drug-induced complication of

antiarrhythmic therapy.

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ORIGINAL CONTRIBUTIONS

At clinically relevant concentrations, quinidine and an orally active lidocaine congener, tocainide, were found to interact in canine Purkinje fibers in a frequency and voltage dependent manner such that:

- At rapid rates of stimulation (as may occur during a tachycardia) or in the presence of extra-systoles, a combination of both drugs produces a greater depression of $\dot{V}_{max}$ and conduction than could be achieved with quinidine alone without adding to the depression achieved by quinidine alone at normal rates of stimulation. This result agrees with the predictions of Hondeghem & Katzung (1980);
- At extremely slow rates of stimulation, the combination prevents the marked prolongation of APD observed with quinidine alone;
- At depolarized potentials, the combination produces the same degree of depression of $\dot{V}_{max}$ and conduction as could be achieved by tocainide alone;

These results were obtained at clinically relevant cycle lengths and membrane potentials and thus, provide a rationale for the enhanced antiarrhythmic efficacy of a combination of two Class I drugs with different kinetics of interaction with the sodium channel and different voltage dependent characteristics.

Exploration of the conditions under which quinidine induces EADs and triggered activity in a consistent and reproducible manner led to the development of an *in vitro* model of quinidine-induced triggered activity:

- Under the same experimental conditions, therapeutic concentrations of quinidine produces two types of triggered activity, viz. arising from low membrane potentials (LMP) or from high membrane potentials (HMP) which exhibit different frequency dependence;

- Acidosis facilitates quinidine-induced triggered activity by a direct prolongation of the APD;
- Therapeutic concentrations of Class Ib drugs were effective in abolishing triggered activity induced by HMP EADs, but not LMP triggered activity;
- Abolition of LMP triggered activity required higher concentrations of Class Ib drugs and long periods of exposure;
- Shortening of APD was not the major mechanism by which Class Ib drugs abolished triggered activity induced by HMP EADs; abolition was dependent on depression of an inward current, presumably sodium.

The contribution of adrenaline in our *in vitro* model of quinidine-induced triggered activity was characterized. Facilitation of triggered activity involved the following specific effects:

- Adrenaline decreases the minimum cycle length for the manifestation of triggered activity, shortens the coupling interval of the triggered action potentials, and transforms single triggered responses into multiple responses;
- Adrenaline induces LMP triggered activity in preparations in which no such activity occurred in its absence;
- Induction of LMP triggered activity is associated with closely coupled ventricular responses and is accompanied by a flattening and prolongation of plateau duration;
- Adrenaline induces rapid activity resembling sustained triggered activity;
- Under circumstances of acceleration in the rate of automaticity, adrenaline can also abolish triggered activity;
- Repolarization abnormalities in Purkinje fibers are highly dependent upon the presence or absence of electrotonic interactions with ventricular muscle.