RATE-DEPENDENT EFFECTS OF PROCAINAMIDE ON INTRAVENTRICULAR CONDUCTION IN ANESTHETIZED DOGS: A QUANTITATIVE TEST OF A MATHEMATICAL MODEL

by

© Wuhua Jing

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Pharmacology & Therapeutics,
McGill University, Montreal, PQ., Canada

September, 1988

RATE DEPENDENT EFFECTS OF PROCAINAMIDE ON INTRAVENTRICULAR CONDUCTION

Dedicated to my mother, father, and brothers

for their love and support

Abstract

The present study was designed to test a mathematical model which quantitatively describes the interval-dependent effects of class I antiarrhythmic drugs on conduction velocity. This mathematical model is developed based on first order recovery from drug effects on I_{Na} or $\dot{V}_{max}$ and a linear relationship between I_{Na} or $\dot{V}_{max}$ and the square of conduction velocity. Both of these assumptions have theoretical and experimental support. We used non-linear least squares curve fitting techniques to assess interval-dependent changes in QRS duration and intraventricular conduction time caused by procainamide. The results showed good agreement with the mathematical model, which was a better descriptor than (previously applied) first order analyses. This study confirms the possibility of quantitative analysis of use-dependent antiarrhythmic drug action on conduction in vivo, and supports in vitro observations suggesting a proportional relationship between the square of conduction velocity and I_{Na} or $\dot{V}_{max}$

RESUME

La presente etude a ete realisee afin de tester un modèle mathématique qui decrit quantitativement les effets dépendants des intervalles, de drogues antiarrhymiques de classe I, sur la vitesse de conduction Le modèle mathematique que nous avons developpe est base sur un retablissement de premier ordre des effets de la drogue sur I_{Na} ou sur $\dot{V}_{max}$ et sur une relation lineaire entre I_{Na} ou $\dot{V}_{max}$ et le carré de la vitesse de conduction Ces hypotheses ont pu être avancees à l'aide des supports theoriques et experimentaux disponibles Nous nous sommes servis de techniques utilisant les courbes non lineaires des moindres carres pour évaluer les changements dependants des intervalles, causes par la procainamide, sur la duree du QRS et sur la conduction intraventriculaire Les resultats obtenus montrent une bonne correlation avec le modèle mathematique presente et celui-ci décrit mieux les resultats experimentaux que le modèle precedent (analyse du premier ordre). Cette etude montre egalement qu'il est possible d'effectuer des analyses quantitatives de l'action de drogues antiarrhymiques dpendante de l'usage sur la conduction in vivo De plus, ce travail confirme les observations in vitro suggerant une relation proportionnelle entre le carre de la vitesse de conduction et I_{Na} ou $\dot{V}_{max}$

TABLE OF CONTENTS

ACKNOWLEDGMENTS

I would like to thank the following people for their contributions and support during the preparation and completion of the thesis.

Dr A C Cuello for giving me the opportunity to pursue my graduate studies in the Department of Pharmacology and Therapeutics.

Dr Stanley Nattel, my research supervisor, for his guidance, enthusiasm, and encouragement during my stay in his laboratory, and for his constructive criticism and careful proof-reading of the thesis, without which the completion of the thesis would not have been possible.

Dr Brian Collier for his advice and encouragement.

Dr Mohsen Nayebpour for his friendship and for sharing with me good times and bad times

Carol Matthews for her skilled technical assistance and friendship.

Dr Serge Mykita for the French translation of the abstract.

Sue Bayly, Mara Solari, Anurag Tandon, Claire O'neill, Ligia Stern, Lorella Garofalo, Ronith Afar, Howard Mount, Jean-Claude Martel for their friendship and for making life enjoyable

Finally, I would like to reserve a special line for a special friend who never says no when asked for help, Dr. Rafael Cabeza, for his fraternal friendship, for his constant help in science and in life through the entire course of my studies, and for his helpful suggestions during the preparation of the thesis

LIST OF FIGURES

LIST OF TABLES

Introduction

1. Initiation and conduction of cardiac electrical activity

1.1 Anatomical features of the cardiac conducting system

The excitation of the heart is mediated by electrical pulses generated from the sinus node and spread through the cardiac conducting system. The cardiac conducting system consists of the sino-atrial (SA) node that generates electrical pulses spontaneously by a mechanism known as pacemaker activity, the atrioventricular (AV) node that connects atria and ventricles and conducts the impulse very slowly to ensure that there is a significant delay between the excitation of the atria and that of the ventricles, and the bundle of His that divides into bundle branches, which in turn conduct the impulse to the surface of the two ventricles where they ramify to Purkinje fibers. The large cells of the His-Purkinje system conduct very rapidly and ensure that the relatively large ventricles are excited almost synchronously.

1.2 Characteristics of the cardiac action potential

The basic element of cardiac electrical activity is the cardiac action potential which is composed of five distinct phases. When the cardiac cell is stimulated, it depolarizes rapidly from resting membrane potential until the cell is 25-40 mV positive inside the cell with respect to the outside (phase 0) Phase 0 depolarization is now known to be due to sodium or calcium inward current through sodium or calcium channels on the cardiac cell membrane. It is responsible for cardiac conduction and is also a major site of antiarrhythmic drug action The maximum rate of rise of phase 0 depolarization ($\dot{V}_{max}$) has been utilized as an index of sodium inward current (Weidmann, 1955; Hondeghem & Katzung, 1977). It is generally accepted that in cardiac cells (Trautwein,

1950; Weidmann, 1952), as in nerve fibers (Hodgkin & Katz, 1949), $\dot{V}_{max}$ contributes directly to determining the cardiac conduction velocity. Phase 0 is followed by repolarization that proceeds relatively fast at first (phase 1) and reaches a slower plateau phase (phase 2), and the cell is then repolarized more rapidly (phase 3) until the negative resting membrane potential is restored (phase 4). The subsequent phase 4 remains quiescent for nonpacemaker cells , but depolarizes spontaneously to reach the threshold from which the next action potential is generated for pacemaker cells.

1.3 Detection of cardiac electrical activity

The detection of cardiac electrical activity relies on the fact that the body in which the heart lies can be considered as a salt solution that conducts electricity. Therefore, the electrical currents generated during the heart beat can be detected from the surface of the body a long distance away from the heart itself by the electrocardiogram (ECG) Each time the heart beats, the ECG exhibits a small slow wave (P wave) which is followed by a fast series of changes (QRS complex) followed by another slow wave (T wave). These waves can be associated with events occurring in the heart itself. The P wave corresponds to atrial excitation, the QRS complex to ventricular excitation or phase 0 depolarization, and the JT interval (the J point is the last deflection point of the QRS complex) to ventricular repolarization QRS duration has been used extensively as an index of ventricular conduction time and JT interval as an index of ventricular action potential duration

Cardiac electrical activity is initiated by a propagating electrical wavefront which activates cardiac cells according to a given sequence. To identify such sequences requires a detailed knowledge of the spatial distribution of membrane activity. To accomplish this, simultaneous recording of electrical activity at a large number of points over the heart is

necessary. The sequence of cardiac electrical activation can thus be mapped. The use of intracellular electrodes to record accurately the intracellular potentials simultaneously from a sufficiently large number of sites has proven to be impractical because of tissue movement, cell damage, and inaccessibility (Spach et al, 1972; 1973). Therefore, extracellular potential recordings have been used. Spach et al. have provided a theoretical rationale and experimental evidence suggesting good agreement between activation as determined from extracellular and intracellular recordings in the two-dimensional structure, Purkinje fibers (Spach et al., 1973), and in multidimensional anisotropic cardiac muscle (Spach et al., 1979). Extracellular electrodes have been extensively employed in cardiac mapping to record potential changes at multiple sites in the heart.

The technique of epicardial mapping in dogs was first reported by Rothberger et al. in 1913 (Rothberger et al., 1913) and Lewis et al. in 1915 (Lewis et al., 1915). This technique was then used to record the excitatory process in the exposed heart of a patient (Barker et al., 1930). This method has been extensively applied to localize accessory pathways associated with the Wolff-Parkinson-White syndrome (Gallagher et al., 1978), to study normal and abnormal atrial activation (Puech et al., 1954; Wellens et al., 1971), to delineate the course of the atrioventricular (A-V) conduction system (Kaiser et al , 1970; Dick et al., 1979), and to identify areas of ischemia and infarction (Durrer et al., 1964) so as to further understand the mechanism of ventricular arrhythmia (Kramer et al., 1985).

Cardiac mapping is a method by which potentials recorded directly from the heart are spatially depicted as a function of time in an integrated manner (Gallagher et al., 1978) The recording can be achieved by employing a mesh sock with an array of electrodes over the heart. Extracellular potentials then can be recorded at many electrode sites. A unipolar recording from the

epicardial surface gives rise to a positive deflection, followed by a rapid intrinsic deflection in the negative direction, with a final return to the baseline (Gallagher et al., 1978). The fast negative deflection, the "intrinsic deflection", in the unipolar electrode lead corresponds to activation at each electrode site (Durrer et al., 1954) Unipolar recordings are generally recorded at frequencies settings of 0.1-1200 Hz The low frequency response tends to make the unipolar electrogram unstable. One of the ways to solve this problem is by using two closely spaced unipolar electrodes, a bipolar electrode, and recording differential voltage between them The configurations of the two unipolar leads recorded in contiguous areas differ only in the detail of the electrogram recorded at the moment of local activation (Gallagher et al , 1978). Therefore, if one of the unipolar leads is electrically inverted and the two leads are algebraically summated, the two unipolar electrograms will thus cancel each other except where voltage differences occur (Gallagher et al., 1978). This voltage difference will produce a differential spike that is coincident in time with the rapid intrinsic deflection of a unipolar electrogram (Durrer et al , 1954), and is referred to as a bipolar electrogram. The bipolar electrogram is recorded at filter frequencies of 5-1200 Hz and provides a sharp deflection that is readily identifiable. Both the peak amplitude (Durrer et al., 1954, Gallagher et al., 1978; Kramer et al., 1985) and the slope of the differential spike have been used as indicator of local activation.

Because of the extensive analysis needed to generate an activation map, computers are used for data handling All the electrogram signals are converted into digital form and transferred to a computer system The timing of electrogram signals by computer analysis has been shown to correlate well with the local activation time expressed relative to a standard ECG reference (Spach & Dolber, 1986). A continuous scan of simultaneously acquired

electrograms can then be performed, showing the local activation time of each electrode The sequence of surface activation is then depicted as isochrone lines. Conduction time to each surface electrode site can be calculated as the difference between the earliest activation time and the activation time at each surface site.

1.4 Membrane properties and cardiac conduction

Cardiac conduction depends on the excitation of certain cardiac cells which in turn excites adjacent areas. Propagation of the action potential depends on both passive and active membrane properties as they relate to the resting potential, subthreshold events and the fulfillment of the requirements for regenerative depolarization. Thus, the passive and active membrane properties of cardiac cells are all involved in providing a mechanism which permits conduction.

1.4 1 Passive membrane properties

Passive membrane properties are characterized by a response from the excitable cardiac membrane that is proportional to the stimulus. These are controlled by the determinants of the resting potential such as intracellular and extracellular ionic activities, energy-requiring pumps that maintain the ionic gradients and the cable properties of cardiac fibers. Specifically, passive electrical properties of excitable cardiac cells are characterized by membrane resistance, membrane capacitance and cable properties.

1 4.1.1 Membrane resistance

The membrane of cardiac cell is a thin, lipid bilayer that separates the aqueous phase inside and outside the cells. The lipid bilayer is a resistive barrier to the flow of ions and charged species. The potential difference

across the membrane of excitable cardiac cells at rest shows that the cellular membrane possesses a resistance to current flow. Sperelakis & MacDonald (1974) showed that in ventricular muscle, the resistivity was much lower longitudinally than transversely. This provides the basis for the important feature of more rapid conduction in the direction longitudinal to the fiber axis than that transverse to it.

Sano et al. (1959) were the first to show that conduction velocity in the direction of myocardial fibers was several times larger than that transverse to them. They also showed that a number of antiarrhythmic drugs seem to increase this difference. Drugs exerted greater suppression on transverse conduction than longitudinal. The same directional difference in conduction velocity was also noted by Kadish et al. (1986) in canine ventricular myocardium. However, they observed that procainamide's depressant effects on conduction velocity were greater in the longitudinal direction This discrepancy of direction-dependent antiarrhythmic drug action on conduction velocity remains unresolved, but other workers (Spach et al, 1987, Bajaj et al. 1987) have also observed preferential effects of sodium channel blockers in the longitudinal direction.

1.4.1.2 Membrane capacitance

The lipid bilayer of the membrane can be polarized Thus, the cardiac cell membrane can act as a capacitor or condenser which can store charge If a current is suddenly applied across the membrane of a cardiac cell, the transmembrane potential is found to reach its new value with an exponentially decaying rise (Arnsdorf, 1984) This is of importance since a sufficiently high capacity may slow conduction velocity. The resistivity of large extracellular fluid can be neglected compared to the internal resistivity The presence of both resistance and capacity of the membrane, and of non zero

internal resistivity makes cardiac cells behave like an electrical cable.

1.4.1.3 Cable properties

The cardiac cell, especially the Purkinje fiber, is more or less cylindrical and is very long in relation to its diameter. The cardiac cell has a low-resistance sarcoplasm and is surrounded by extracellular fluid. These compartments are separated by a relatively resistive and capacitative cell membrane. These structures resemble a telegraph cable. The classical cable equations have therefore been applied to cardiac structures. Hodgkin and Rushton (Hodgkin & Rushton, 1946) first showed that a modified cable equation fit experimental observations in a nerve axon. Weidmann (1952) applied one-dimensional cable theory to cardiac Purkinje fibers and found the experimental data to be well described by cable equations. The mathematical equations of one-dimensional cable theory describe the transmembrane current flow as composed of two membrane components: a capacitive current through the membrane capacitor (C_m) and an ionic current through the membrane resistor (r_m) The transmembrane ionic flow also equals the amount of current lost due to membrane leakage as longitudinal current flows through the myoplasm. Mathematical analysis can be performed based on the cable equations to derive characterizing terms that can then be assessed experimentally (Arnsdorf, 1984)

1.4 2 Active membrane properties

Active membrane properties are characterized by a response from the excitable cardiac membrane that is out of proportion to the stimulus. These include the critical amount of tissue that must be raised above threshold to overcome the repolarizing effects of adjacent resting tissues and to result in regenerative depolarization ("liminal length"), and the voltage and time

dependent ionic conductances which are responsible for depolarization and repolarization.

Lapicque (1907) first recognized in nerves that the current required to attain threshold was greater for stimuli of short than for stimuli of long durations. This current strength-duration relationship has been used to analyze the characteristics of, and drug effects on, excitability Fozzard & Schoenberg (1972) introduced the concept of "liminal length", which is the length of tissue required to be raised above threshold so that the inward depolarizing current from that region is greater than the repolarizing influence of adjacent tissues. This concept shows that cable properties are important in defining the conditions under which regenerative depolarization can occur.

1.4.3 Cardiac conduction

Cardiac conduction depends on propagation of the action potential When threshold is attained, sodium rushes into the cell down the electrochemical gradient, and the resulting intracellular positive charges offset the negative charges stored on the inside of the membrane. The local transmembrane voltage is thus more positive than in neighboring parts of the cell so a driving force for longitudinal current in the cytoplasm is established The circuit is completed by a capacitive current flow across the membrane and finally, by current flow in the extracellular space. Thus, conduction velocity is strongly dependent on passive and active membrane properties.

1.4.3.1 Determinants of conduction

As described above, conduction velocity depends on many factors It is determined by cable properties of the cardiac fiber, by threshold potential and resting potential, and most importantly by the maximal rate of phase 0

depolarization ($\dot{V}_{max}$) In general, the more positive the threshold is, the less excitable the membrane will be to a stimulus. Thus, if threshold is raised to a less negative value, cardiac conduction will be slowed. Raising the resting membrane to more positive values will also inactivate sodium channels, leaving fewer sodium channels available for phase 0 depolarization. As a consequence, cardiac conduction will be slowed. Most changes in conduction velocity primarily result from changes in phase 0 sodium current, as reflected by the rate of depolarization. The maximal rate of phase 0 depolarization indicates the rapidity and electrical strength with which one region of the membrane depolarize the adjacent region, in turn determining the rapidity of cardiac conduction

Arnsdorf & Bigger studied the effect of procainamide (1976) and lidocaine (1975), in concentrations equivalent to clinical antiarrhythmic plasma levels, on determinants of cardiac conduction. They found that both drugs shifted the non-normalized strength-duration curve upward, indicating that the tissue was less excitable. They showed that procainamide did so by making the threshold less negative while having no effects on resting membrane potential. On the other hand, lidocaine had no effects on threshold but decreased membrane resistance in the subthreshold range. Both drugs significantly decreased $\dot{V}_{max}$ but neither of them altered C_m.

1.4 3.2 Relationships between conduction velocity, $\dot{V}_{max}$ and I_{Na}

Active membrane properties can be considered the source or battery which supplies current to neighboring units. The maximal rate of rise of phase 0 depolarization is proportional to phase 0 inward current for a very small unit of membrane. The relationship between $\dot{V}_{max}$ and transmembrane current is less certain as units combine together, since it is greatly influenced by the state of neighboring membranes and the characteristics of longitudinal resistance.

One-dimensional cable analysis suggests that $\dot{V}_{max} \propto I_{total}/C_m$, indicating that $\dot{V}_{max}$ correlates well with the intensity of sodium current if C_m is constant and I_{total}, the total ionic current, is equivalent to the sodium current (Arnsdorf, 1984). The relationship between $\dot{V}_{max}$ and conduction velocity is more complicated. Hunter et al. (1975) gave an approximate solution of one-dimensional cable equations, suggesting that conduction velocity was roughly proportional to the square root of $\dot{V}_{max}$

The electrical spread of current first fills the capacitance of adjacent quiescent units. This results in a slow exponentially rising increase in transmembrane voltage which precedes the rapid depolarization phase and is called the "foot" of the action potential. Solutions of cable equations suggesting that conduction velocity correlate inversely with the time constant of the foot (τ_{foot}) were confirmed experimentally by Dominguez & Fozzard (1970).

2. Frequency dependent effects of antiarrhythmic drugs

2.1 Origination of the concept of frequency-dependence

Most of the clinically effective antiarrhythmic drugs decrease the maximum rate of depolarization of the cardiac action potential ($\dot{V}_{max}$) and slow myocardial conduction These effects are known to be due to their ability to inhibit the cardiac sodium or calcium inward current in fast and slow channel tissues, respectively. The alteration of sodium or calcium channel availability by antiarrhythmic drugs is strongly dependent on cardiac rate, being most evident at fast heart rates, and much less pronounced or even absent at slow heart rates. This phenomenon has been referred to as "frequency-dependent" or "rate-dependent" effects of antiarrhythmic drugs. Other commonly used terms relating to time-dependent properties of

antiarrhythmic drugs such as "use-dependent" and "interval-dependent" block have also been applied to characterize different aspects of their frequency-dependent effects. "Use-dependent block" refers to drug-induced reduction of sodium channel availability developing with successive action potentials. It describes the time-dependent onset of drug action and the absence of drug-induced block when tissue is completely rested. "Interval-dependent block" is related to time-dependent recovery from antiarrhythmic drug-induced sodium channel blockade.

2.2 Work leading to the formation of the modulated receptor hypothesis

2.2.1 Initial observations of frequency dependence

The first observation of frequency-dependent properties of antiarrhythmic drugs was made by Johnson and McKinnon in 1957. They reported that quinidine causes only a minimal decrease in $\dot{V}_{max}$ of guinea pig ventricular action potentials when the driving rate is 0.1 Hz but leads to a progressive decrease in $\dot{V}_{max}$ as the driving rate increases. This important finding was later confirmed by Heistracher in 1971. He showed that in quinidine-treated fibers, there is a clear time course of development of drug effects on $\dot{V}_{max}$ $\dot{V}_{max}$ in the first response following a resting period of several minutes is not decreased. It then decreases progressively with each subsequent response until a new steady state value is achieved. The extent to which the maximal rate of rise falls at the new steady state increases, and the time necessary to reach the new steady level decreases, with an increasing rate of stimulation. Furthermore, he also observed that the effects of quinidine and quinidine-like drugs (eg. procainamide) are reversible even in the presence of these drugs. If stimulation is interrupted for a sufficient period of time, the maximal rate of rise of the first action potential after

the pause in stimulation returns to the control value.

These two studies suggested that one of the most commonly used antiarrhythmic drugs, quinidine, has strong frequency-dependent inhibitory effects on $\dot{V}_{max}$, an in vitro index of sodium inward current, and alters the recovery kinetics of $\dot{V}_{max}$.

2.2.2 Further in vitro and in vivo studies related to frequency dependence

It is well known that in nerve tissue and cardiac muscle fibers, sodium inward current is responsible for the rising phase of the action potential Hodgkin and Huxley (1952) first showed that in squid giant axon, depolarization leads to a transient increase in sodium conductance, which allows sodium ions to move down their electrochemical gradient Draper and Weidmann (1952) found that the rapid phase of depolarization of cardiac action potential was also generated by an increase in sodium conductance Maximal rate of rise of the cardiac action potential, $\dot{V}_{max}$, was then proposed by Weidmann (1955) as an index of phase 0 sodium influx and of maximum sodium conductance of the cardiac fiber membrane. Extensive investigations were undertaken on the effects of quinidine and lidocaine, two sodium channel blockers, which are commonly used antiarrhythmic agents and are quite different in their kinetic effects on $\dot{V}_{max}$.

2.2.2.1 Quinidine

Ample in vitro observations have shown that quinidine decrease $\dot{V}_{max}$ in calf ventricular false tendon (Weidmann, 1955), guinea pig ventricular muscle fibers (Johnson, 1956; Johnson & McKinnon, 1957, Heistracher, 1971) , and isolated rabbit atria (Vaughan Williams, 1958; West & Amory, 1960) and ventricles (Gettes et al., 1962). Quinidine also caused decreases in conduction velocity (Vaughan Williams, 1958) or increases in conduction time

(West & Amory, 1960). The depressant influence of quinidine on depolarization or conduction was shown to be directly related to the frequency of depolarization in a dose-dependent fashion. West & Amory (1960) evaluated the effects of quinidine on $\dot{V}_{max}$ and conduction time simultaneously at various interstimulus intervals ranged from 10000 msec to 100 msec in <u>in vitro</u> preparations of rabbit atrium. In the absence of the drug, $\dot{V}_{max}$ and conduction time were not changed until the interstimulus interval approached 250 msec or less, where the effective refractory period was presumably encountered. However, in the presence of quinidine, depressant effects on $\dot{V}_{max}$ and conduction were substantially greater at short interstimulus intervals. Rate-dependent depression of $\dot{V}_{max}$ by quinidine was also shown in guinea pig papillary muscle fibers (Heistracher, 1971; Tritthart et al., 1971).

While there are many reports of the effect of quinidine on intracellular potentials of isolated cardiac muscle, there is comparably little in the literature on its effect <u>in vivo</u>. Prinzmetal et al. (1967) first investigated quinidine's action on electrical behavior in the canine heart <u>in vivo</u> with simultaneous recordings of intracellular potentials from the left ventricle. They observed that quinidine caused a considerable increase in QRS duration, associated with a decrease in maximal upstroke velocity ($\dot{V}_{max}$). Wallace et al. (1966) monitored quinidine's effect on H-S interval (interval between activation of the His bundle and the base of the ventricular septum), PM-S interval (interval between activation of the right anterior papillary muscle and ventricular septum), and QRS duration, indicators of ventricular activation time, in awake dogs with chronically implanted electrodes. Quinidine elicited time-dependent slowing of ventricular conduction. The magnitude of changes in H-S interval, PM-S interval and QRS duration were substantially enhanced as the driving rate was increased from 100 beats/min. to 150 beats/min. Thus, it was evident that quinidine not only causes

frequency-dependent depression of $\dot{V}_{max}$ and conduction velocity in vitro, but also slows intraventricular conduction in vivo in a rate-dependent fashion

2.2.2.2 Lidocaine

Unlike quinidine, the data on lidocaine's effect on $\dot{V}_{max}$ and conduction had been contradictory. Neither Davis & Temte (1969) nor Bigger & Mandel (1970a) found any depressant effects of lidocaine on $\dot{V}_{max}$ and conduction time in canine ventricular muscle and Purkinje fibers at concentrations below 5 mg/L when the preparation was paced at frequency of 95/min and 60-120/min, respectively . One study (Bigger and Mandel, 1970b) showed that, at a pacing cycle length of 800 msec, lidocaine increased $\dot{V}_{max}$ at concentrations below 5 mg/L. Thus, a different mode of action of lidocaine from that of quinidine and similar compounds, which interfere directly with depolarization, on the cardiac membrane was suggested (Davis & Temte, 1969, Bigger & Mandel, 1970a;1970b).

This conclusion was later challenged by Singh and Vaughan Williams (1971), who studied the effects of lidocaine on rabbit atrial and ventricular muscle. They found that lidocaine decreased $\dot{V}_{max}$ as extracellular potassium concentration was raised from 3.0 mM to 5.6 mM. They suggested that lidocaine might also slow the depolarizing phase of cardiac action potential, and that differences of action might be due to mechanisms other than effects on $\dot{V}_{max}$. Similar effects of lidocaine on canine Purkinje fibers were later observed by Rosen at al. (1976).

Using voltage clamp techniques to investigate the effect of lidocaine on the early transient inward current as reflected by the maximal rate of change of phase 0 of the action potential ($\dot{V}_{max}$), Weld and Bigger (1975) reported that in sheep Purkinje fibers the recovery from lidocaine-induced sodium channel blockade proceeds in a monoexponential fashion with a time constant

of 100 msec , suggesting a time-dependent interaction between lidocaine and cardiac sodium channels.

2.2.2.3 Lidocaine versus quinidine

Chen and Gettes (1976) compared the effects of lidocaine and quinidine on $\dot{V}_{max}$ in guinea pig papillary muscle. They observed that, as does quinidine, lidocaine produces depressant effects on $\dot{V}_{max}$. The decrease of $\dot{V}_{max}$ by lidocaine is also rate-dependent , but the rate must exceed 2 Hz before the rate dependent effect becomes noteworthy, whereas the depressant effect of quinidine is rate-dependent above 0.1 Hz (Johnson & McKinnon, 1957; West & Amory, 1960, Chen & Gettes, 1976; Heistracher, 1971). Similar to the finding by Weld and Bigger (1975) , they also observed that the $\dot{V}_{max}$ value recovers in a monoexponential manner (Chen et al., 1975) with a time constant of 120 msec. Furthermore, they found that lidocaine, but not quinidine, slows the recovery kinetics of $\dot{V}_{max}$ (Chen et al., 1975). The recovery kinetic parameters of lidocaine were a function of membrane potential. The preparation recovered from drug induced depression of $\dot{V}_{max}$ slower at more positive resting membrane potentials The absence of changes of recovery kinetics produced by quinidine, in contrast to the findings by Johnson and McKinnon (1957) and Heistracher (1971), probably occur because the coupling intervals used by Chen et al. were less than 500 msec. This recovery time interval was not long enough to observe substantial recovery from quinidine-induced $\dot{V}_{max}$ depression. Subsequent studies (Grant et al., 1980; Hondeghem & Katzung, 1980; Weld et al , 1982; Nattel, 1987a) confirmed that quinidine also slows recovery kinetics

2.2.2.4 Other agents possessing antiarrhythmic properties

Besides quinidine and lidocaine, which were studied extensively, a

number of other compounds that possess antiarrhythmic effects were also shown to have frequency-dependent actions. The reduction of $\dot{V}max$ by Diphenylhydantoin (DPH), an agent with lidocaine-like action, was substantially augmented by increases in frequency in rabbit atria (Jensen & Katzung, 1970; Singh & Vaughan Williams, 1971) and ventricle (Singh & Vaughan Williams, 1971). Droperidol, a neuroleptic drug known to exert antiarrhythmic effects, was studied in auricles, papillary muscles and Purkinje fibers of different species (Carmeliet et al., 1976). Similar to quinidine and lidocaine, droperidol significantly decreased $\dot{V}_{max}$ and conduction velocity. The former effect was more pronounced at lower membrane potentials and the inactivation curve relating $\dot{V}_{max}$ to membrane potential was shifted to more negative membrane potentials. The recovery time, defined as the time needed for $\dot{V}_{max}$ to recover its full amplitude, was markedly prolonged in the presence of the drug.

Although frequency dependent effects of antiarrhythmic drugs had been observed both <u>in vitro</u> and <u>in vivo</u>, the mechanism of this phenomenon was not clear. The mechanism of differences in action between quinidine and lidocaine was still a matter of dispute. Chen & Gettes (1976) ascribed the frequency dependent effects of quinidine to a resetting of the sodium potassium pump, and of lidocaine to a slowing of recovery kinetics. It was not until 1977 when Hille and Hondeghem & Katzung simultaneously postulated the "Modulated Receptor Hypothesis" that understanding of the mechanism of the frequency-dependent properties of antiarrhythmic drugs was greatly improved.

3. Molecular models for sodium channel blocking drug action

3.1 Membrane expansion theory

In 1949, it was first discovered that local anesthetics inhibit

electrical conduction in neurons by decreasing sodium conductance (Hodgkin & Katz, 1949). Seeman (1972) reviewed subsequent experimental observations and proposed that local anesthetics might act through electrically stabilizing the membrane by fluidizing and disordering the components within the membrane (Seeman, 1972). As a consequence, the membrane would be expanded and membrane-associated enzymes and proteins could be inhibited. Pathways for facilitated fluxes of solutes across membranes, such as the sodium channel, would be depressed, presumably because of the conformational changes brought about by the membrane-expanding effects. This "membrane expansion theory" was later extended by Lee (1975). He postulated that sodium channels in nerve membranes are surrounded by lipid molecules in the gel-like liquid phase. The addition of local anesthetics triggers a change in the surrounding lipids to the gel phase, allowing the sodium channel to close with resulting local anesthesia. However, these ideas, which suggest that local anesthetics and antiarrhythmic drugs block sodium channels by a nonspecific effect on the cell membrane, failed to explain the frequency (Johnson & McKinnon, 1957) and voltage dependent (Weidmann, 1955) properties of these drugs, and the competitive antagonism of combinations of two sodium channel blockers (Clarkson & Hondeghem, 1985).

3.2 Modulated Receptor Hypothesis

3.2.1 Gating mechanism of sodium channels--Hodgkin & Huxley model

Understanding of antiarrhythmic drug action evolved from the modulated receptor hypothesis. In 1952, Hodgkin and Huxley quantitatively described electrical activity in nerve tissue, and concluded that transmembrane current through sodium channels plays an essential role in conduction and excitation. They postulated that sodium channels exist in three primary states: the rested

state (R), most prevalent at negative membrane potentials, an open or activated state (A), through which sodium channels open to allow for the influx of sodium inward current upon depolarization; and an inactivated state (I) during which the sodium channel is closed at depolarized membrane potentials.

3.2.2 Development of the concept of drug-receptor interaction for local anesthetics

Drug-receptor interaction in ionic channels of nerve tissue was originally proposed by Armstrong (1966) to explain the blockade of K channels by internal quaternary ammonium derivatives. Such models were then utilized to explain the sodium channel blocking effects of quaternary (Strichartz, 1973) and tertiary amine (Courtney 1975) local anesthetic compounds.

Strichartz (1973) studied the effects of a quaternary derivative of lidocaine, QX-314, on sodium currents in myelinated nerve. He found that sodium channels are most quickly blocked by internally administered QX-314 when the channels are repetitively depolarized Drug-induced inhibition increases when the nerve membrane is subject to a train of depolarizing pulses. Both the inactivation gate (h gate) and activation gate (m^3 gate) must be open for the inhibition to increase. Finally, the drug-induced inhibition is reversible under conditions which open both h and m^3 gates such as small membrane depolarizations. Thus, he showed, using voltage clamp techniques, that the influence of the drug is markedly dependent on the previous history of use of sodium channels. It was evident that the inhibition of sodium currents is enhanced by membrane depolarizations of sufficient magnitude to open the channels, a phenomenon referred to as "use-dependence" of sodium channel blockade. These results suggest that QX-314 must interact directly with sodium channels to account for the voltage-sensitive block. A simple

model to explain drug-induced sodium channel block, which takes all the above features into consideration, was constructed by the author. Strichartz postulated that QX-314 binds to, and dissociates from open sodium channels. QX-314 cannot bind to or dissociate from the closed channels, but complexed channels can open and close in the normal manner. It was assumed that voltage sensitive inhibition could arise from either drug binding itself, if it is directly influenced by membrane potential or by voltage-dependent steps which provide and remove substrates for drug binding These steps refer to the opening and closing of free and complexed sodium channels which precede or follow the QX-314 binding reaction. Furthermore, a scheme of the structure of a sodium channel was proposed, suggesting that the binding site for QX-314 is located within the sodium channel between the selectivity filter and the outer mouth of the sodium channel, with the m and h gate towards the axoplasmic surface of the channel internal to the drug binding site.

Courtney (1975) observed similar effects for an exceptionally hydrophilic amine lidocaine analog GEA 968. Repetitive opening of the sodium channels by depolarizing pulses enhanced the voltage sensitive inhibition observed in the presence of GEA 968. This inhibition could be partially reversed by repetitive opening of the sodium channels produced by depolarizing pulses which were preceded by large hyperpolarizing prepulses. Furthermore, he found that with GEA 968, use dependent sodium channel blockade recovered with a time constant averaging 10 sec. The inactivation curve of GEA 968-induced sodium channel blockade was found to be shifted to more negative membrane potentials. In other words, more polarized membrane potentials were required to permit recovery from sodium channel inactivation to occur. Thus, it was hypothesized by Courtney that the GEA 968 molecule binds to open sodium channels and, in doing so, simultaneously blocks the channel and shifts the curve relating channel inactivation to more negative membrane potentials.

Unless strong hyperpolarizing prepulses are given, sodium channels would become trapped in the drug-associated inactivated state during repetitive stimulation. This hypothesis extended Strichartz's model by adding the idea of shifted voltage dependence of sodium channel inactivation.

These observations suggested that all inhibitory effects of sodium channel blocking drugs are explainable assuming binding of local anesthetics to a single receptor. Cumulative frequency dependent and voltage dependent block could be seen as manifestations of a single inhibitory mechanism (Courtney, 1975).

3.2.3 Hille's modulated receptor hypothesis

To further understand the mechanism of antiarrhythmic drug action, Hille in 1977 studied the effects of neutral, amine, and quaternary local anesthetic compounds on the sodium inward current of the node of Ranvier by using the voltage clamp technique He found that benzocaine, a neutral compound, produced a full inhibition of sodium inward current on the first test pulse and showed no further reduction of sodium influx upon continuous stimulation Lidocaine, an amine form of local anesthetic, showed some diminution of sodium influx on the first pulse and a further decrease on fast repeated stimulation The quaternary molecules, QX-572 and RAD 251 I (quaternary derivatives of lidocaine), showed almost no effect on the first pulse and a considerable but slowly developing decrease in sodium current on repetitive stimulation

Building on these findings, Hille proposed that there is a single specific receptor in the sodium channel for all anesthetics. When the receptor is bound by the drug molecule, the sodium channel forms (R, A and I) may be modified to yield the drug-receptor complexed forms (R*, A* and I*) The modified forms themselves can undergo transitions between resting, open and inactivated states. However, these transitions of the modified channels may

have rates and equilibria different from those for normal channels. Furthermore, the reaction of a drug with one form may also have different rates and equilibria from the reaction with another form. Therefore, this model was first named the "modulated receptor hypothesis" to emphasize the modulation of the drug-receptor interaction by sodium channel state.

It is noted that there is another important aspect of this model. Hille postulated that there are two pathways for a drug to access the receptor in the sodium channel. The hydrophilic pathway is a direct route for charged or less lipid-soluble compounds to pass by the open gates and through the channel to the receptor, but is available only when the gates of the channel are open. Thus, it is the only pathway for quaternary anesthetics. The hydrophobic pathways are available at all times to lipid-soluble molecules because lipid soluble drug forms can bypass the gates and are thought to come and go from the receptor via a hydrophobic region of the membrane by diffusion through the membrane. The hydrophobic pathway renders the lipid soluble compounds' recovery time constants much faster than for charged drug forms. Hence, the apparent differences in the action of neutral, amine, and quaternary molecules observed by Hille can be largely explained by the differences in the rates of drug-receptor interactions.

3.2.4 Hondeghem and Katzung's modulated receptor hypothesis

Almost simultaneously, Hondeghem and Katzung in 1977 reviewed the effects of commonly used antiarrhythmic drugs on the $\dot{V}_{max}$ of the cardiac action potential under conditions of different rates and rhythms of stimulation, and compared these with drug effects in nerve tissue. They found a striking similarity between nerve and cardiac excitation. Therefore, the Hodgkin and Huxley description was utilized to frame a fundamental hypothesis of antiarrhythmic drug interaction with cardiac sodium channels.

A theoretical model was produced to predict the effects of antiarrhythmic drugs on $\dot{V}_{max}$ of the cardiac action potential and provide a general model to describe the mechanism of antiarrhythmic drug action. The resulting "Modulated Receptor Model" postulated that antiarrhythmic drugs bind to a common receptor site on or very close to the cardiac transmembrane sodium channel. The affinity of the receptor for drug binding is modulated by sodium channel state. Antiarrhythmic drugs usually have a much higher affinity to bind to the receptor when the sodium channel is in the activated (A) or inactivated (I) state , whereas they have a much lower affinity for the receptor binding when the sodium channel is in the resting state (R). Each sodium channel blocker has a characteristic association and dissociation rate constant for channels in each of these state. The drug associated channels do not conduct, and show transitions between drug associated resting (R*), activated (A*) and inactivated (I*) channels in a voltage dependent fashion. However, the voltage dependence is altered such that the state transition behaves as if the membrane potential is less negative.

Like Hille, Hondeghem and Katzung (1977) hypothesized that, despite the heterogeneous chemical properties among antiarrhythmic drugs, they interact with the same receptor site on cardiac sodium channels. The sodium channels were considered to consist of a hydrophilic channel, which provide a hydrophilic pathway for drug access, whereas the hydrophobic lipid membrane affords a hydrophobic pathway for drug molecules to gain access to the receptor. The differences in actions of antiarrhythmic drugs can be attributed to differential binding to the channel receptor which is a function of the state of the channel and the lipid solubility of the individual drug.

An important aspect of Hondeghem and Katzung's model is its explanation of the disparate effects of antiarrhythmic drugs on different parts of the heart. The differences can be viewed as a consequence of different action

potential configurations in various portions of the heart. According to the model, drug interactions with sodium channels are time- and voltage-dependent. Therefore, different action potential configurations could result in significantly different numbers of sodium channels in various states. The drug pool concentrations in each sodium channel state would be dramatically altered

The "Modulated Receptor Hypothesis" was expressed in a set of differential equations to quantitatively predict antiarrhythmic drug action on sodium channels. It was found that the predictions of the computer model generated from the "Modulated Receptor Hypothesis" remarkably concurred with experimental observations of the use and voltage dependent effects of quinidine and lidocaine. It also predicted the changes in $\dot{V}_{max}$ when quinidine and lidocaine are used in combination; drug combinations could provide steady state block that could not be attained by either drug alone (Hondeghem & Katzung 1980)

3.3 Starmer's model

The modulated receptor hypothesis views the frequency- and voltage-dependent effects of antiarrhythmic drugs as a result of a variable-affinity receptor governed by the the sodium channel state and modified inactivation kinetics in drug-complexed channels. An alternative model was proposed by Starmer et al. (1984) to consider drug-induced block as a consequence of drug binding to a constant-affinity channel receptor, with receptor access regulated by the channel gates. Conducting channels leave the receptor binding sites unguarded and accessible to drugs, whereas nonconducting channels will convert the gates to closed conformations that restrict drug access to unbound receptors and trap drugs in drug-complexed channels. The computer simulations generated from this model are in quantitative agreement with experimental

results of time- and voltage- dependent drug action on cardiac sodium channels.

Despite the differences of the basic concepts of these two models described above, they both provide accurate quantitative analyses of antiarrhythmic drug action and constitute basic models in attempting to understand underlying molecular mechanisms.

3.4 Determinants of frequency dependence

Different antiarrhythmic drugs have distinctive kinetics of blocking action (Hondeghem & Katzung, 1977). The differences in the kinetics of frequency dependent effects of antiarrhythmic drugs arise from varying intrinsic physico-chemical properties and factors that affect these properties.

3.4.1 Molecular weight

Since use-dependent block results from the binding of an antiarrhythmic agent to its receptor site on sodium channels via a hydrophilic pathway, the sodium channel (Hille, 1977), factors such as molecular weight and size may be important determinants of drug-channel association and dissociation rates Courtney (1979) showed that in rabbit atrium, the rate of onset of frequency dependent block in $\dot{V}_{max}$ produced by sodium channel blockers correlates well with the molecular weight of the drug molecule. Smaller local anesthetics produce faster frequency-dependent blockade. In another <u>in vitro</u> study of interval-dependent effects of several structurally differing antiarrhythmic drugs in guinea pig myocardium, Courtney (1980a) showed that the molecular weight of the antiarrhythmic drug applied also plays an essential role in determining the recovery kinetics of frequency-dependent blockade. These results were confirmed by Campbell (1983) who demonstrated proportional

relationships between onset and recovery time constants and molecular weights of class I antiarrhythmic drugs. Sada and Ban (1981) reported a significant correlations between the recovery time constants and the molecular weights of various structurally related beta-adrenoceptor blocking agents which produced frequency-dependent depression in $\dot{V}_{max}$ in guinea pig papillary muscles It was assumed that, according to the modulated receptor hypothesis, drugs with larger molecular weight or size would less readily access the receptor site through the sodium channel, and thus be associated with a longer onset and recovery time constant. A similar linear relationship was also found between the recovery time constant induced by four calcium channel antagonists and the molecular weight of the compounds (Uehara & Hume, 1985).

3 4.2 Lipid solubility and degree of ionization

Although the correlation between the onset and recovery time constant and the molecular weight or size of a drug is significant, frequency dependence of block cannot be fully explained by molecular weight alone. Some drugs, eg lidocaine (234 MW) and procainamide (235 MW), have almost the same molecular weight and yet procainamide has a much slower time constant (lidocaine τ 150 ms, procainamide τ=2.6 sec) (Courtney, 1980a). Thus, other physico-chemical characteristics of the drug have to be taken into consideration in accounting for the determinants of the kinetics of antiarrhythmic drug action.

The very low lipid solubility of procainamide might explain why it is a kinetically slower drug than lidocaine. As a matter of fact, when taking both molecular weight and lipid solubility into account, Courtney (1980a) observed a much better correlation with recovery time constant than by molecular weight characteristics alone. In a later study by Courtney (1983), the concentrations of amide-linked drugs required for a given blocking action varied inversely

with the lipid distribution capability of each drug. Nattel and Bailey (1983) compared the electrophysiological effects of quinidine with those of lidocaine and disopyramide and also found that the greater lipophilicity and lipid/water partition coefficient (an indicator of the lipid solubility of a compound) of lidocaine is related to its faster onset of action and washout than those of quinidine and disopyramide. Courtney (1980b) and Sada & Ban (1981) showed that the lipid solubility of drug molecules correlated well with the potencies of local anesthetics in nerve, and the structurally related beta-adrenoceptor blocking drugs in guinea pig ventricular muscle, respectively.

The lipid solubility of a drug molecule is closely related to the degree of ionization of the compound. The permanently charged form of a drug is insoluble in fat, while a neutral compound is much more lipophilic. Hence, the kinetics of recovery for drugs that are predominantly charged are slower than for drugs that are predominantly neutral (Hille, 1977). Schwarz et al. (1977) reported a longer time constant of use dependent block on sodium current for the permanently charged QX-314 than for lidocaine in frog skeletal muscle. Gliklich et al. (1978) compared the effects of lidocaine and those of several derivatives including QX-314 and QX 472, which are two quaternary derivatives, and compound 6603, which is a tertiary analogue with a pKa of 9.81, on $\dot{V}_{max}$ in canine cardiac Purkinje fibers. They found that when administered by superfusion (drugs acting extracellularly), these four agents exerted qualitatively similar depressant effects on $\dot{V}_{max}$ at high drug concentrations, but the rate of onset of action of the quaternary derivatives was considerably slower than that of lidocaine. On the other hand, intracellular injection resulted in a marked decrease or attenuation of $\dot{V}_{max}$. These effects were reversible with time for lidocaine but not for its permanently charged derivative QX-314. It was speculated that even though some 80% of lidocaine molecules are charged at intracellular pH, the concentration gradient greatly

favors efflux from the fiber of the uncharged form during intracellular injection. As the uncharged molecules cross the membrane and are removed from within the fiber, charged molecules lose protons in order to maintain the charged-uncharged ratio In this manner, the intracellular lidocaine concentration rapidly decreases However, this mechanism does not operate when a molecule, such as QX-314, is permanently charged. It was therefore concluded that drug effects on $\dot{V}_{max}$ result from the charged form acting from the inner surface, in agreement with findings by Strichartz (1973) and Hille (1977) in nerve tissue

In an attempt to determine whether the charge of antiarrhythmic drugs influences their effects on cardiac fibers, Gintant et al. (1983) investigated the effects of charged and uncharged forms of local anesthetic drugs on sodium inward currents in canine Purkinje fibers , using changes in $\dot{V}_{max}$ during phase 0 as an indicator of changes in peak sodium inward current. A series of lidocaine tertiary analogues with different pKa, as well as permanently charged quaternary analogues and the neutral compound benzocaine, were chosen to provide different proportions of both drug forms. They found that only the quaternary analogues and tertiary amine analogues existing predominantly in the charged form cause use dependent block. Furthermore, the onset and recovery time constants were the longest for permanently charged quaternary molecules and the shortest for 6211 (93% uncharged). Benzocaine showed no use dependent block, but it is possible that the association and the dissociation of benzocaine with the sodium channel is so fast that use dependent block does not occur even at the most rapid stimulation rates possible for the preparation. This was the first systematic study in cardiac tissues relating molecular charge of antiarrhythmic agents with their effects on sodium inward currents. These concepts were further confirmed by Sanchez-Chapula et al. (1983) who showed a significant use dependent block for lidocaine and a

minimal use dependent block for benzocaine on the sodium inward current measured by voltage clamp techniques in rat single ventricular cells They suggested that the small molecular size and predominantly neutral form of benzocaine at physiological pH may contribute to the lack of use dependent effects of the compound, or to an extremely fast onset and recovery rate that were not detectable by the technique.

Therefore, it appears that molecular weight and lipid solubility of antiarrhythmic drugs can account for a significant part of their rate dependent properties. The rate of block development and the rate of recovery from block can be predicted on the basis of the ionic charge and the molecular weight of local anesthetic drugs. quaternary compounds block rather slowly, while neutral compounds block much more quickly For tertiary compounds the rate of block development appears intermediate and can be altered significantly by changes in pH (Schwarz et al., 1977) In a quantitative structure activity study of antiarrhythmic properties in a series of lidocaine and procainamide derivatives, Ehring et al (1987) showed that the affinity of a drug for the open sodium channel state is related to the compound's lipid solubility, while the rate of dissociation from inactivated channels is correlated with both the molecular weight and charge of the drug molecule

3.4.3 Environmental pH

Most antiarrhythmic drugs are weak bases. Thus, these drugs exist in both cationic and neutral forms in a ratio determined by the environmental pH As described above, the degree of ionization of drug molecules dramatically influence the potency and the kinetics of frequency dependent block produced by local anesthetics. External acidosis or lowering environmental pH promotes the cationic drug form which less readily crosses the membrane. Since the cationic drug form obtained by lowering the pH is less accessible to the

receptor on the sodium channels, slower kinetics of action are anticipated in the presence of acidosis.

Schwarz et al. (1977) studied the effect of pH on the use dependent block of sodium channels in frog skeletal muscle, using voltage clamp techniques They observed that with lidocaine and procaine, increasing external pH significantly shortened the onset time constant, whereas changes in intracellular pH had little effects on use dependent block. This was confirmed by Bean et al. (1983) who reported that increasing the pH sped up the onset of lidocaine block of the sodium inward current measured by voltage clamp techniques in rabbit Purkinje fibers. Schwarz et al. (1977) also observed a substantial lengthening of the recovery time constant by lowering extracellular pH. Since cationic drug forms reach the drug receptor only from the intracellular side of the membrane (Strichartz, 1973; Hille, 1977) and lowering of external pH would result in more cationic drug forms that are less readily able to cross the membrane, the question arose as to how an increase in external protons influence drug molecules which react with the receptor. Schwarz proposed a kinetic model suggesting that the major effect of lowering pH is the production of protons that can get to the receptor site via the external mouth of the sodium channel. Thus, protonation of the drug-receptor complex will occur and antiarrhythmic drug molecules will be trapped in the blocking position for a longer time. Grant et al. observed that when the extracellular pH was reduced from 7.4 to 6.9, the time constant of recovery from block was increased by 100% for lidocaine (1980), and by 66% for quinidine (1982) in guinea pig ventricular myocardium. Bean et al. (1983) also reported a decrease in the recovery time constant from 810 msec to 450 msec when pH was increased from 7.0 to 8.1 for lidocaine-induced block of sodium inward current in rabbit Purkinje fibers.

The effects of pH changes on the potency of drug action are more

complicated. Schwarz et al. (1977) reported a decrease in the steady state block by lidocaine when external pH was decreased. Grant et al (1982) observed little change in the potency of quinidine upon changes of external pH, whereas Nattel (1981) found an increase in depressant effects on $\dot{V}_{max}$ for both lidocaine and quinidine in the presence of external acidosis. Since external acidosis creates more cationic drug forms that are less readily to access the receptor and thus reduce the net drug concentrations in the fiber, this effect will be expected to reduce the drug potency (Schwarz et al., 1977). On the other hand, external acidosis causes protonation of drug-bound-receptor complex which will prolong drug action and as a consequence increase drug potency (Schwarz, 1977; Grant et al., 1980, 1981, Bean et al., 1983). Therefore, it is believed that the effect of pH changes on antiarrhythmic drug potency depends on the balance between these two factors (Hondeghem & Katzung, 1984).

4. Subsequent work related to the modulated receptor model

Molecular models (Hille, 1977; Hondeghem & Katzung, 1977, Starmer et al., 1984) of antiarrhythmic drug interactions with cardiac sodium channels have greatly extended our understanding of the cellular mechanism of antiarrhythmic drug action. Numerous subsequent studies have been undertaken in nerve and in cardiac tissue to investigate the frequency dependent effects of antiarrhythmic drugs in the light of the modulated receptor hypothesis. Electrophysiological and biochemical work have provided observations that are consistent with the model and allowed for further insight into the mechanisms of antiarrhythmic drug action.

4.1 In nerve tissue

4 1.1 Electrophysiological studies

Rimmel et al. (1978) studied the rates of block by procaine and benzocaine as well as the procaine-benzocaine interaction at the the node of Ranvier According to the modulated receptor hypothesis, benzocaine, which stays permanently uncharged over a wide range of pH, passes to the common receptor through a hydrophobic pathway and therefore has faster onset and recovery kinetics than procaine which exists predominantly in the cationic form at physiological pH, and is expected to access the receptor site through a hydrophilic pathway. Rimmel et al. found that, when these two drugs were used in combination, the decrease produced in $\dot{V}_{max}$ was as much as that by benzocaine alone. Furthermore, when benzocaine was taken away from the benzocaine-procaine mixture, there was a temporary partial relief of block followed by a characteristic procaine induced inhibition. When a hyperpolarizing prepulse was used, block by benzocaine was relieved faster and to a greater extent than the block produced by procaine. This is expected only if benzocaine competes with procaine for the same receptor, but binds and leaves the receptor faster than procaine. These results provide evidence for a common receptor site as suggested by the modulated receptor hypothesis. Similar results were obtained later for the interaction of benzocaine and lidocaine (Schmidtmayer & Ulbricht, 1980), and benzocaine and the indole alkaloid ervatamine (Pichon et al., 1981), an alkaloid known to block the sodium channels of cockroach and squid axons in a frequency dependent fashion.

4 1 2 Biochemical studies

Because of extensive electrophysiological investigation, much is known regarding the effects of local anesthetics on sodium channels in nerve membranes, suggesting a common receptor site for local anesthetics' action.

However, there is no direct evidence identifying the receptor site on the sodium channel. The development of radiolabelled neurotoxins which interact specifically with the sodium channel has provided a new approach to study the drug-receptor interaction model at the molecular level. Voltage-sensitive neuron sodium channels have been shown to have at least three distinct receptor sites for neurotoxin binding (Catterall, 1980). The inhibitors tetrodotoxin (TTX) and saxitoxin bind at receptor site 1 and inhibit ion flux through the sodium channels (Colquhoun et al., 1972; Ritchie & Rogart, 1977, Henderson et al., 1973). The alkaloids veratridine, batrachotoxin (BTX), and aconitine act at receptor site 2 and cause persistent activation of sodium channels (Catterall, 1980). The polypeptide scorpion toxins and sea anemone toxin bind at receptor site 3 (Catterall, 1980) These toxins slow sodium channel inactivation, and enhance persistent activation of sodium channels caused by toxins acting at site 2 through an allosteric mechanism. Using a tritiated form of batrachotoxin, [^{3}H]BTX, Catterall showed that a number of widely used class I antiarrhythmic drugs competitively inhibit [^{3}H]BTX binding at neuron toxin binding site 2, and thus block neurotoxin-activated sodium channels in neuroblastoma cells (Catterall, 1981) and rat brain synaptosomes (Postma & Catterall, 1984). Binding phenomena were seen at concentrations that effectively block sodium channels in electrophysiological studies The competitive inhibition of [^{3}H]BTX binding by local anesthetics appears to be through an allosteric mechanism. It is likely that there is a common receptor site for antiarrhythmic drugs which is located in the sodium channel and is distinct from neurotoxin receptor site 2 so that local anesthetics cause an allosteric interaction and alter batrachotoxin binding indirectly

4.2 In cardiac tissue

4.2.1 Electrophysiological studies

4 2.1.1 In vitro work

A common mode of action to all the sodium channel blockers is the depression of phase 0 of the cardiac action potential. Information about drug effects on cardiac sodium channels in vitro can be obtained by either using $\dot{V}_{max}$ as an indirect measure of sodium inward current, or by employing voltage clamp techniques which directly measure the sodium inward current.

Although the validity of $\dot{V}_{max}$ as an index of sodium inward current has been a subject of dispute, it nevertheless provides information about drug behavior on the cardiac sodium channel and is a practical in vitro method to study antiarrhythmic drug action on cardiac sodium current under physiological conditions Abundant data for a variety of sodium channel blockers has been acquired regarding the time dependent inhibition by antiarrhythmic drugs of sodium inward current by evaluating their effects on $\dot{V}_{max}$

Sada et al. (1979) showed that procainamide caused a frequency- and dose-dependent reduction of $\dot{V}_{max}$ in guinea pig papillary muscles. The time dependent recovery from procainamide-induced reductions in $\dot{V}_{max}$ proceeded in a monoexponential manner with a time constant averaging 4 seconds. Their experimental data was incorporated into a computer simulation based on the modulated receptor hypothesis and found to be consistent with model predictions. - Interval-dependent effects of procainamide on $\dot{V}_{max}$ in guinea pig myocardium were also reported by other investigators (Courtney, 1980a; Buchanan et al., 1985) In vitro work has indicated a simple exponential recovery process from procainamide-induced decrease in $\dot{V}_{max}$ with a time constant averaging from 1 to 4 seconds (Courtney, 1980a; Varro et al., 1985; Nattel, 1987a; Ehring et al., 1988).

Interval dependent block and first order recovery from drug-induced

reduction of $\dot{V}_{max}$ were also reported for lidocaine (Hondeghem & Katzung, 1980, Courtney, 1980a; Oshita et al., 1980; Grant et al., 1980), quinidine (Hondeghem & Katzung, 1980; Grant et al., 1982), mexilitine (Hohnloser, 1987, Campbell, 1983) and other sodium channel blockers (Courtney, 1980a, Oshita et al., 1980; Campbell, 1983) in guinea pig papillary muscles The time constant for each drug was found to be in the same range in different studies. Varro et al. (1985) were the first to quantitatively characterize the frequency dependent effects of several class I antiarrhythmic drugs in canine Purkinje fibers. The time constants obtained from their studies were comparable to those reported from the studies using guinea pig ventricular myocardium Nattel (1987a) examined the interval dependent reduction of $\dot{V}_{max}$ and prolongation of conduction time by lidocaine, mexilitine, procainamide, amitriptyline and quinidine. The recovery kinetics were first order and the time constants were in the same range as those obtained by Varro et al (1985). Moreover, he also found that for each drug, the time constant of recovery from drug induced depression of $\dot{V}_{max}$ is nearly identical to that from drug induced conduction slowing. Weld et al (1982) employed voltage clamp techniques to control membrane potential and apply conditioning pulses but used $\dot{V}_{max}$ to indirectly measure drug effects on the sodium inward current in ovine Purkinje fibers. They showed that quinidine produced a use and voltage dependent depression of $\dot{V}_{max}$ with an exponential recovery process

It has been difficult to obtain a reliable measurement of phase 0 sodium inward current (I_{Na}) in cardiac tissue under physiological temperatures by voltage clamp techniques because of difficulties in maintaining voltage control in the face of a large I_{Na} and distinguishing I_{Na} from the capacitance artifact (Grant et al., 1984, Fozzard et al., 1985). To study the time dependent inhibition of I_{Na} by sodium channel blockers, voltage clamp experiments have been performed on cardiac tissue at low temperatures.

Sanchez-Chapula et al. (1983) noted a use dependent inhibition of I_{Na} by lidocaine in rat single ventricular cells at 24°C. Recovery from lidocaine induced I_{Na} diminution showed first order kinetics with a recovery time constant averaging 500 msec. This time constant is much slower than those reported for lidocaine from <u>in vitro</u> studies at 37°C using $\dot{V}_{max}$ as an index of I_{Na} (Hondeghem & Katzung, 1980; Courtney, 1980a; Sada et al., 1980; Grant et al , 1980, Campbell, 1983; Varro et al., 1985; Nattel, 1987a). Similar use dependent changes in I_{Na} were characterized by Bean et al. (1983), who studied lidocaine-induced block at 17°C in rabbit Purkinje fibers. Thus, it appears that sodium channel blocking antiarrhythmic drugs exert frequency dependent inhibition of $\dot{V}_{max}$ and I_{Na} The time dependent recovery from antiarrhythmic drug blockade can be expressed by a single exponential process with a characteristic time constant

The modulated receptor hypothesis was also tested and shown to account for the effects of drug mixtures on cardiac sodium channel blockade. In an elegant experiment in guinea pig ventricular myocardium, Clarkson & Hondeghem (1985) showed that lidocaine competitively antagonized bupivacaine-induced sodium channel blockade. Bupivacaine is a popular local anesthetic drug known to block sodium channels both in nerve and cardiac tissue and to have relatively slow recovery kinetics (τ=1-2 sec.) (Clarkson & Hondeghem, 1985) as compared to those of lidocaine (τ=150 msec). In the presence of high concentrations of these two drugs, the drug that has fast recovery kinetics (lidocaine) displaced the drug with relatively slow recovery kinetics (bupivacaine), and caused a net reduction in sodium channel blockade at certain stimulation frequencies This can be explained only if these two drugs bind to a common receptor site on the cardiac sodium channel as proposed by the modulated receptor hypothesis. This study provided further insight into the electrophysiological effects of drug mixtures, as well as a critical test

of the hypothesis that local anesthetic and antiarrhythmic drugs block cardiac sodium channels by binding to a common receptor site.

4.2.1.2 In vivo work

While frequency dependent effects of antiarrhythmic drugs on the sodium inward current and $\dot{V}_{max}$ have been well documented in vitro as described above, much less is known about the interval dependent effects of antiarrhythmic drugs on cardiac conduction in vivo. Amitriptyline is a tricyclic antidepressant known to produce both sinus tachycardia and ventricular arrhythmia after overdoses in man. Nattel (1985) studied its frequency-dependent effects on $\dot{V}_{max}$ in canine Purkinje fibers in vitro, and compared this to its effects on QRS duration, an index of ventricular conduction time, in the intact dog heart in vivo He showed that amitriptyline produced a dose dependent prolongation of QRS duration that was markedly frequency dependent. A similar rate dependent depression in the maximum rate of rise of the action potential ($\dot{V}_{max}$) was observed for canine Purkinje fibers superfused with amitriptyline in vitro The time course of recovery both in vitro and in vivo was found to be well fitted by a single exponential process. Davis et al. (1986) used His-to-Ventricular conduction interval (HV interval) as well as QRS duration as indicators of ventricular conduction time to study the use dependent effects of lidocaine on ventricular conduction in vivo. They reported that lidocaine produced a rate- and dose-dependent prolongation of both HV interval and QRS duration. The two parameters were qualitatively changed in the same direction, although the former is a more accurate measure of conduction time in the His-Purkinje system. The time constant reported for lidocaine from their study was similar to the previously reported time constant of recovery from lidocaine induced depression of $\dot{V}_{max}$ (Hondeghem & Katzung, 1980; Courtney, 1980; Oshita et al., 1980, Grant et al.,

1980; Campbell, 1983, Varro et al., 1985; Nattel, 1987a) in vitro. The steady state effects of lidocaine on HV interval were markedly enhanced, and the recovery time constant increased by five fold during acidosis, consistent with in vitro findings by Grant et al. (1980). Bajaj et al. (1987) evaluated the frequency- and orientation- dependent actions of two other class I antiarrhythmic drugs, mexilitine and quinidine, both alone and in combination, on intraventricular conduction in vivo. Mexiletine induced frequency dependent increases in conduction time at cycle lengths of 600 msec or less, and these changes were significantly greater in orientations along myocardial fibers. Quinidine, on the other hand, increased the conduction time at all cycle lengths tested (250 to 1500 msec) without a significant orientation dependent effect The time constant for recovery from frequency dependent conduction slowing by mexiletine was 223 ± 23 msec, in agreement with the time constant obtained in vitro (Varro et al., 1985), while recovery from frequency dependent conduction depression by quinidine was incomplete over the mean pauses obtainable in their in vivo study, indicating a long recovery time constant for quinidine. The time course of recovery in the presence of both drugs was shown to be made up of a faster mexiletine component and a slower quinidine one fitted by a biexponential function.

Antiarrhythmic drug effects on conduction have also been shown to be qualitatively frequency dependent in man (Morady et al., 1985; Gang et al., 1985) Morady et al. (1985) demonstrated the rate dependent effects of intravenous lidocaine, procainamide and amiodarone on intraventricular conduction in patients. Similar results were obtained by Gang et al. (1985) for procainamide.

4.2.2 Biochemical studies

Recently, Sheldon et al. applied the concept of neurotoxin binding on

nerve sodium channels to cardiac tissue and demonstrated that there is also a [^{3}H]BTXB (Batrachotoxin benzoate, an analogue of BTX) binding site in rat cardiac myocytes (Sheldon et al., 1986). Similar to the studies in nerve, they (Sheldon et al., 1987) showed that class I antiarrhythmic drugs competitively inhibited the [^{3}H]BTXB binding on rat myocyte sodium channels and block [^{3}H]BTXB-induced sodium activation through an allosteric mechanism. It was demonstrated that the inhibition by antiarrhythmic drugs of [^{3}H]BTXB binding is saturable, reversible, and stereospecific and occurs at pharmacologically relevant concentrations, with similar rank orders of potency as reported for in vivo and in vitro electrophysiological studies. These results further confirm one assumption of the modulated receptor hypothesis- that the binding of antiarrhythmic drugs to a common receptor site results in their pharmacological activity

5. Basis of the generation of the current hypothesis

5.1 Quantification of frequency dependent effects of antiarrhythmic drugs

Antiarrhythmic drugs have been shown to alter sodium channel availability in a frequency dependent manner. These observations have been incorporated into two important molecular models describing antiarrhythmic drug action, one explaining frequency dependence on the basis of the relationship between drug affinity and sodium channel state (Hondeghem & Katzung, 1977), and the other relating frequency-dependence to changing drug access to a constant affinity receptor (Starmer et al., 1984). While these models have important clinical implications (Hondeghem & Katzung, 1984), the nature and significance of these models remain to be fully elucidated. To establish the clinical relevance of these models requires the demonstration that antiarrhythmic drug effects on conduction in vivo have a time dependency

that is quantitatively predictable from the interval dependence of effects on V_{max} in vitro

Quantitative analysis of sodium channel blockers (Nattel, 1985; Davis et al., 1986, Bajaj et al., 1987) and calcium channel blockers (Talajic & Nattel, 1986) has shown that their kinetics of action on conduction in vivo are similar to previously studied kinetics for blockade of the corresponding inward currents (Uehara & Hume et al., 1985) or their indices ($\dot{V}_{max}$) in vitro (Nattel, 1985, Chen et al., 1975; Varro et al., 1985; Nattel, 1987a). No theoretical models have provided a basis to explain the quantitative relationship between drug effects on conduction velocity and the recovery time interval So far, all the quantitative studies of antiarrhythmic drug kinetics in vivo have empirically used first-order kinetic models to evaluate time-dependent recovery from drug effects on conduction, in analogy to in vitro observations showing a first order recovery from drug effects on $\dot{V}_{max}$ and I_{Na}

5.2. Controversy existing in current studies

The relationship between measures of phase 0 sodium inward current and conduction velocity is not totally understood. Approximate solutions of equations describing propagation in one-dimensional cable suggest a linear relationship between $\dot{V}_{max}$ or I_{Na} (normalized to C_f, the foot capacitance of the action potential) and the square of conduction velocity in fast channel tissue (Hunter et al., 1975; Walton & Fozzard, 1983). This nonlinear relationship between $\dot{V}_{max}$ and conduction velocity has been demonstrated in vitro in canine Purkinje fibers (Nattel, 1987a) and guinea pig myocardium (Buchanan et al., 1985) for the effects of a number of class I antiarrhythmic drugs If recovery from drug-induced changes in phase 0 current is first order, and changes in conduction velocity are proportional to the square root

conduction slowing would not be expected. This raises the question of why there has been good agreement between first-order analysis and measurements of interval-dependent conduction slowing in vivo.

6. Goal of the present studies

Nattel (1987b) developed a mathematical model which predicts the recovery of drug-induced conduction changes assuming a linear relationship between drug-induced alterations in measures of phase 0 inward current and the square of conduction velocity, and accommodating a quantitative analysis of interval dependent effects of antiarrhythmic drugs on conduction in vivo. The model was originally derived using $\dot{V}_{max}$ as an indicator of phase 0 sodium current, but total phase 0 sodium current, normalized by the capacitance of the foot of the action potential (I_{Na}/C_f), could be used equally well. The model predicts that the time-dependent recovery of drug-induced conduction slowing will fit a first order approximation at lesser magnitudes of drug effect, but will increasingly deviate from the first-order relationship as drug effects increases. These predictions have been tested quantitatively and been found to hold for the effects of lidocaine on conduction in canine Purkinje fiber false tendon in vitro (Nattel 1987b).

The purpose of the present studies is to test critically the ability of the model, incorporating a linear relationship between I_{Na} or $\dot{V}_{max}$ and the square of conduction velocity, to account for the time-dependent recovery from antiarrhythmic drug effects on conduction in vivo. The mathematical model can only be critically tested if most of the recovery process can be observed. We therefore selected procainamide as the antiarrhythmic drug for evaluation, because its recovery time constant (Varro et al., 1985, Nattel, 1987a, Courtney, 1980; Sada et al., 1979; Ehring et al , 1988) is sufficient short

for nearly complete recovery to occur over pause durations attainable in vivo. We avoided drugs with more rapid recovery kinetics than procainamide because of the consequent difficulty we anticipated in differentiating between early diastolic drug unbinding and recovery from voltage-dependent (phase 3) block. The model describes drug effects on conduction in terms of changes in conduction time, and is valid as long as the conduction pathway remains constant. In initial experiments, we used total QRS duration as the simplest available measure of ventricular conduction time. Subsequently, epicardial activation mapping using computer-based analysis of simultaneously acquired electrograms from 56 epicardial locations was used to further test the model.

Methods

1. General methods

Mongrel dogs of either sex weighing 7-15 kg were anesthetized with morphine (2 mg/kg im.) and alphachloralose (100 mg/kg iv) Small dogs were selected in order to facilitate the production of atrioventricular (AV) block by injecting formalin as previously described (Nattel 1985). All dogs were ventilated via an endotracheal tube at a rate of 10/min with a tidal volume obtained from a nomogram. Catheters were inserted into the left femoral artery and both femoral veins and kept patent by heparinized saline solution (0.9%) Arterial blood gases were measured to ensure adequate oxygenation (SaO_2 > 90%) and physiological pH (7.38-7.45). A right thoracotomy was performed in the third intercostal space and a pericardial cradle created

A bipolar, Teflon coated, stainless steel electrode was inserted into the right ventricle. Constant current pacing stimuli were delivered by a programmable stimulator and a stimulus isolator using 4 msec square-wave pulse at twice diastolic threshold

2. Production of atrioventricular (AV) block

In order to control and evaluate the ventricular rhythm over a wide range of pacing frequencies, it is imperative to induce complete atrioventricular (AV) block to prevent the pacemaker influence from the SA node. Complete AV block was produced by the injection of small quantities (average 0.15 ml) of formalin directly into the AV node region using the method of Steiner and Kovalic (1968).

A standard right thoracotomy was made through the third intercostal space to expose the region of the superior vena cava, right atrium, and root of the aorta. A tissue clamp was used to retract the right atrial appendage to

expose the groove between the right atrium and the aortic root. This provides a useful approach to the bundle of His which lies superficial and can be reach by passing a needle only a few millimeters into the myocardium. A 25-gauge hypodermic needle of 1.5 cm length was bent approximately 60° at the base and attached to a 1 ml syringe filled with 40% formaldehyde. The needle was inserted into the atrial tissue at the groove between the right atrium and aorta and directed parallel to the aorta inferiorly and dorsally to a depth of 0.5 to 1 cm. As the needle was advanced, aspiration was carried out to preclude entry into the atrial cavity. If no blood could be aspirated, 0.1 ml of 40% formaldehyde was injected and the needle withdrawn.

Throughout the procedure, the electrocardiogram was monitored. If AV block was not observed within 2 min, or blood was withdrawn during aspiration, the procedure was repeated. Experiments with more than five injections of formaldehyde were rejected for analysis. Immediately after AV block was produced, artificial pacing was performed. The right ventricle was paced at a frequency at 1 Hz, except when specific pacing protocols were used to evaluate frequency-dependent drug action.

3 Data recording

3.1 ECG recording

A Mingograf paper record (Siemens, Can., Ltd.) was used to monitor the six standard surface electrocardiographic recordings, arterial pressure and stimulus artifacts. Electrocardiographic recordings were obtained at 250 mm/sec paper speed.

3.2 Activation mapping

An array of 56 bipolar electrodes with 2 mm interpolar distance in a

mesh sock (Bard Electrophysiology Inc., Billerica, Mass) was applied to the heart. A bank of operational amplifiers was used to amplify and filter each signal with a bandpass of 30 to 400 Hz. The signals were digitized with 12 bit resolution and a 1 kHz sampling rate, and transmitted via duplex fiber optic cables into a Compaq 286 microcomputer (Compaq Computer Corp , Houston, Texas). Software routines were used to amplify, display, and analyze each electrogram signal, as well as to generate maps showing activation times at each electrode site. Interpolation techniques were used to produce isochrone maps of epicardial activation, but only measured activation times (not interpolated data) were used for quantitative analysis. Each electrogram was analyzed using computer-determined peak amplitude criteria (Kramer et al., 1985), and was reviewed manually to exclude low-amplitude signals with indiscrete electrograms Activation time was expressed relative to a standard surface ECG reference point. For each test interval an 8 second window of data was acquired, timed to include the complex resulting from the test stimulus and (whenever possible) the last complex of the basic train The data was stored temporarily on a hard disk, and later down-loaded on high density (1.2 Mbyte) diskettes for subsequent analysis Electrogram review and map generation was performed off line after the completion of each experiment Hard copy of isochrone maps and activation times for each test activation was obtained using an IBM inkjet printer, and data from each map was also saved on high density diskette. Hardware and software for the mapping system were obtained from Biomedical Instrumentation Inc., Markham, Ontario, Canada

The stimulating electrode was positioned adjacent to a right ventricular epicardial electrode. Conduction time to each surface electrode site was calculated as the difference between the activation time at the epicardial site adjacent to the stimulating electrode and the activation time at each surface site. Constancy of the activation pattern was evaluated in two ways:

(1) by observing the pattern of isochronal activation (qualitatively); and subsequently (2) by comparing the relative conduction time to each electrode site. Quantitative analysis of the latter was obtained by normalizing the conduction time at each electrode site to the conduction time at the site of latest activation. This provides a numerical index of the relative time of activation at each point on the epicardial surface, which should remain constant for different complexes if the activation pattern is unchanged. For beats displaying varying degrees of drug-induced conduction slowing, a constant pattern of activation would be reflected by unchanging relative activation times at each electrode site.

4. Experimental procedure

4.1 Evaluation of frequency dependence

Steady state drug effects on QRS duration and JT interval were determined after at least 30 seconds of continuous pacing at selected basic cycle lengths between 300 msec and 2000 msec. The interval dependence of drug effects on QRS duration and ventricular activation times was evaluated by stimulating the ventricles at a basic cycle length (S_1S_1) of 300 msec and applying test pauses (S_1S_2 interval) of selected duration. A basic train of 40 stimuli was used to ensure constant steady state block prior to the test interval. The activation resulting from the S_2 was recorded at 250 mm/sec paper speed in experiments using QRS duration as an index of overall conduction time.

4.2 Experiment protocol

Changes in QRS duration and JT interval over a range of basic cycle lengths, and alterations in QRS duration and activation time as a function of

S_1S_2 coupling intervals, were measured under control conditions. Loading and maintenance dose infusion regimens were then applied to produce a series of stable, selected concentrations for procainamide. Procainamide was the drug chosen for this study because its in vitro recovery time constant is approximately 2.6 sec (Courtney, 1980), allowing us to observe almost complete recovery from frequency-dependent sodium channel blockade within the maximal pause range obtainable (generally 4-8 sec), and to avoid contamination from voltage dependent phase 3 block. The loading and maintenance infusion regimens that we used are summarized in Table 1. Doses were selected to produce stable drug concentrations which would create a range of drug-induced QRS prolongation of 25-75% at a basic cycle length of 300 msec. Electrophysiological studies were begun 20 minutes after the onset of each maintenance infusion. The time course of recovery from drug-induced conduction slowing was then evaluated, using the same protocol as under control conditions. Blood samples for procainamide concentration measurements were drawn prior to and after electrophysiological studies to confirm the stability of drug concentrations during each drug infusion. Plasma procainamide concentration was measured by reverse-phase high performance liquid chromatography (HPLC).

5. Data analysis

5.1 Mathematical model

The mathematical model was developed with the following two assumptions. The first assumption is theoretically based on the analysis of cable properties (Hunter et al., 1975; Walton & Fozzard, 1983) as experimentally confirmed in vitro (Walton & Fozzard, 1983, Buchanan et al., 1985; Nattel, 1987a), and the second one is predicted by the modulated receptor hypothesis

(Hondeghem & Katzung, 1977) and verified by a vast number of in vitro observations (Chen et al., 1979; Grant et al., 1980; Varro et al., 1985; Nattel, 1987)

(1) A proportional relationship between the peak inward ionic current or $\dot{V}_{max}$, and the square of conduction velocity (CV).

(2) First order recovery of drug-induced changes in I_{total} or $\dot{V}_{max}$.

If I is used to represent I_{total} or $\dot{V}_{max}$, then the following equations will apply

$$I \alpha (CV)^2 \qquad (1)$$

$$\frac{d(\text{delta } I)}{dt} = k(\text{delta } I) \qquad (2)$$

Where (delta I) = normalized drug-induced reduction from control in the measured index of phase 0 current at recovery time t, for instance, delta $I=(I_t-I_c)/I_c$, and k is the rate constant characterizing the first order process. With the further definition that CT = L/CV, where CT = conduction time between two points under analysis and L = path length, the above equations can be combined as previously shown (Nattel, 1987b), resulting in a differential equation of the form:

$$\frac{d(\text{delta CT})}{dt} = \frac{k}{2} \cdot \frac{(CT)\ (CT + CTc)}{(CTc)^2} \cdot (\text{delta CT}) \qquad (3)$$

Where CT = conduction time at time t, CT_c = control conduction time, (delta CT) = $(CT - CTc)/CT_c$ (i.e. drug-induced relative change in conduction time at recovery time t), t = recovery time and k = recovery rate constant as defined above. The above equation (3) can be rearranged as:

$$\frac{d(\text{delta CT})}{dt} = \frac{k}{2} \cdot (1 + (\text{delta CT})) \cdot (2 + (\text{delta CT})) \cdot (\text{delta CT}) \qquad (4)$$

This equation (4) implies that the rate of change in drug-induced conduction slowing is solely determined by the instantaneous magnitude of drug

effects on conduction (delta CT) and the rate constant (k) for changes in the of sodium current. For small magnitude of drug effect, (delta CT) tends to 0 and changes in conduction time should approximate a first-order function with rate constant k. For large changes in conduction time, the curve relating (delta CT) to recovery time interval should deviate increasingly from the terminal exponential as conduction time increases Figure 1 shows a series of recovery curves for a range of procainamide concentrations predicted by the quadratic model. On a logarithmic scale, the terminal portion of the curve is linear, and deviations from linearity become evident as drug-induced conduction slowing exceeds 20%. The terminal linear portion of the log plot, which was fitted by linear least-squares regression (dashed lines), has a slope (as predicted by the model and shown in the figure) equal to the rate constant (k) for changes in I_{total} Other specific predictions of the quadratic model regarding model logarithmic plots of delta CT against coupling interval include: (1) the slope of the terminal linear portion should be indepeendent of drug dose and the magnitude of drug action; (2) the nonlinearity of the overall curve should be more apparent for doses of the drug producing larger effects; and (3) the coupling interval at which nonlinearity appears should be greater for larger doses

The model was originally developed (Nattel, 1987b) using $\dot{V}_{max}$ as the index of sodium current in equations (1) and (2) This was done because of the theoretical (Walton & Fozzard, 1983) and experimental (Walton & Fozzard, 1983; Buchanan et al., 1985; Nattel, 1987a) evidence suggesting that, at least when varied by antiarrhythmic drugs, $\dot{V}_{max} \alpha (CV)^2$ (equation 1a) and the large amount of experimental work suggesting that

$$\frac{d\ (\text{delta } \dot{V}max)}{dt} = k(\text{delta } \dot{V}max) \qquad \text{(equation 2a)}$$

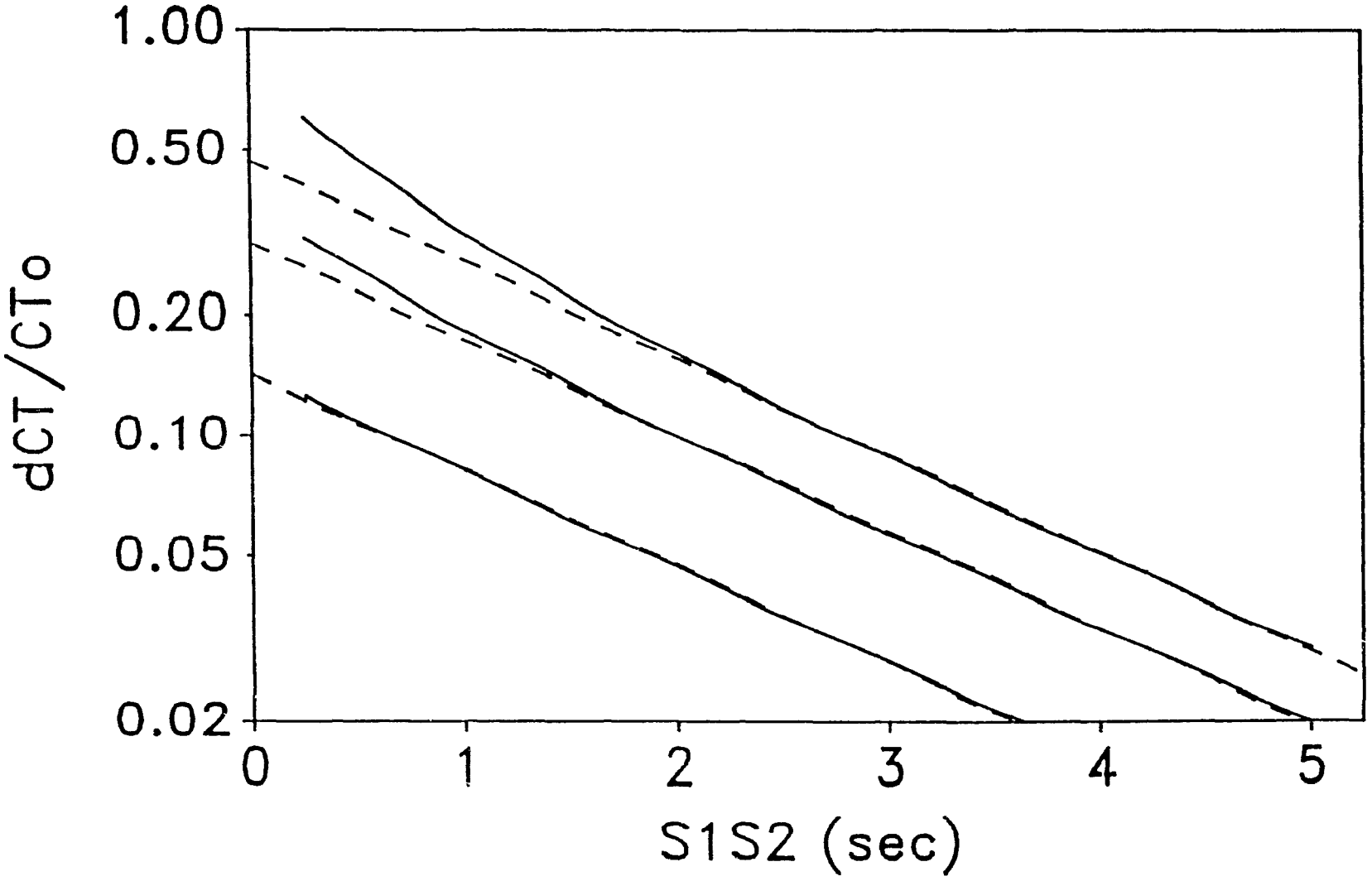
for a host of sodium channel blocking drugs (for reviews see Hondeghem &

FIGURE 1· Expected recovery of procainamide-induced conduction changes if conduction velocity is proportional to the square root of phase 0 sodium current, based on the mathematical model described in the text

A recovery time constant of 2 sec (rate constant = 0.5 sec^{-1}) was assumed, and curves calculated (solid lines) for drug concentrations producing 1, 2, and 3% conduction time increases at a coupling interval of 5 seconds. Drug-induced increases in conduction time normalized to control conduction time (dCT/CT_0) are plotted logarithmically against the coupling interval. CT_0 represents the conduction time at the longest coupling interval studied (in this case, $CT_0=CT_c$), and $dCT=(CT-CT_0)$. The terminal portion of each curve was fitted by linear least-square regression (dashed lines). The slopes of the fitted lines are 0 54, 0 54, and 0 55 sec^{-1} respectively, and the correlation coefficients of the lines are all 0.998.

FIG. 1

MODEL–PREDICTED CURVES



Katzung, 1977; 1984; Grant et al., 1984). There is thus strong theoretical and experimental support for the validity of equations (1) and (2) when formulated in terms of $\dot{V}_{max}$ (as in equations 1a and 2a), and equations (3) and (4) should follow. While there is dispute about the relationship between $\dot{V}_{max}$ and phase 0 sodium current (I_{Na}) (Cohen et al., 1984), the validity of the model depends on the correctness of assumptions (1) and (2), and not necessarily on the relationship between the index of sodium current used ($\dot{V}_{max}$) and I_{Na} (phase 0 sodium current). In any case, the model could be derived in an identical fashion if we assume that I_{total} is primarily composed of I_{Na} at the time of $\dot{V}_{max}$ (Hondeghem, 1978).

$$I_{Na} \alpha CV^2 \qquad (1b)$$

and

$$\frac{d\,(\text{delta INa})}{dt} = k(\text{delta INa}) \qquad (2b)$$

Walton and Fozzard (1983) as well as Arnsdorf (1984) have provided evidence that I_{Na} (we assume $I_{total} = I_{Na}$, see Discussion) normalized to the capacitance of the foot of the action potential is linearly related to the square of conduction velocity, and while observations of the kinetics of antiarrhythmic drug action on I_{Na} are very limited, Bean et al. (1983) and Sanchez-Chapula et al (1983) have shown first order recovery of I_{Na} in voltage-clamp rabbit Purkinje fibers and rat single ventricular cells, respectively. Using equations (1b) and (2b), equations (3) and (4) can be derived in an identical fashion.

5.2 Comparison between experimental results and the mathematical model

In experiments evaluating QRS duration, the QRS duration resulting from a test stimulus (S_2) with a selected S_1S_2 coupling interval was recorded at 250 mm/sec paper speed. QRS duration was used as an indicator of total ventricular activation time, and drug-induced changes in QRS duration of the complex resulting from S_2 were studied as a function of S_1S_2 coupling

interval. Measurements of QRS duration were made from a point at the first rapid voltage deflection to the last rapid deflection. Corresponding points were used for QRS duration measurement of all the QRS complex at each drug concentration. The QRS duration of the complex resulting from the last S_1 of the basic train was measured to assure a constant level of drug-induced block prior to the test pulse (S_2). The QRS resulting from the last S_1 of the basic train was constant for each drug infusion, with a standard deviation for all test runs averaging 3.0 ± 1.3% of the mean value. If changes in QRS morphology were observed, the experiment was rejected.

When epicardial mapping was applied, values from the first and last electrode sites to be activated were used for evaluation of the model. Conduction time were calculated for each activation from the difference between the earliest epicardial activation time (at the site next to the stimulating electrode) and the activation time at the last excited site. All analyses for a given infusion were performed using data from the same electrodes, and all electrograms were reviewed to assure that corresponding points on the local electrograms were identified by the computer algorithm to define local activation. QRS duration measurements were accurate to ± 2 msec, and activation time measurements to ± 0.5 msec.

The experimental recovery curves relating drug-induced changes in QRS duration and epicardial activation time to S_1S_2 coupling interval were analyzed in two ways:

(1) Changes in QRS duration and conduction time were plotted on a log-linear scale as a function of coupling interval, as in Figure 1. According to the mathematical model, and as illustrated in Figure 1, the terminal portion of each log-linear plot should be parallel with a slope equal to the rate constant (k) for sodium channel depression. In addition, other specific predictions include: (1) the terminal slope should be independent of dose, (2)

the non-linearity of the overall curve should become more apparent with largest doses that produce increasing magnitude of drug effect; (3) the coupling interval at which deviation from the terminal line becomes obvious should increase with increasing drug dose

(2) Curve-fitting techniques were developed to fit recovery data to the quadratic model and to a simple exponential relationship. The instantaneous slope of the curve predicted by the quadratic model (eq 4) is determined by the rate constant k and the magnitude of drug-induced conduction slowing (delta CT) at that time The entire recovery curve can therefore be characterized by k and a single value of delta CT at a given coupling interval (t_x) Any exponential curve can similarly be characterized by the equation delta CT_t (delta CT_x e^{kt_x}) e^{-kt}, where delta CT_x is the drug induced conduction slowing (delta CT) at a specific time t_x, and delta CT_t is delta CT at any time t Thus, both the quadratic model and exponential curves are determined by the same three varibles - the rate constant (k) and the magnitude of drug effect (delta CT) at a single time point t_x

We wrote software routines in BASIC (see Appendix) and executed them on an IBM AT compatible computer (10 MHz clock) to generate curves using either the quadratic or exponential model for any values of k, delta CT_x, and t_x. The ability of any quadratic model or exponential curve to fit a set of experimental data was determined by the sum of squared deviations (SS) of experimental points from the putative curve. The program stepped through a range of values of k and delta CT_x, using step sizes of 0.01 sec^{-1} for k and 0 001 for delta CT_x Since t_x can be any point in the recovery process, it was set at the longest coupling interval studied in each experiment. Thus, the corresponding CT_x would equal CT_o (CT_x=CT_o), the drug-induced conduction slowing, at the longest coupling interval studied, and delta CT represents (CT-CT_o). The curve with least sum of squared deviations (LSS) represented the

best-fit quadratic model or exponential curve for that set of data (Sachs, 1984). A nested design was used to test all delta CT_x values for each value of k, and a range of values was selected for testing such that at least two values of delta CT_x and two of k above and below those of the best-fit curve had a SS larger than the LSS curve

Inspection of equation 4 and 1 suggests that an exponential fit should provide a reasonable approximation to data that perfectly obeyed the quadratic model. Figure 2 shows the best-fit regression line to a log linear plot of a set of points generated by the mathematical model, as well as the best monoexponential curve fit to a linear plot of the same data (using the method described above). While it is clear that the theoretical points are not perfectly described by a first order (log-linear or monoexponential) relationship, the deviation from the first order behavior is subtle and easily masked by experimental error Our comparison between the model and the first order approximation was therefore based on a nonparametric statistical comparison between the LSS of the best-fit quadratic model curves and exponential curves determined in the same way using the same determining variables and the same set of data in each experiment A significantly lesser LSS should indicate superiority of a given model in describing the observed results.

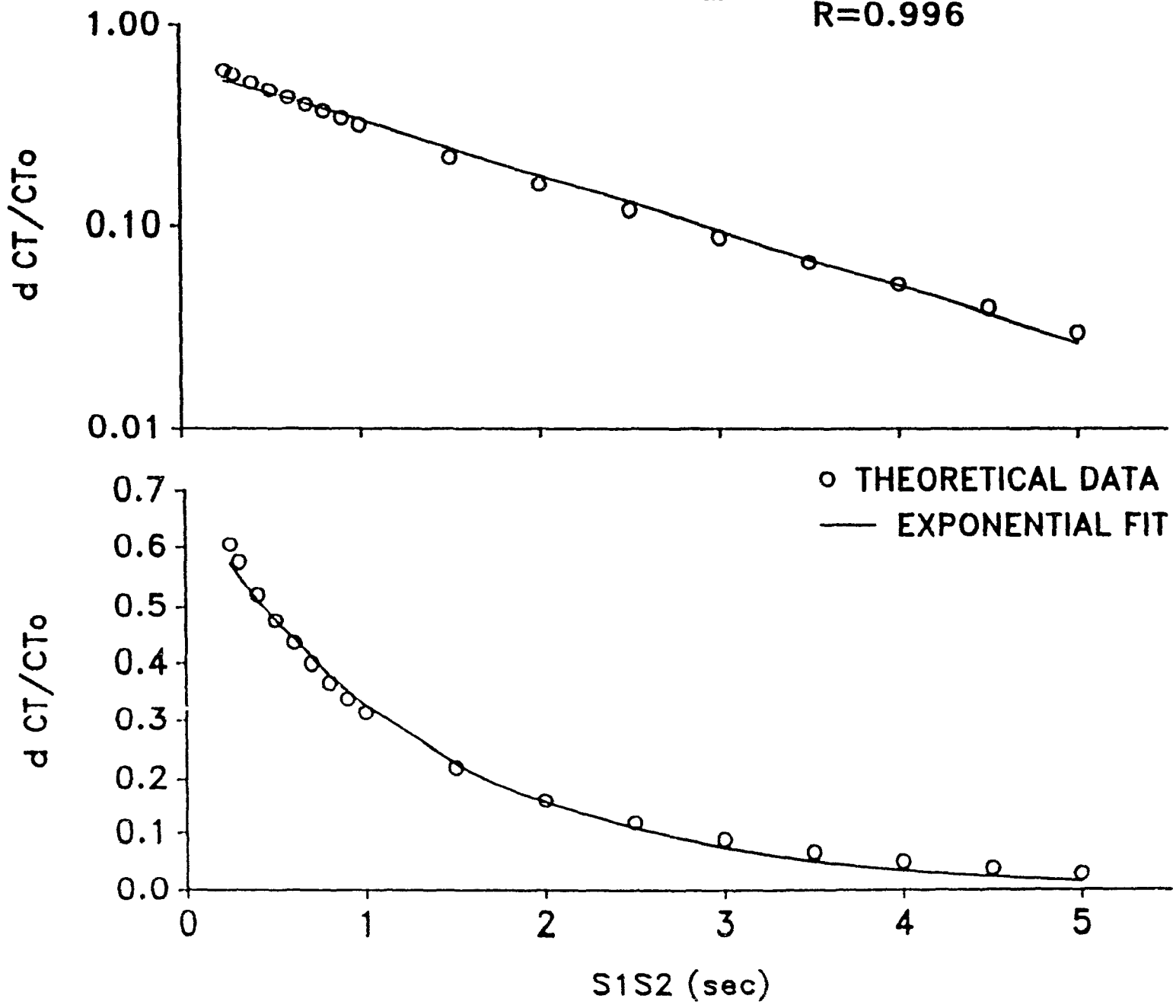
5.3 Statistical methods

Group data are presented as mean ± SD Comparison between group means were made by two-way analysis of variance with Scheffe's test (Sachs, 1984) The frequency dependence of steady state drug effects was evaluated by two way analysis of variance with an F test for interaction (Sachs, 1984) Student's t-test was used for analysis when only two groups of results were compared (Sachs, 1984). A two-tailed probability of less than 5% was taken to indicate

FIGURE 2. Approximation of theoretical behavior by a first order model

Open circles represent recovery curves assuming the behavior described by the mathematical model The solid lines show the best first-order fit by linear regression of the log-linear plot (top) or by non-linear least squares regression to the arithmetic plot (bottom). CT_o represents the conduction time at the longest coupling interval studied (in this case, CT_o=CT_c), and $dCT=(CT-CT_o)$ While the fits are clearly imperfect, the deviations of theoretical data from the first-order approximation are subtle. Experimental data resulting from behavior described by the mathematical model would therefore be expected to be reasonably fitted to a first order relationship, although agreement should be better with curve fits based on the equations described in the text

FIG. 2


R=0.996
1.00
0.10
0.01
d CT/CTo
THEORETICAL DATA
EXPONENTIAL FIT
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
d CT/CTo
0
1
2
3
4
5
S1S2 (sec)

Results

1. Frequency-dependent effects of procainamide on QRS duration and repolarization

Our loading and maintenance dose regimen produced stable procainamide concentrations (Table 1). No measurable concentrations of N-acetyl procainamide were detected. Procainamide increased QRS duration in a frequency and concentration dependent fashion (Figure 3, table 2). Drug-induced changes in QRS duration were a monotonic function of basic cycle length, and were increased an average of 3-fold by increasing ventricular activation frequency from 30 to 200/min (Figure 3). In contrast, the JT interval was not significantly altered by procainamide at any drug concentration or pacing frequency (Table 2)

2. Interval dependent changes in QRS duration and conduction time

Under control condition, QRS duration was increased over a narrow range of coupling intervals (averaging 50 msec) just beyond the effective refractory period. Over a wide range of coupling intervals, ranging from 211 ± 46 msec to 3.9 ± 1.9 sec, QRS duration was constant and equal to the QRS duration at the basic cycle length.

In the presence of procainamide, changes in coupling interval produced substantial alterations in QRS duration. As the coupling interval was increased, the procainamide-induced conduction changes gradually diminished. Over a mean maximal pause interval of 5.8 ± 2.0 sec, the effect of procainamide on QRS duration decreased by 85 ± 9% with respect to its effect at the shortest coupling interval.

Changes in conduction of the complex initiated by a test stimulus (S_2) depended on the preceding (S_1S_2) coupling interval. Figure 4 shows the

TABLE 1: Procainamide infusion regimens and resulting plasma concentrations.

Loading doses were administered over fifteen minutes. The maintenance dose was begun immediately after completion of loading infusion. All doses are in terms of the hydrochloride salt, which was administered as a solution in isotonic saline. Twenty minutes after the end of the maintenance dose, a blood sample (pre-study) was obtained for subsequent measurement of plasma procainamide concentration. Electrophysiological studies were then performed after which a blood sample (post-study) was obtained for drug assay and the next loading dose was begun. A total of 9 dogs were studied for the study of the frequency-dependence of QRS duration, with data available from dose 1 in 6, dose 2 in 5, and dose 3 in 5.

TABLE 1

[illegible] INFUSION REGIMENS AND RESULTING PLASMA CONCENTRATIONS

	LOADING DOSE	MAINTENANCE DOSE	PLASMA CONCENTRATIONS (mg/L)	
			Pre-study	Post-study
DOSE 1	50 mg/kg	12 mg/kg/hour	37.5 ± 14.6	31.5 ± 14.6
DOSE 2	50 mg/kg	24 mg/kg/hour	68.2 ± 14.4	60.2 ± 11.9
DOSE 3	50 mg/kg	48 mg/kg/hour	98.5 ± 15.8	118.2 ± 43.4

FIGURE 3: Procainamide-induced QRS prolongation as a function of steady-state pacing cycle length (BCL), as determined during maintenance infusion 1, 2, and 3.

For each dose of procainamide, drug-induced increases in QRS duration relative to control values (% delta QRS) are augmented by decreasing the basic cycle length (increasing frequency) of activation. Asterisks indicate the statistical significance of the difference between drug effects at a given basic cycle length and effects at a basic cycle length of 2000 msec during the same drug infusion, by analysis of variance with a range test. Results are shown as the mean ± standard deviation, from 6 experiments with infusion 1, and 5 each infusion 2, and 3.

FIG. 3

PERCENTAGE CHANGES IN QRS AS A FUNCTION OF BASIC CYCLE LENGTH

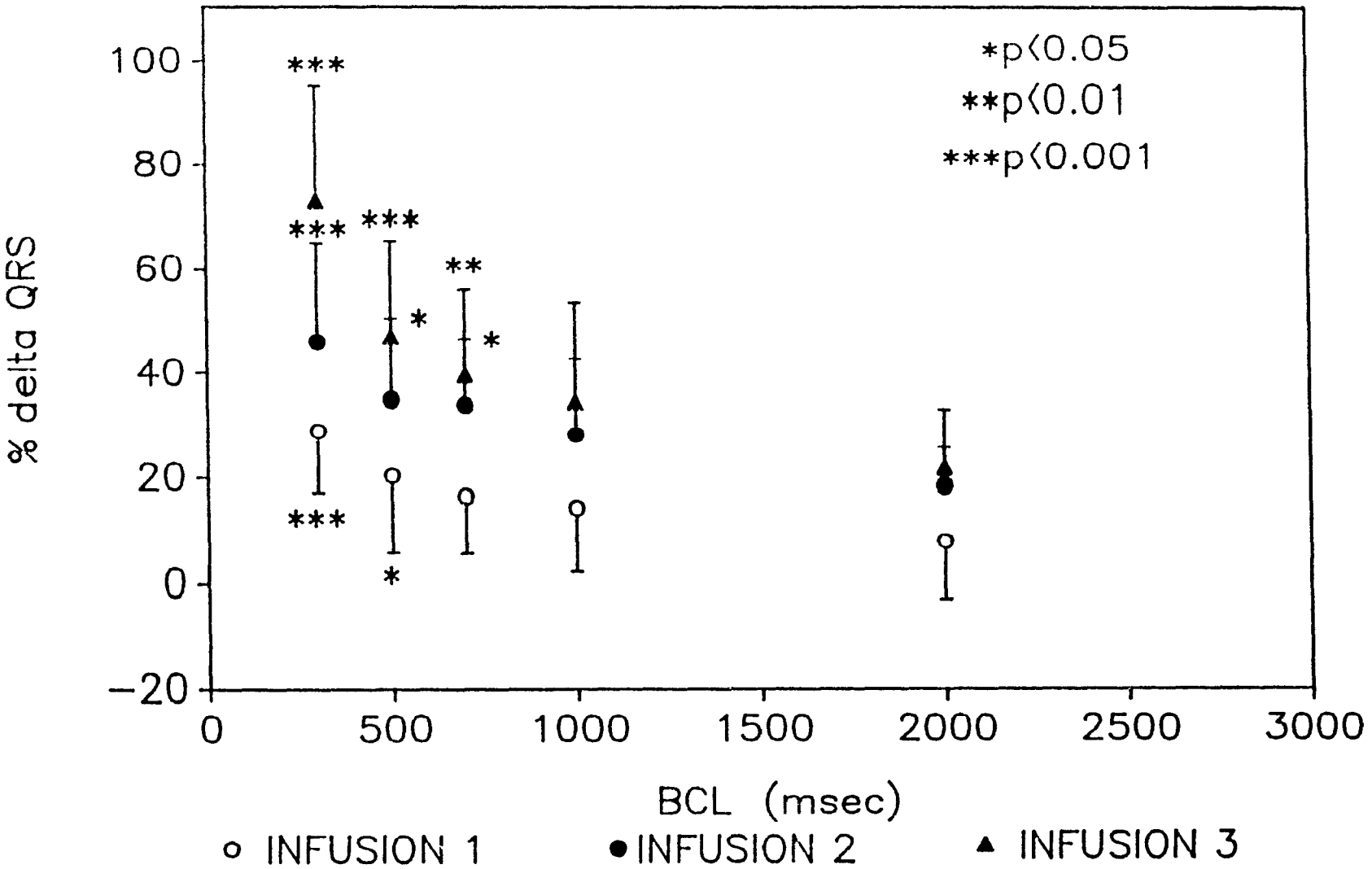


TABLE 2: Changes in QRS duration and JT interval, produced by procainamide infusion

Abbreviations. BCL Basic cycle length in msec, JT duration from the [illegible] QRS complex to the end of T wave, F_{int} F value from interaction analysis (for details, see below), NS non-significant, CTL control

Results are shown for 6, 5 and 5 experiments with doses [illegible] respectively Values for QRS and JT intervals shown are in msec and the values are represented as mean ± SD

[a]p indicates statistical significance of the interaction between cycle length and drug effects analyzed by a two-way ANOVA with an F test for interaction. Procainamide significantly increased QRS duration from control [illegible] at all cycle length and for all doses JT interval was not significantly altered by any procainamide infusion at any cycle length

TABLE 2

CHANGES IN QRS DURATION AND JT INTERVAL PRODUCED BY PROCAINAMIDE INFUSION

QRS DURATION (msec)

PCL	300		500		700		1000		2000			
	CTL	DRUG	CTL	DRUG	CTL	DRUG	CTL	DRUG	CTL	DRUG	F_{int}	p^a
[illegible] 1	6	87	69	83	67	79	68	78	70	75	31 9	$2\ 0*10^{-8}$
	±16	±21	±17	±21	±16	±20	±17	±20	±16	±19		
[illegible] 2	64	92	65	88	64	85	64	81	65	77	7 7	$1\ 2*10^{-3}$
	±17	±24	±19	±24	±17	±23	±19	±20	±18	±22		
[illegible] 3	66	113	68	99	66	92	66	89	67	82	32 7	$1\ 6*10^{-7}$
	±15	±26	±16	±22	±14	±24	±16	±23	±16	±21		

JT INTERVAL (msec)

PCL	300		500		700		1000		2000			
	CTL	DRUG	CTL	DRUG	CTL	DRUG	CTL	DRUG	CTL	DRUG	F_{int}	p^a
[illegible] 1	139	143	159	168	176	184	194	210	209	237	2.0	NS
	±29	±27	±31	±40	±37	±46	±43	±59	±61	±89		
[illegible] 2	147	143	160	178	185	194	204	222	224	237	1 019	NS
	±26	±16	±33	±29	±35	±32	±41	±43	±56	±57		
[illegible] 3	145	148	164	185	191	209	212	229	228	272	2.5	NS
	±26	±35	±37	±36	±34	±48	±39	±56	±53	±99		

relationship between coupling interval and the logarithm of changes in QRS duration in a representative experiment. For all doses, points showing a QRS or conduction time increase less than 20% were well fitted by a simple log-linear relationship For points showing greater magnitudes of effect, experimental values deviated increasingly from the terminal log-linear relationship The coupling interval at which deviation from the terminal line was noted was substantially greater for infusions producing a greater magnitude of drug effect. The terminal log-linear fit resulted in measured time constants which were independent of dose, and averaged 2.10 ± 1.09 sec for dose 1, 2.04 ± 0.58 sec for dose 2 and 2.33 ± 0.42 sec for dose 3

Changes in QRS duration and conduction time were well-fitted by the mathematical model. For all experiments, the model curve fitted the data better than a monoexponential analysis, as illustrated in Figure 5 As predicted by the theoretical considerations illustrated in Figure 2, the difference between model and exponential fits were subtle, including an underestimate by the exponential fit at the shortest and longest coupling intervals, and an overestimate for intermediate values The sum of squares was significantly less for the mathematical model (Table 3), indicating a better fit of the experimental data. The rate and time constants from the terminal linear portion of the log (delta CT)-S_1S_2 curve were similar to values obtained from the model fit, but were significantly different from values obtained by a monoexponential analysis

3. Analysis of conduction pattern

3.1 Qualitative analysis

Under control conditions, both activation time and pattern remained unchanged over a wide range of S_1S_2 intervals tested In the presence of

FIGURE 4 Recovery of procainamide-induced conduction slowing at two steady-state plasma concentrations (open symbols) during a representative experiment.

Increase in QRS duration produced by procainamide (dQRS) are normalized to control QRS duration ($dQRS/QRS_0$) and plotted logarithmically as function of the S_1S_2 recovery interval for each test pulse. For test responses showing <20% conduction slowing, there is a log-linear relationship with recovery time As conduction times slow further, they deviate increasingly from the terminal linear relationship Increasing drug concentration displaces the recovery curve upwards (to greater magnitudes of drug effect), make the nonlinearity of the recovery curve more obvious, and shifts the point at which nonlinear behavior becomes manifest to longer coupling intervals In this experiment, deviation from linearity occurred at coupling intervals of 500 and 1000 msec at the lower and higher drug concentration, respectively. The slope of the terminal linear relationship indicates the recovery rate constant, according to the mathematical model (for details see text), and should be independent of concentration

FIG. 4

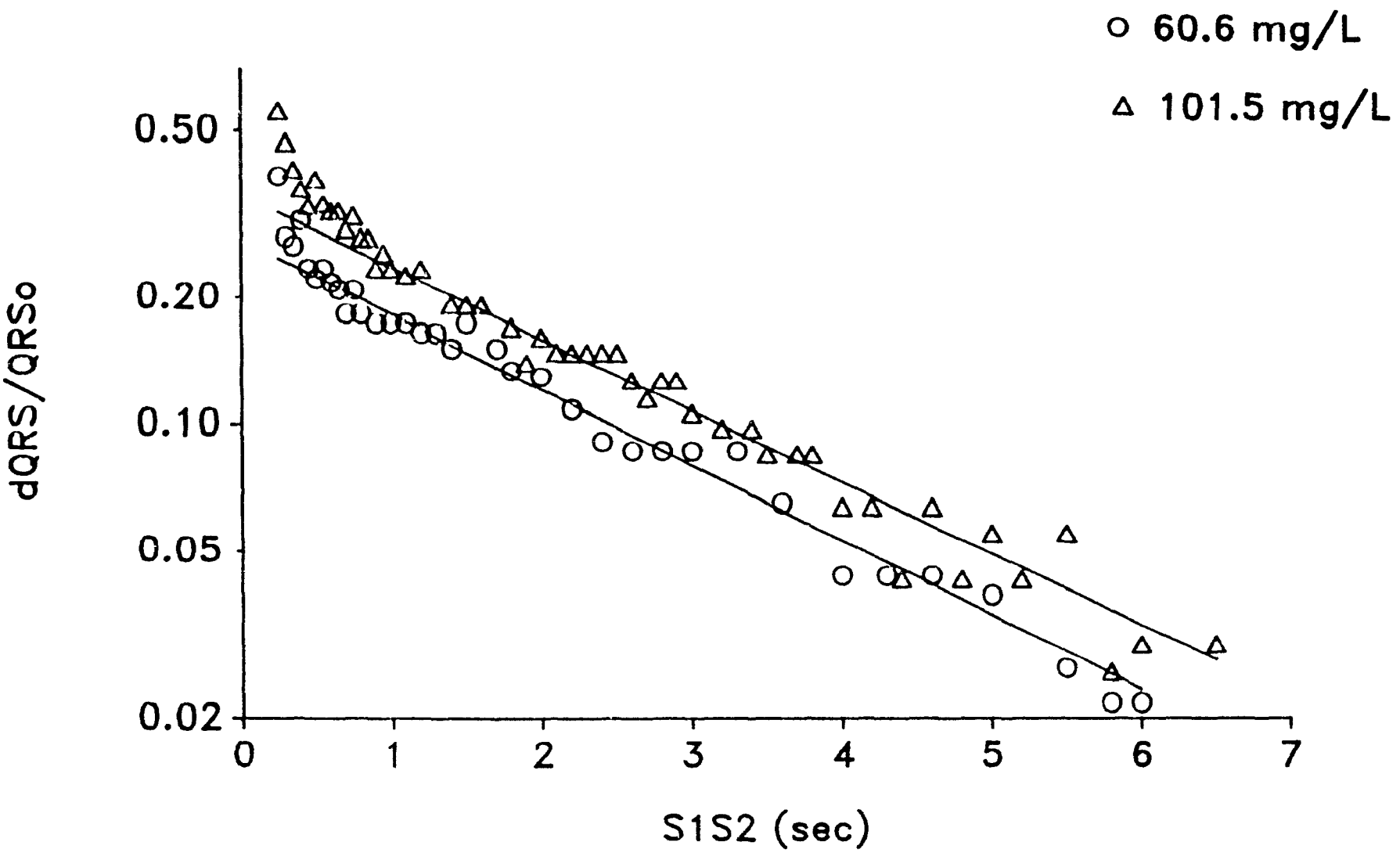

○ 60.6 mg/L
△ 101.5 mg/L
dQRS/QRSo
0.50
0.20
0.10
0.05
0.02
0
1
2
3
4
5
6
7
S1S2 (sec)

FIGURE 5. Examples of curve-fitting to conduction time measurements from a representative experiment using the mathematical model (left) and a simple monoexponential relationship (right).

The techniques for determining the best-fit curve were identical for both curve-fitting approaches (see Methods section), and conduction time was measured by epicardial activation mapping. CT_o represents the conduction time at the longest coupling interval studied, and $dCT=(CT-CT_o)$. While the differences between the fits are subtle, in all cases the model fitted the experimental data better as indicated by a smaller sum of squared deviations of experimental data from the best-fit curve. In this experiment, the least sum of squared deviations was 0.0051 for the model fit compared to 0.0093 for the exponential fit.

FIG. 5

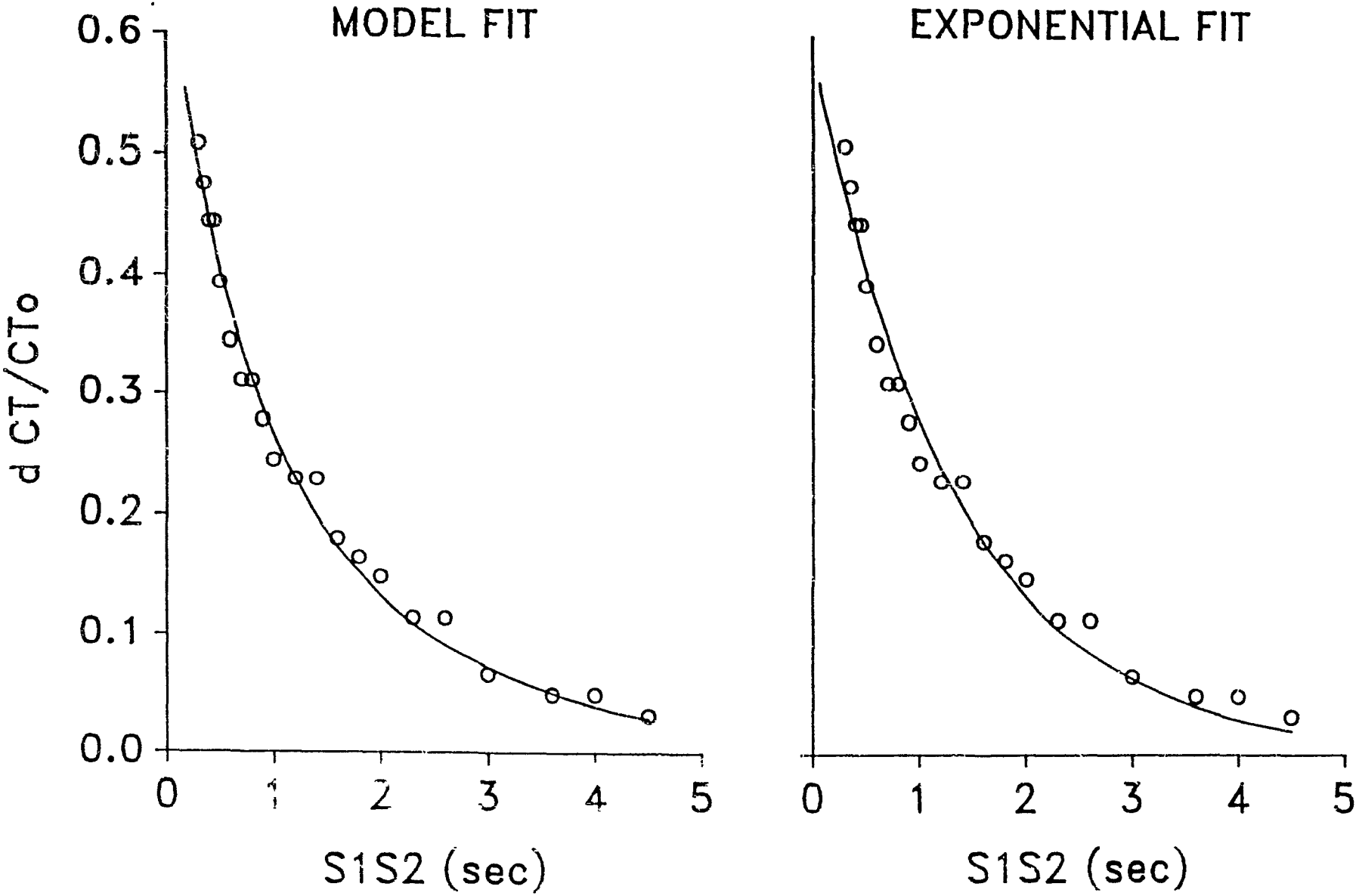

MODEL FIT
EXPONENTIAL FIT
d CT/CTo
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
S1S2 (sec)
S1S2 (sec)

TABLE 3 Kinetic characteristics of recovery from procainamide-induced block

Abbreviations: k, the kinetic rate constant of procainamide (for definition see Methods), τ, time constant of recovery from procainamide-induced block, SS, sum of squares of differences of experimental data from model or exponential curve fit at the same coupling interval. (A smaller sum of the squares indicates a better curve fit to the observed results).

[a] Linear terminal portion of curve relating the logarithm of changes in QRS duration or conduction time to coupling interval (see Figure 1). According to the mathematical model (see text), the slope of this line should equal the rate constant for changes in phase 0 sodium current

[b] Best-fit curves to the mathematical model described in the Methods section and to a monoexponential relationship were obtained using iteration and curve fitting techniques as described in detail in Methods.

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, for differences comparing exponential fit results to values obtained either from the terminal linear portion of the log (delta CT) - S_1S_2 curve or the model fit, results for k and τ obtained from the terminal line and model curves were not significantly different from each other

Values for k and τ were compared by analysis of variance with a multiple range test, while the sum of squares for the model was compared to the sum of squares for the exponential fit by paired t-test. Results shown are for 9 experiments studying QRS duration, and 5 additional experiments studying epicardial activation

TABLE 3

KINETIC CHARACTERISTICS OF RECOVERY FROM PROCAINAMIDE-INDUCED BLOCK

	k (/sec)	τ (sec)	SS
a. Results using QRS duration as an indicator of conduction time in 9 dogs			
Terminal line[a]	-0 49 ±0 16	2 24 ±0 65	----
Model fit[b]	-0 51 ±0 17	2 14 ±0 54	0 027 ±0 02[illegible]
Exponential fit[b]	-0 63*** ±0 17	1 66*** ±0 37	0 034[illegible] ±0 032
b Results using conduction time from epicardial mapping in 5 dogs			
Terminal line[a]	-0 64 ±0 14	1 63 ±0 36	----
Model fit[b]	-0 64 ±0 11	1.58 ±0 25	0 0048 ±0 0021
Exponential fit[b]	-0 82*** ±0 16	1 24*** ±0 21	0 0072[illegible] ±0 0037

procainamide, activation times were dependent on the preceding coupling interval, but overall conduction pattern remained unchanged Figure 6 shows the similarity of isochrone maps in a representative experiment at a basic cycle length of 300 msec (both control and procainamide), after a 6 second pause (control), and after a 4 5 sec pause (procainamide) which was sufficient to allow for virtually complete recovery of drug-induced sodium channel block in the presence of procainamide At the basic cycle length of 300 msec, activation was clearly slowed by procainamide, but the overall pattern of activation resembled that under control conditions.

3 2 Quantitative analysis

The results of quantitative analysis of activation pattern are illustrated in Figure 7 The relative time during a complex at which each site is activated is determined by dividing the conduction time to that site (calculated as the difference between the activation time at this site and the earliest activation time) by the overall conduction time for the complex (calculated as the difference between the earliest and the latest activation time) Results for a beat during steady state drug effect (at a cycle length of 300 msec) are then compared to results for a beat showing maximum recovery. If the activation patterns of the two beats are equivalent, each site should be activated at the same relative conduction time for either complex, and resulting points should fall along the line of identity as shown in Figure 7 for a representative experiment For five experiments using activation mapping, similar results were obtained The regression lines fitting data plotted as in Figure 7 were close to the line of identity an each case, and had a mean slope of 1.00 ± 0.05, an intercept of 0.01 ± 0.05, and a correlation coefficient of 0.99 ± 0.02.

FIGURE 6: Isochrone activation maps under control condition (left) and in the presence of procainamide (right).

The scale of 10-msec isochrone colors is shown at the left of each map. Electrode positions are shown by the white dots, and the positions of the coronary arteries are shown diagrammatically. Results during steady state pacing at a cycle length of 300 msec are shown at the top, and results after a pause of 6 seconds (control) and 4.5 seconds (procainamide) are at the bottom. Activation patterns were not significantly altered by the pause under control conditions. In the presence of procainamide, there is substantial conduction slowing at the basic cycle length (top right), which is almost completely reversed after a 4.5 sec pause (bottom right). The conduction slowing by procainamide is uniform, leaving the sequence of activation unchanged.

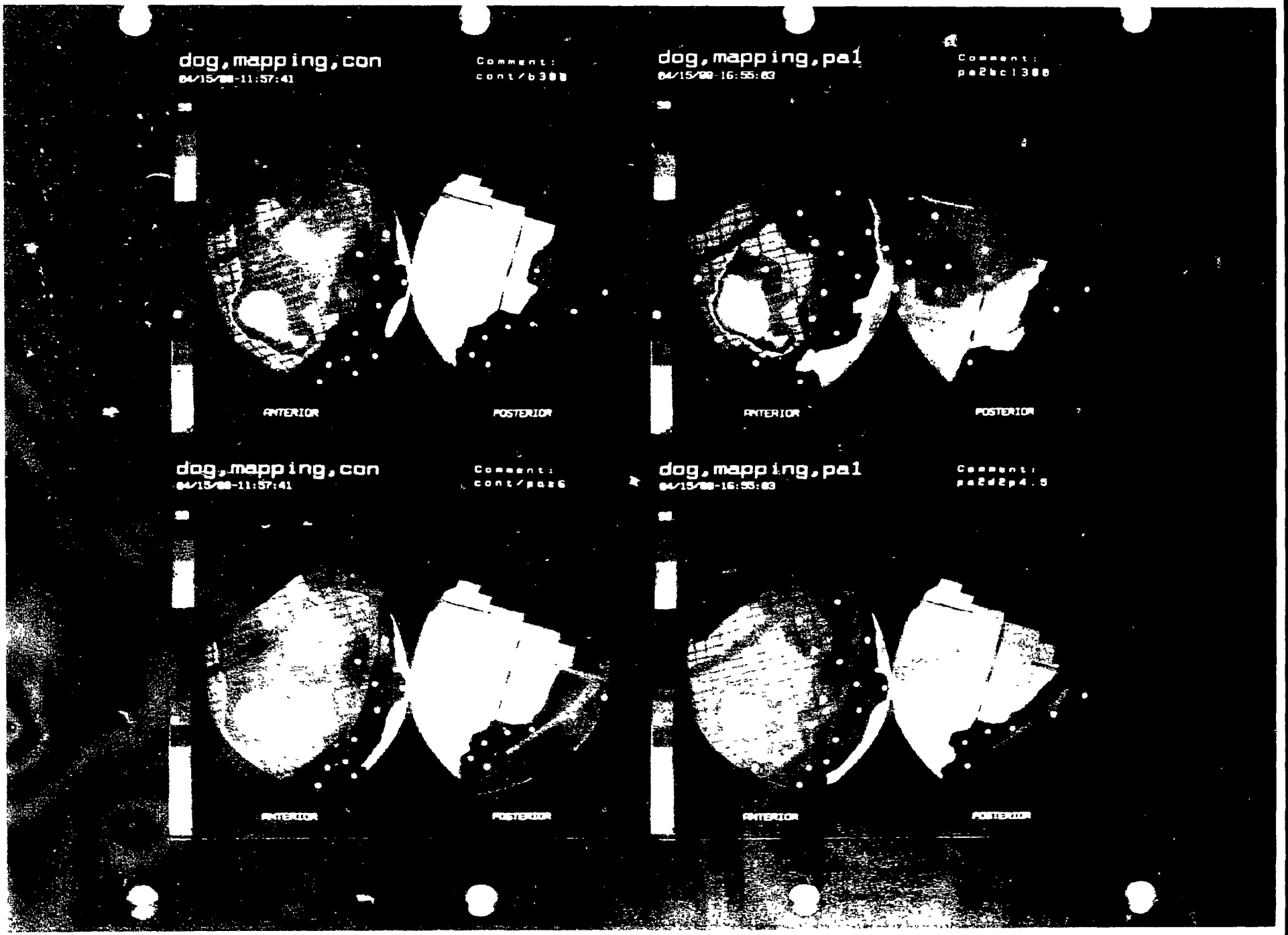
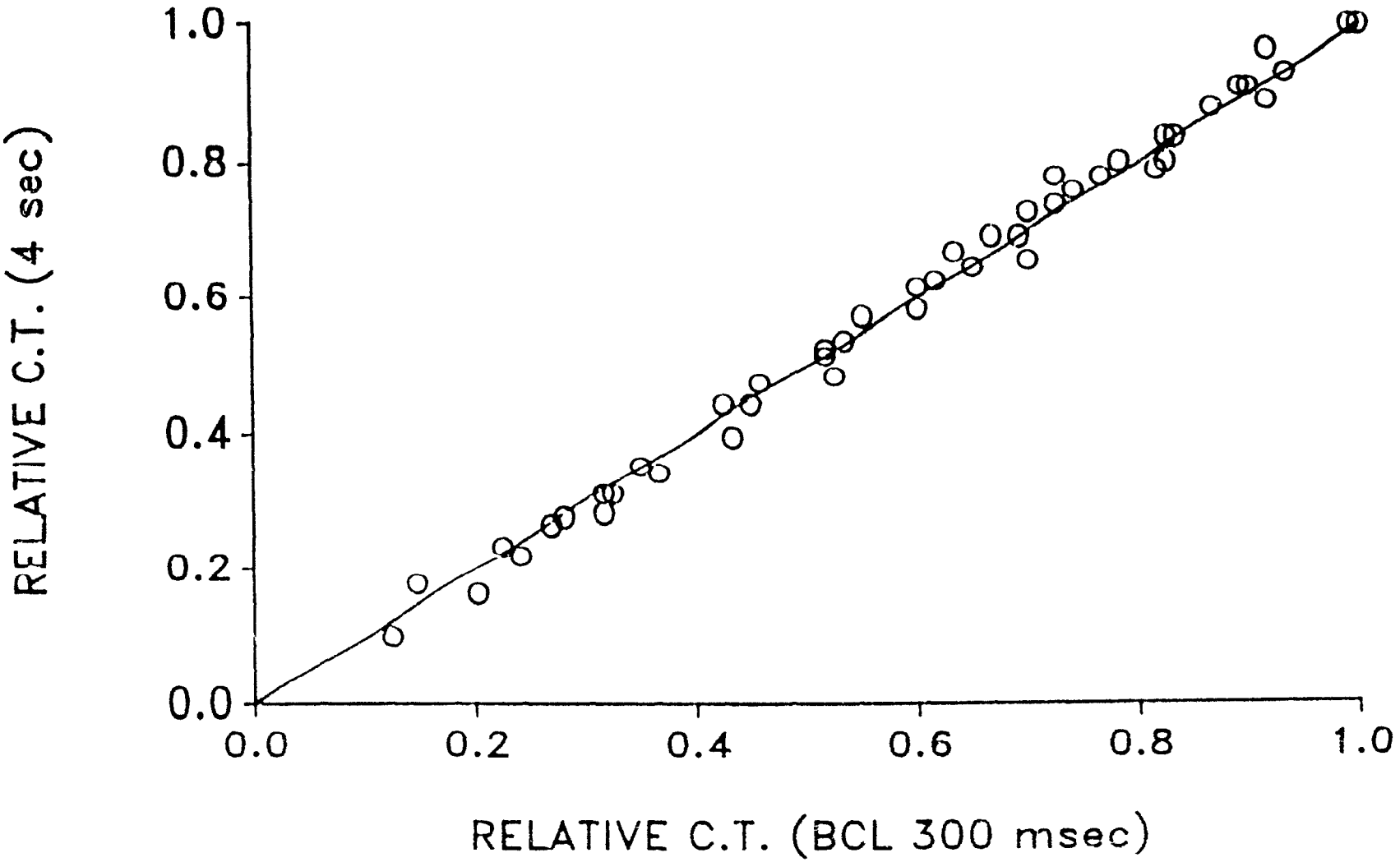

dog,mapping,con
Comment:
cont/b300
ANTERIOR
POSTERIOR
dog,mapping,pa1
Comment:
pa2bc1300
ANTERIOR
POSTERIOR
dog,mapping,con
Comment:
ANTERIOR
POSTERIOR
dog,mapping,pa1
Comment:
ANTERIOR
POSTERIOR

FIGURE 7 Relative conduction times to various epicardial electrogram sites in the presence of procainamide during steady state pacing at a basic cycle length of 300 msec (BCL 300) , compared to conduction times at the same sites after a 4 sec pause (4 sec).

Relative conduction time was obtained by dividing the conduction time at each site by the overall (longest) conduction time This provides an index of the time at which each site was activated relative to the total activation time Each point indicated relative conduction time to a given site at steady state drug effect compared to the corresponding value after the pause. During steady state pacing procainamide increased overall conduction time by 35% To achieve complete reversal of drug effect by a 4 sec pause, each site was activated at virtually the same point within the activation sequence This is shown by the close adherence of experimental points to the line of identity (solid line)

FIG. 7


1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
RELATIVE C.T. (4 sec)
RELATIVE C.T. (BCL 300 msec)

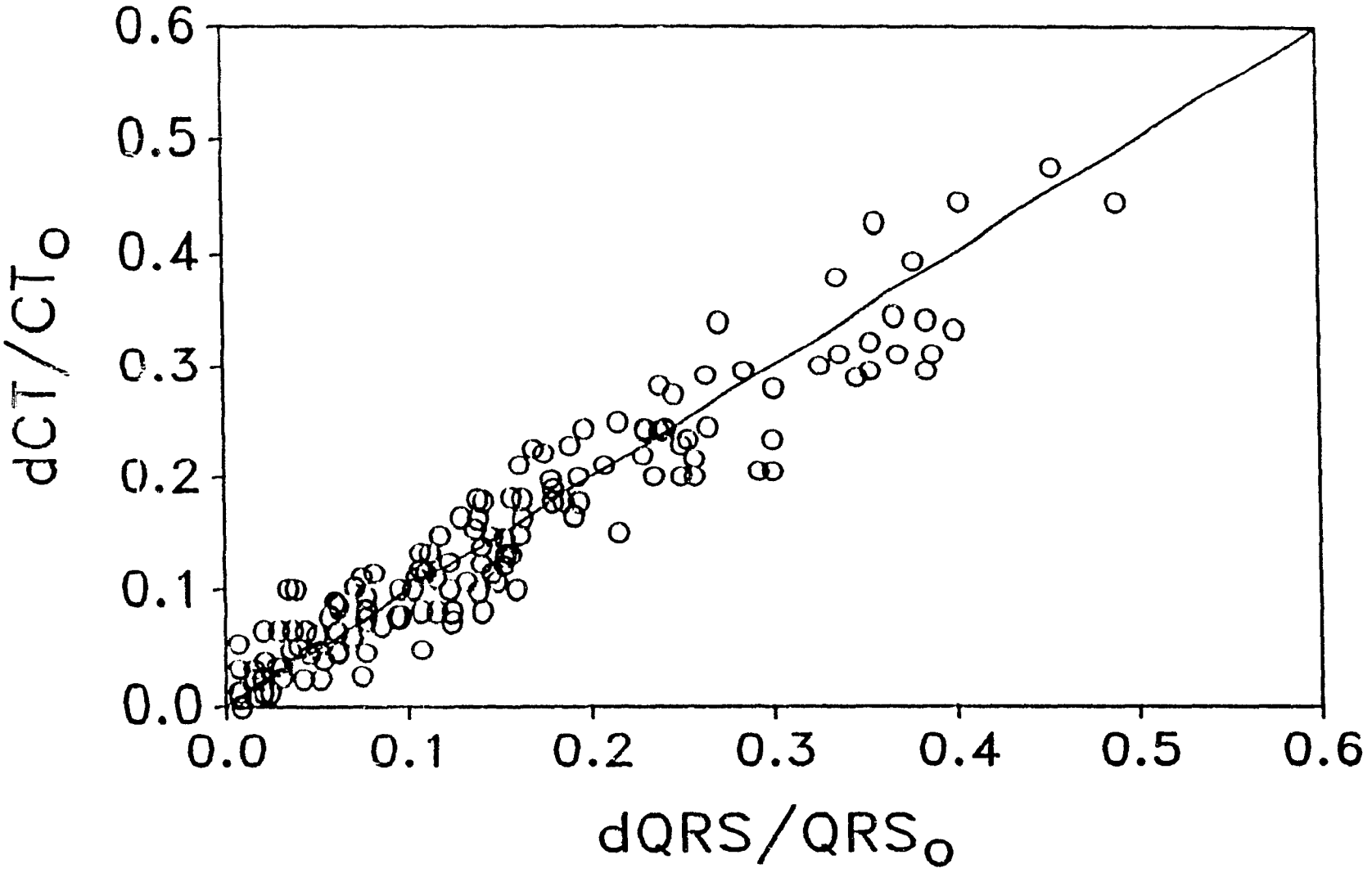
4 Analysis of the relationship between QRS measurement and activation mapping

The results obtained using QRS duration as an index of conduction time were qualitatively similar to results using epicardial activation mapping, although the latter was quantitatively more accurate To analyze the concordance between these methods, drug-induced increases in conduction time with corresponding changes in QRS duration in 4 experiments in which both were measured simultaneously were compared. Changes in these two indices were closely related (correlation coefficient = 0 95), and fell close to the line of identity (Figure 8)

FIGURE 8: Procainamide-induced changes in conduction time measured by epicardial mapping (dCT/CT_o) compared to simultaneously measured changes in QRS duration ($dQRS/QRS_o$), as determined over a wide range of coupling intervals during 4 experiments.

The two indices of drug-induced conduction slowing were closely related to one another (r=0 95), and the resulting points fall close to the line of identity. CT_o represents the conduction time at the longest coupling interval studied, and $dCT=(CT-CT_o)$.

FIG. 8



Discussion

The objective of the present study was to quantitatively examine the frequency-dependent effects of procainamide on intraventricular conduction in vivo, incorporating an underlying theoretical assumption of a linear relationship between peak sodium inward current (I_{Na}) or $\dot{V}_{max}$ and the square of myocardial conduction velocity. Changes in conduction velocity are important electrophysiological effects of antiarrhythmic drugs, and figure significantly in the antiarrhythmic and arrhythmogenic actions of such compounds (Zipes, 1984). Local anesthetics are known to decrease cardiac conduction velocity by reducing phase 0 peak sodium inward current or the maximum rate of rise of action potential. Since sodium channel blockade by antiarrhythmic drugs is rate dependent, drug-induced conduction slowing should also be frequency-dependent.

Frequency dependent effects of antiarrhythmic drugs have important clinical implications (Hondeghem & Katzung, 1984). They allow for the possibility of selectively blocking extrasystoles and other high frequency arrhythmias while having little effect on cardiac tissue at normal heart rate. However, full understanding of the clinical relevance of frequency dependent properties requires that interval-dependent effects of antiarrhythmic drug in vivo be quantitatively predictable from rate dependent drug-induced depressant effects on $\dot{V}_{max}$ or I_{Na}. In the current study, we tested the interval dependent effects of procainamide on parallel changes of QRS duration and conduction time in vivo in the face of expectations resulting from the relationship between conduction velocity and $\dot{V}_{max}$. The present model predicts 1) a first order time dependent recovery from drug-induced changes in conduction time or QRS duration for small drug-induced conduction time changes (<20%); 2) a consistent deviation from the terminal log-linear relationship

between conduction time or QRS duration and recovery time interval as changes in conduction time increase (>20% Figure 4); 3) deviation from the terminal log-linear relationship at longer coupling interval as the magnitude of drug effects increase; 4) the ability of a mathematical model based on the proportional relationship between $\dot{V}_{max}$ or I_{Na} and the square of conduction velocity to fit the experimental data (Figure 5); 5) a lesser least sum of squares for the model-fitted data compared to monoexponential curve fits (Table 3), and 6) the agreement in rate and time constants between model-derived data and results obtained from the terminal log-linear relationship (Table 3) The results from the present study are qualitatively and quantitatively in agreement with these predictions, providing further evidence for a linear relationship between $\dot{V}_{max}$ or I_{Na} and the square of conduction velocity in vivo

Relationship between $\dot{V}_{max}$ and I_{Na}

The mathematical model tested in the present study involves assumptions about the relationship between $\dot{V}_{max}$, I_{Na} and conduction velocity. The relationship between $\dot{V}_{max}$ and sodium inward current has been the subject of great debate The use of the maximum rate of rise of the action potential ($\dot{V}_{max}$) as a measure of the sodium conductance in excitable membrane was first proposed by Hodgkin and Katz (1949) in nerve tissue. They showed that at the time of $\dot{V}_{max}$, the total ionic current (I_{total}) across the cell membrane is directly proportional to $\dot{V}_{max}$ Since Draper and Weidmann (1952) first provided evidence that the rapid phase of depolarization of cardiac action potential is generated by an increase in sodium conductance (g_{Na}), maximum upstroke velocity ($\dot{V}_{max}$) has also been used to characterize the maximum cardiac sodium channel availability (Weidmann, 1955). As $\dot{V}_{max}$ depends on the total ionic currents across the membrane, which consist of sodium inward current, outward

potassium current triggered upon depolarization and background currents, the use of $\dot{V}_{max}$ to measure sodium current requires knowledge about the relative magnitudes of the nonsodium currents.

Under normal conditions, the total ionic current in nerve at the time of $\dot{V}_{max}$ consists mainly of sodium current, and $\dot{V}_{max}$ will predominantly represent I_{Na} (Hodgkin & Katz, 1949). However, Cohen and Strichartz (1977) showed that in situations such as exposure to drugs or conditions that block I_{Na} and alter potassium and leakage currents, nonsodium currents may contribute significantly to the total ionic current and affect the validity of $\dot{V}_{max}$ as an accurate measure of sodium inward current in nerve tissue. They employed a theoretical simulation based on the Hodgkin and Huxley equations (1952) and showed that the use of $\dot{V}_{max}$ as a measure of I_{Na} could erroneously suggest a voltage dependent action of tetrodotoxin on maximum sodium conductance under conditions in which nonsodium currents contribute substantially to the total ionic current (I_{total}) (Cohen & Strichartz, 1977). Therefore, Cohen and Strichartz (1977) concluded that $\dot{V}_{max}$ is not a valid index of sodium inward current (I_{Na}) and sodium channel conductance (g_{Na}), at least in nerve tissue and possibly also in cardiac tissue. Hondeghem (1978) argued that these "nerve" results do not bear on the use of $\dot{V}_{max}$ as a measure of sodium inward current in cardiac membrane. He stated that, in contrast to the situation in nerve tissue, the outward potassium current is much smaller in heart and shows inward-going rectification (McAllister et al., 1975). Consequently, the potassium current constitutes only a minimal fraction of the total current at the time of $\dot{V}_{max}$. In addition, the other outward currents (eg i_x) activate very slowly and are largely deactivated at holding potentials negative to -50 mv. As a result, during the upstroke of the cardiac action potential, the only significant current flowing is sodium current. It was estimated that I_{Na} always comprises at least 98% of the total ionic current at the time of $\dot{V}_{max}$.

(Hondeghem, 1978).

Using the equations and constants based on a model describing excitation in cardiac ventricular membranes (Beeler & Reuter, 1977), Hondeghem (1978) developed a computer simulation of cardiac excitation in the ventricle to determine the relationship between parameters involved in cardiac conduction. He showed a linear relationship between $\dot{V}_{max}$ and peak sodium inward current (I_{Na}), and between $\dot{V}_{max}$ and sodium channel conductance (g_{Na}). The same relationship was obtained for the computer simulations in Purkinje fibers (Walton & Fozzard, 1979) based on the model by McAllister et al (1975).

These studies stimulated broad discussions about the meaning of $\dot{V}_{max}$ measurements. In a reply to Hondeghem's study, Strichartz and Cohen (1978) challenged the conclusion of a linear relationship between $\dot{V}_{max}$ and g_{Na} in cardiac tissue. They first performed a computer simulation in nerve tissue, keeping the potassium outward current and leakage currents at zero. They showed that even in the absence of potassium and leakage currents $\dot{V}_{max}$ is not proportional to g_{Na} in nerve tissue. They concluded that in nerve tissue, the relationship between $\dot{V}_{max}$ and g_{Na} is not only dependent on the nonsodium currents, but also on the inherent kinetics of the sodium conductance system. In terms of cardiac tissue, they questioned the computer simulation by Hondeghem (1977) which is not based on the voltage clamp results, but rather on assumed kinetics of sodium currents. According to Strichartz and Cohen (1978), the linear relationship between $\dot{V}_{max}$ and g_{Na} reported by Hondeghem was fortuitous and requires conditions that are restricted to special situations (e.g. assuming a 50-fold ratio of inactivation to activation time constants over the rising phase of the action potential). All the studies mentioned so far could not be evaluated experimentally because of difficulties in measuring the large phase 0 sodium inward current and separating it from the

capacitance current by the voltage clamp techniques then available.

Improvements in methods for measuring I_{Na} under voltage clamp conditions have made direct comparison possible (Colatsky & Tsien, 1979; Colatsky, 1980; Cohen et al., 1981). In 1984, Cohen et al. (1984) studied the maximum upstroke velocity of action potentials in short rabbit Purkinje fibers, and compared it to sodium currents measured under voltage clamp at the temperature of 17.5 ± 1.0°C. It was found that with sodium channel blockade by tetrodotoxin or inactivation with steady depolarization, the maximal upstroke velocity is a nonlinear measure of sodium inward current and available sodium conductance. $\dot{V}_{max}$ is much less sensitive to TTX than is I_{Na}. They concluded that the analysis of $\dot{V}_{max}$ can be very misleading and that caution has to be used in applying $\dot{V}_{max}$ to the quantitative analysis of sodium channel properties. However, doubts still exist since the voltage clamp experiments by Cohen et al. (1984) were performed at unphysiologically low temperature (17°C). Moreover, their comparisons between $\dot{V}_{max}$ and I_{Na} were made at different holding potentials (more depolarized membrane potential for I_{Na}) and different external sodium concentrations (lower external Na for the measure of I_{Na}) in order to obtain measurable I_{Na} (Hondeghem, 1985; Courtney, 1985). In any case, $\dot{V}_{max}$ remains a valuable index of sodium channel availability and the controversy about the relationship between $\dot{V}_{max}$ and I_{Na} cannot be resolved until $\dot{V}_{max}$ and peak sodium current (I_{Na}) can be measured under identical physiological conditions.

Based on cable theory, Walton and Fozzard (1983) utilized three mathematical models describing uniform conduction in a telegraph cable and simulated individual experimental action potential upstrokes. These models were originally developed by themselves and by Hunter et al. (1975). Approximate solutions of the equations generated based on those mathematical models (Walton & Fozzard, 1983) suggested that maximal upstroke velocity

($\dot{V}_{max}$) is directly proportional to peak inward ionic current (I_{total}) normalized by capacitance (C_f) that is filled during the upstroke (I_{total}/C_f), and that conduction velocity was directly related to the square root of either $\dot{V}_{max}$ or I_{total}/C_f.

The development of the present mathematical model involves only two requirements: (1) a total ionic current (I_{total}) and/or $\dot{V}_{max}$ that varies directly with the square of conduction velocity; and (2) first-order recovery from drug effects on I_{total} and/or $\dot{V}_{max}$. For the first of these assumptions (1), changes in $\dot{V}_{max}$ produced by antiarrhythmic drugs, including procainamide, have been shown empirically to vary with the square of changes in conduction velocity (Nattel, 1987a; Buchanan et al., 1985). The relationship between I_{total}/C_f and the square of conduction velocity, however, has not been tested empirically. Procainamide does not alter membrane capacitance (Arnsdorf & Bigger, 1977) and therefore, at least theoretically, I_{total} should be proportional to the square of conduction velocity in the presence of procainamide. The second requirement assuming first order recovery has been shown for $\dot{V}_{max}$ by many investigators (Chen et al , 1979, Grant et al , 1980, Nattel, 1987a). First order recovery from lidocaine's effects on sodium inward current (I_{Na}) at room temperature has also been shown (Bean et al , 1983, Sanchez-Chapula et al., 1983). There is thus substantial theoretical and experimental support for the two assumptions on which the model is based, whether $\dot{V}_{max}$ or I_{total} is used as the index of sodium current for development of the mathematical model. One major advantage in using $\dot{V}_{max}$ is that there are, as discussed above , numerous <u>in vitro</u> studies estimating the time constant for recovery of procainamide's effects on $\dot{V}_{max}$. These can be used for comparison with values obtained in <u>in vivo</u> experiments

Although the relationship between $\dot{V}_{max}$ and sodium current does not seem to play an important role in the assessment of the present model as discussed

above, the model does, however, rely on the fact that the total ionic current (I_{total}) flowing through the membrane is primarily composed of sodium current (I_{Na}) at the time of upstroke of conducted cardiac action potential. The outward repolarizing potassium current (i_x) is turned on slowly and is relatively small at the time of upstroke The background potassium current (i_{k1}) also shows inward-going rectification and is thus negligible compared to the large amount of peak sodium inward current (I_{Na}) (Hondeghem, 1985).

Drug-induced changes in passive electrical properties could affect the relationship between conduction velocity and sodium current. Buchanan et al. (1985) showed that increasing potassium concentration first speeds, and then slows conduction velocity, in spite of a consistent decrease in $\dot{V}_{max}$. They ascribed this deviation from the predicted linear relationship between $\dot{V}_{max}$ and the square of conduction velocity to potassium-induced supernormal conduction. Cable analysis has shown that the passive electrical properties controlling conduction are little changed by procainamide (Arnsdorf & Bigger, 1977)

Applications of cable theory to the present study

The propagation of the impulse depends on the flow of electric current along the cardiac muscle fibers from active to resting regions of the heart. In some regions such as Purkinje fibers, this process involves flow along a cylindrical fiber and only one spatial dimensional is involved. The use of "cable theory" relies on the fact that cardiac Purkinje fiber can be viewed as a cylindrical fiber immersed in a very large volume of conducting solutions (extracellular fluids), and the propagation of cardiac electrical pulse can be treated as electrical conduction through a one-dimensional telegraph cable. Thus, Ohm's law and a series of mathematical treatments can be applied to characterize the relationship among the parameters involved in myocardial

conduction, and to provide the theoretical basis for the mathematical model tested in this study (Noble, 1979).

Myocardial conduction may be a more complex issue and involves the multidimensional spread of current over and through a more complex geometrical network, such as the walls of atria and ventricles. Unlike nerve or skeletal muscle fibers, cardiac muscle fibers are not composed of single cylinders. Each fiber is composed of a large number of closely apposed cells, each of which is surrounded by a complete envelope of membrane. However, there are regions (the nexuses) at which the membranes come into very close contact (McNutt and Weinstein 1973). The anatomical feature of these contact regions suggests that they are specialized to allow conduction of ions and other small molecules between cells as verified by Barr et al. (1966).

Sperelakis and MacDonald (1974) have shown a lower longitudinal than transverse resistivity in cat ventricular muscle. The present model assumes that the cardiac fibers are arranged as a bundle of electrically connected cylindrical cable fibers, with faster conduction longitudinal to fiber direction compared to conduction transversely. The anisotropic properties of cardiac muscles and the directionally determined effects of antiarrhythmic drug action (Sano et al., 1959; Bajaj et al., 1987; Kadish et al., 1986, Spach et al., 1987) support this assumption. The cable equations describing one-dimensional propagation on which the present model is based may thus be relevant to conduction through the more complex structures in the whole heart.

Relationship to previous electrophysiological studies of procainamide

1. Recovery kinetics

The recovery time constants measured for changes in $\dot{V}_{max}$ produced by procainamide <u>in vitro</u> have ranged from about 1 to 4 seconds (Varro et al.,

1985, Nattel, 1987a, Courtney, 1980a; Sada et al., 1979; Ehring et al., 1988). The range of values is not surprising in view of the variety of tissue types, superfusion solutions, and modeling techniques used The mean recovery time constant estimated from the present experimental data are in the same range as previous in vitro values A simple monoexponential analysis fit our data less well, and provided a significantly shorter time constant than that obtained by the kinetic analysis of either the model or the terminal linear portion of the log (delta CT)-S_1S_2 curve, the latter representing the time constant characterizing the time dependent recovery from drug-induced sodium channel blockade (Table 3).

2 Procainamide effects on effective refractory period (ERP)

In the current study, we used JT interval as an in vivo index of action potential duration (APD). Previous reports on procainamide's effects on ERP or action potential duration were variable. In vitro studies with procainamide have shown either small, statistically non-significant (Sada et al , 1979; Kadish et al , 1986) increases or no change (Varro et al., 1985) in action potential duration and effective refractory period. Morady et al. (1986) and Giardina et al (1973), however, reported that procainamide produced significant prolongation of ERP and QT interval in patients, respectively. Greenspan et al (1980) showed that at plasma concentration of 13.6 ± 8.6 mg/L, procainamide increased the QT interval by less than 25%.

We did not find evidence for procainamide-induced changes in repolarization time based on measurements of JT interval (Table 2). It is not certain whether the concentration of procainamide required to exert significant effects on ERP in the intact dog heart is much higher than that in man However, our study was not designed to evaluate procainamide's effects on repolarization, and we cannot exclude the possibility that procainamide

delayed repolarization slightly, below our threshold of detection In addition, we were not able to find measurable active metabolite of procainamide, N-acetylpreocainamide (NAPA), a procainamide metabolite known to prolong action potential duration or ERP while having no effects on sodium channel availability.

3. Procainamide concentrations

The procainamide concentrations we evaluated were in the range of 30-100 mg/L (Table 1) which are higher than commonly accepted therapeutic concentrations (4-10 mg/L) (Morady et al., 1985; 1986, Gang et al , 1985) The usual "therapeutic" range of plasma procainamide concentrations increase QRS duration in man by less than 10% (Giardina et al , 1973), and substantially larger doses (producing concentrations in the range that we studied) are frequently needed to control recurrent ventricular tachyarrhythmias in patients (Greenspan et al , 1980) Gang et al (1985) reported that in patients with atrial pacing cycle length of 851 ± 163 ms, a therapeutic procainamide dose (15 mg/kg or 10 0 ± 3 mg/L) was able to prolong HV interval by 19%, and QRS duration by 8%. Greenspan et al (1980) observed a less than 25% increase in QRS duration at a procainamide concentration of 13 6 ± 8.6 mg/L.

Morady et al. (1985) were the first to show the rate-dependent effects of a number of antiarrhythmic drugs in human beings They found that at a serum level of 8.2 ± 1.9 mg/L (therapeutic concentration), procainamide produced frequency-dependent prolongation of QRS duration, with an increase in QRS duration by 42 ± 11 % at the shortest pacing cycle length of 250 ms It is very possible that larger drug concentrations may be required in dogs to produce the same magnitude of drug effects as seen in patients Our results are consistent with the magnitude of changes in $\dot{V}_{max}$ and conduction velocity

in canine tissue exposed to comparable procainamide concentrations in vitro (Varro et al , 1985, Nattel, 1987a; Sada et al , 1979; Kadish et al, 1986, Rosen et al , 1973) Varro et al (1985) reported a nearly 20% decrease in V_{max} at pacing cycle length of 300 msec and at a procainamide concentration of 30 mg/L in guinea pig papillary muscle At the same drug concentration and pacing rate, Nattel (1987a) showed that procainamide caused a 17.8 % and 10.7% decrease respectively in $\dot{V}_{max}$ and conduction velocity in canine Purkinje fibers Kadish et al (1986) noted that 20 mg/L procainamide caused a 23% reduction of $\dot{V}_{max}$ in directions either longitudinal or transverse to fiber axis , and an 11 6 % and 6% reduction respectively in longitudinal and transverse conduction velocity at a pacing cycle length of 1 sec in canine ventricular myocardium Rosen et al (1973) showed that 30 mg/L procainamide produced a 24% decrease in $\dot{V}_{max}$ at a cycle length of 1000 msec

Procainamide was used in these experiments as a prototype fast channel blocker, in order to test a model of use-dependent drug effects on conduction Since the model predicts effects based on the magnitude of drug action rather than drug concentration and the underlying rate of the recovery process, it should be relevant to the actions of other antiarrhythmic drugs on ventricular conduction

Action of N-acetylprocainamide

Procainamide pharmacokinetics studies in man (Dreyfuss et al , 1972; Reidenberg et al , 1975) show that procainamide is mainly metabolized to a major metabolite, N-acetylprocainamide NAPA N-acetylprocainamide has been reported to be biologically active in studies of animal arrhythmia models (Drayman & Hoffman, 1981) and of arrhythmia patients (Josephson & Singh, 1986) NAPA may be present in significant concentrations in the plasma during procainamide therapy and exerts antiarrhythmic effects that may be partly

responsible for the antiarrhythmic effect of procainamide (Reidenberg et al., 1975; Bagwell et al., 1975). Unlike its parent compound procainamide, which substantially reduces $\dot{V}_{max}$ and depresses conduction, electrophysiological studies have shown that N-acetylprocainamide has little effect on myocardial conduction and maximal rate of rise of the action potential, and is much more selective than procainamide in prolonging action potential duration and the refractory period (Jaillon & Winkle, 1979; Singh et al., 1986). Changes of action potential duration and the refractory period produced by NAPA constitute its primary antiarrhythmic drug effects (Jaillon & Winkle, 1979; Singh et al., 1986). The lack of effect on phase 0 sodium current along with a delaying action on repolarization makes NAPA a class III antiarrhythmic agent (Singh et al., 1986).

Greenspan et al. (1980) showed that in the treatment of arrhythmia patients with large dose of procainamide, drug efficacy did not appear to correlate with plasma NAPA levels, which ranged from 1.4 to 51.4 mg/L during long term oral therapy. In one patient an effective procainamide concentration was associated with a NAPA level of 0. In other patients where arrhythmias could still be induced despite relatively high procainamide and NAPA levels, arrhythmias were subsequently controlled with the addition of procainamide infusion without a change in NAPA level. In the present study no measurable NAPA concentration was detected after the procainamide administration. Had NAPA been formed and appeared in plasma in the present study, it might have prolonged the effective refractory period and increased the likelihood of voltage dependent block.

Voltage-dependent block and tonic block

The model is designed to predict pure frequency-dependent antiarrhythmic drug-induced blockade. If there were voltage-dependent block as well, model

predictions would not necessarily apply. Voltage-dependent block is usually expected in diseased tissue, but our experiments were conducted in dogs with normal heart. Phase 3 block is voltage-dependent block occurring during the fast repolarization phase (phase 3) of the action potential where sodium channel inactivation is not fully reversed. It frequently occurs either at fast pacing rates when the diastolic interval is insufficient for complete repolarization, or after significant prolongation of action potential duration that results in the take-off of the next activation at a depolarized membrane potential. Consequently, considerable enhancement of drug-induced conduction slowing will occur. Phase 3 block could result in excess conduction slowing for very premature activations, thereby confounding the interpretation of pure frequency dependent depression of conduction velocity examined in this study.

Phase 3 block is insufficient to account for the deviation of our experimental data from a simple first-order relationship, as these deviations (as shown in Figure 4) occurred at coupling interval as great as 1 second. The short basic cycle length (300 msec) at which recovery kinetics were studied, the lack of effect of procainamide on the JT interval (Table 2), and the much shorter JJ interval than 300 msec at a basic cycle length of 300 msec suggest that recovery from phase 3 block is unlikely to have played any important role in our observations.

The model requires that the recovery from frequency-dependent conduction slowing be followed to completion, or that there be an absence of tonic block. Assuming a recovery time constant of 2.6 second for $\dot{V}_{max}$ block by procainamide (Courtney, 1980a), we should have observed an average of 89% recovery over a mean pause interval of 5.8 sec achieved in our experiments if there were no tonic block. Our observation of 85 ± 9% recovery from QRS prolongation caused by procainamide is consistent with a negligible amount of tonic block.

Relationship to previous <u>in vivo</u> studies of use-dependent antiarrhythmic drug effects

Previous studies have shown that conduction slowing in vivo by amitriptyline (Nattel, 1985), lidocaine (Davis et al , 1986), and mexiletine (Bajaj, 1987) can be empirically described as a monoexponential function of recovery interval. The recovery time constants of these agents in vivo were in the same range as those obtained for $\dot{V}_{max}$ changes produced by the same drugs <u>in vitro</u> (Nattel, 1985; Chen et al., 1979, Varro et al , 1985, Nattel, 1987a) There is no data available in the literature regarding the time dependent effects of these agents on I_{Na} at physiological temperatures (in the range of 37°C), although lidocaine's effects on I_{Na} in rabbit Purkinje fibers at 17°C (Bean et al., 1983), and in rat single ventricular cells (Sanchez-Chapula et al , 1983) at 24°C recovers in a first order fashion

While the apparent first order relationship in vivo is only an approximation, the difference between the monoexponential analysis and the mathematical model for the time dependent recovery kinetics can only be detected by careful statistical evaluation (Table 3) and is readily masked by experimental error As suggested by Bajaj and co-workers (1987) and predicted by our mathematical analysis, a monoexponential model will tend to underestimate the recovery time constant at larger magnitudes of drug effect This was confirmed by our experimental results (Table 4) The degree of underestimate is relatively small if the recovery process is followed to completion. In the present study, a first order analysis resulted in time constants that were 20% less than those resulting from the mathematical model, leaving them in the range of recovery time constants for procainamide effects on $\dot{V}_{max}$ reported in the literature. If only a small portion of the recovery process at short coupling interval is observed, however, the underestimate can be much more substantial. This is because the deviation of drug effects from

the first order relationship would have occupied a larger percentage of the recovery process observed

Index of conduction time and evaluation of conduction pattern

In the present study, we initially utilized QRS duration as an in vivo index of total ventricular conduction time. The results obtained using QRS duration were similar to those obtained by using epicardial activation mapping. Our study showed that changes in these two indices were closely related (Figure 8)

QRS duration has been used extensively as a measure of overall ventricular activation time in human studies (Morady et al., 1985; 1986; Gang et al., 1985) as well as in in vivo animal models (Nattel 1985; Davis et al., 1986) Davis et al (1986) showed that lidocaine produced concordant changes in QRS duration and HV interval Our observation that QRS duration changes correspond well with directly measured alterations in conduction time by epicardial activation mapping (provided that morphology remains constant) suggests that the former is a valid index for quantitative analyses of antiarrhythmic effects on ventricular conduction in the normal heart. This supports previous observations of time-dependent recovery of drug-induced prolongation in experimental animals (Nattel 1985) and studies of the kinetics of changes in QRS duration produced by antiarrhythmic drugs in man (Greenspan et al., 1980, Morady et al., 1985; Gang et al.,1985). Since QRS duration can be measured non-invasively with commonly available equipment, our analysis and observations provide a basis for further quantitative analysis of the kinetics of antiarrhythmic drug action in man.

The present model is valid only if the conduction pattern through the whole heart remains constant. If changes in the pattern of impulse propagation occur, they can alter the results in two important ways. First, changes in the

direction of activation will alter path lengths and therefore affect the assumptions underlying the incorporation of conduction time in the model (Nattel 1987b). Furthermore, antiarrhythmic drug action is directionally determined, being greater when conduction is in the the longitudinal than the transverse direction (Bajaj et al., 1987, Kadish et al., 1986; Spach et al., 1987). This appears to be due to effects of the propagation pattern on the action potential upstroke and consequently antiarrhythmic drug uptake (Spach et al., 1988). A stable conduction pattern was evident in the current study as reflected by the constant QRS morphology. The qualitative and quantitative analyses of epicardial activation also showed that the pattern of conduction was not altered (Figures 6, 7) , even in the presence of concentrations of procainamide that produced important frequency-dependent changes in conduction velocity. The doses of procainamide that we used were not high enough to produce conduction block and Wenckebach periodicity, which have been associated with directional differences in propagation produced by sodium channel blocking agents (Spach et al , 1988)

Recovery kinetics of drug-induced changes in conduction in Purkinje fibers versus in ventricular myocardium

The speed of transmission of the electrical impulse is an important factor in cardiac excitation since it is the function of the conducting system to ensure the mechanical events are correctly timed One of the critical determinants of cardiac conduction velocity is the size of cardiac fibers. Mathematical analysis of one-dimensional cable theory suggests that the cardiac conduction velocity varies approximately with the square root of the radius of a cardiac fiber (Noble, 1979) Thus, as would be expected, the conduction velocity is the fastest in Purkinje fibers (4 m/sec) that have the largest fiber size (about 50 μm in radius), and relatively slower in atrial

and ventricular muscles where fibers are smaller (Noble, 1979). As cardiac conduction in the ventricle involves electrical propagation in Purkinje fibers as well as ventricular muscle, the question arises as to whether the recovery kinetics of antiarrhythmic drugs are different in the Purkinje fiber system versus ventricular muscle. If the time constants of antiarrhythmic drugs are different in the Purkinje fibers and ventricular muscle, one might expect a biexponential recovery process of time-dependent antiarrhythmic drug-induced changes in intraventricular conduction. However, in the current literature no studies on recovery kinetics of antiarrhythmic drugs in Purkinje fibers and ventricular muscle have been conducted in the same species. Most of the recovery kinetic studies in vitro were performed either in canine Purkinje fibers (Varro et al , 1985, Nattel, 1985; 1987), or in guinea pig papillary muscle (Chen et al., 1979; Sada et al., 1979; Courtney, 1980a; Hohnloser et al., 1982; Campbell 1983; Grant et al., 1980; 1982). This makes a direct comparison of the recovery kinetics in Purkinje fibers versus ventricular muscle difficult. Nevertheless, the time constants of various sodium channel blockers, including procainamide, obtained from the two conducting systems in different species are of the same order. The agreement between experimental data and the present model does not exclude the possibility that other theoretical models could potentially fit the experimental results as well.

Significance of our results

We have shown that the recovery from procainamide-induced ventricular conduction slowing in vivo in the intact dog heart is predicted by our mathematical model This is the first critical quantitative analysis of frequency-dependent actions of sodium channel blocking drugs on conduction in vivo We have also demonstrated that, on a theoretical basis, apparent first-order recovery from drug-induced conduction slowing is expected if

recovery from sodium channel blockade is first order, and if sodium current is proportional to the square of conduction velocity. A first order analysis will tend to underestimate the recovery time constant, and is only an approximation of the true recovery process as can be demonstrated by careful analysis of recovery from significant degrees of drug-induced conduction slowing. These considerations suggest that previous observations of the time dependence of drug-induced conduction slowing in vivo (Nattel, 1985, Davis et al., 1986, Bajaj et al., 1987; Talajic & Nattel, 1987), which showed time constants similar to those for $\dot{V}_{max}$ (Nattel, 1987a; Chen et al., 1979; Varro et al., 1985; Nattel, 1985) or calcium current (Uehara & Hume, 1985) in vitro, are more than fortuitous and have a theoretical underpinning.

The frequency-dependent effects of antiarrhythmic drugs have great potential clinical implications (Hondeghem & Katzung 1984). At fast heart rate with short diastolic interval, less recovery from drug-induced sodium channel blockade can occur between beats than at slower heart rate. The shorter the cycle length, the greater the steady-state sodium channel blockade, and thus the greater depression in $\dot{V}_{max}$ and cardiac conduction velocity. This property is beneficial since it can enable antiarrhythmic drugs to selectively block high frequency tachycardias while having little effect on conduction at normal heart rate. An early extrasystole can also be viewed as a very rapid single beat tachyarrhythmia. Thus, a sufficiently early extrasystole would be expected to be suppressed by a sodium channel blocker with a relatively fast onset rate of block.

Recently, Hondeghem (1987) discussed the applications of the modulated receptor hypothesis with respect to the importance of time- and voltage dependent block of sodium channel blockers in contribution to their antiarrhythmic and arrhythmogenic actions, and their relevance in some clinical conditions. He showed there exists an optimal time constant of

recovery of drug that could provide maximum steady state block for any desired end diastolic block. This generates important ideas for drug selection and a rationale for multi-drug therapy, which have to be tested in further studies.

The development of biophysical models of subcellular antiarrhythmic drug action (Hondeghem & Katzung, 1977; Starmer et al., 1984) allows for new insights into their mechanisms of electrophysiological and antiarrhythmic actions (Hondeghem & Katzung, 1984; Hondeghem, 1987). Quantitative studies of the relevance of these ideas to drug effects in vivo provide an important link between basic theory and potential clinical implications. The current study demonstrates the applicability of this approach in describing in a critical and quantitative fashion the effects of an important antiarrhythmic drug on ventricular conduction in vivo.

Bibliography

Armstrong CM: Time course of TEA^+-induced anomalous rectification in squid giant axons. J Gen Physiol 1966;50:491-503

Arnsdorf MF: Cable properties and conduction of the action potential **Physiology and Pathophysiology of the Heart.** N Sperelakis (ed.) Martinus Nijboff Publishing, Boston/The Hague/ Dordrecht/Lancaster 1984;109-140

Arnsdorf MF, Bigger JT Jr: The effect of lidocaine on components of excitability in excitability in long mammalian cardiac Purkinje fibers. **J Pharmacol Exp Ther** 1975;195:206-215

Arnsdorf MF, Bigger JT Jr: The effect of procaine amide on components of excitability in long mammalian cardiac Purkinje fibers. Circ Res 1976;38:115-122

Bagwell EE, Walle T, Drayer DE, Reidenberg MM, Pruett JK Correlation of the electrophysiological and antiarrhythmic properties of the N-acetyl metabolite of procainamide with plasma and tissue drug concentrations in the dog. **J Pharmacol Exp Ther** 1975;197:38-48

Bajaj AK, Kopelman HA, Wikswo JP, Cassidy F, Woosley RL, Roden D Frequency and orientation-dependent effects of mexiletine and quinidine on conduction in the intact dog heart. Circulation 1987,75 1065-1073

Barker PS, Macleod AG, Alexander J: The Excitatory process observed in the exposed human heart. Am Heart J 1930;5:720-742

Barr LM, Dewey M, Berger W: Propagation of action potentials and the structure of the nexux in cardiac muscle. J Gen Physiol 1965;48:797-823

Bean BP, Cohen CJ, Tsien RW: Lidocaine block of cardiac sodium channels. J Gen Physiol 1983,81 613-642

Beeler GW, Reuter H: Reconstruction of the action potential of ventricular myocardial fibers. J Physiol (London) 1977;269 177-210

Bigger JT, Mandel WJ: Effect of lidocaine on the electrophysiological properties of ventricular muscle and Purkinje fibers. J Clin Invest 1970b,49.63-77

Bigger JT, Mandel WJ: Effect of lidocaine on conduction in canine Purkinje fibers and at the ventricular muscle-Purkinje fiber junction. J Pharmacol Exp Ther 1970a;172:239-254

Buchanan JW, Saito JT, Gettes LS: The effects of antiarrhythmic drugs, stimulation frequency, and potassium-induced resting membrane potential changes on conduction velocity and dV/dt_{max} in guinea pig myocardium Circ Res 1985,56 696-703

Campbell TJ: Importance of physico-chemical properties in determining the kinetics of the effects of Class I antiarrhythmic drugs on maximum rate of depolarization in guinea-pig ventricle. Brit J Pharmacol 1983,80 33-40

Carmeliet E, Xheonneux R, Van Glabbeek A, Reneman R Electrophysiological effects of droperidol in different cardiac tissues. Naunyn Schmiedeberg's Arch Pharmacol 1976,293 57-66

Catterall WA: Neurotoxins that act on voltage-sensitive sodium channel excitable membranes. Ann Rev Pharmacol Toxicol 1980,20 15-43

Catterall WA: Inhibition of voltage-sensitive sodium channels in neuroblastoma cells by antiarrhythmic drugs Mol Pharmacol 1981,20 356-362

Chen CM, Gettes LS, Katzung BG: Effect of lidocaine and quinidine on steady state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. Circ Res 1975;37 20-29

Chen CM, Gettes LS: Combined effects of rate, membrane potential, and drugs on maximum rate of rise ($\dot{V}_{max}$) of action potential upstroke of guinea pig papillary muscle. Circ Res 1976,38 464-469

Clarkson CW, Hondeghem L: Evidence for a specific receptor site for lidocaine, quinidine, and bupivacaine associated with cardiac sodium channels in guinea pig ventricular myocardium. Circ Res 1985,56 496-506

Cohen CJ, Bean BP, Colatsky T, Tsien RW: Tetrodotoxin block of cardiac sodium channels in rabbit Purkinje fibers: Interaction between toxin binding and channel gating J Gen Physiol 1981;78.383-411

Cohen CJ, Bean BP, Tsien RW· Maximal upstroke velocity as an index of available sodium conductance Circ Res 1984;54:636-651

Cohen IS Strichartz GR· On the voltage-dependent action of tetrodotoxin Biophys J 1977,17·275-279

Colatsky TJ Voltage clamp measurements of sodium channel properties in rabbit cardiac Purkinje fibers. J Physiol (London) 1980;305·215-234

Colatsky TJ, Tsien RW Sodium channels in rabbit cardiac Purkinje fibers. Nature 1979,278.265-268

Colquhoun D, Henderson R, Ritchie JM: The binding of labelled tetrodotoxin to non-myelinated nerve fibers. J Physiol 1972;227:95-126

Courtney KR Mechanism of frequency-dependent inhibition of sodium currents in frog myelinated nerve by the lidocaine derivative GEA 968. J Pharmacol Exp Ther 1975,195:225-236

Courtney KR Fast frequency-dependent block of action potential upstroke in rabbit atrium by small local anesthetics. Life Sci 1979;24:1581-1588

Courtney KR: Interval-dependent effects of small antiarrhythmic drugs on excitability of guinea-pig myocardium. J Mol Cell Cardiol 1980a,12 1273-1286

Courtney KR: Structure-activity relations for frequency-dependent sodium channel block in nerve by local anesthetics J Pharmacol Exp Ther 1980b;213·114-119

Courtney KR: Quantifying antiarrhythmic drug blocking during action potentials in guinea-pig papillary muscle. J Mol Cell Cardiol 1983,15 749-75

Courtney KR: Additional comments on Circ. Res 54 636-651, 1984 Circ Res. 1985;57·194-195

Dangman KH, Hoffman BF: *In vivo* and *in vitro* antiarrhythmic and arrhythmogenic effects of N-acetyl procainamide J Pharmacol Exp Ther 1981,217 851-862

Davis J, Matsubara T, Scheinman MM, Katzung B, Hondeghem LM: Use-dependent effects of lidocaine on conduction in canine myocardium application of the modulated receptor hypothesis in vivo Circulation 1986,74 205-214

Davis LD, Temte JV: Electrophysiological actions of lidocaine on canine ventricular muscle and Purkinje fibers Circ Res 1969,24 639-655

Dick M, II, Norwood WI, Chipman C, Castaneda AR: Intraoperative recording of specialized atrioventricular conduction tissue electrograms in 47 patients. Circulation 1979;59:150-160

Dominguez G, and Fozzard HA. Influence of extracellular K^+ concentration on cable properties and excitability of sheep cardiac Purkinje fibers Circ Res 1970;26 565-574

Draper MH, Weidmann S Cardiac resting and action potentials recorded with an intracellular electrode J Physiol (London) 1951;115:74-94

Drevius J, Bigger JT Jr, Cohen AI, Schreiber EC. Metabolism of procainamide in rhesus monkey and man Clin Pharmacol Ther 1972,13:366

Durrer D, Van Der AAW, Buller J. Epicardial and intramural excitation in chronic myocardial infarction. Am Heart J 1964;68·765-776

Durrer D, Van der Tweel LH: Spread of activation in the left ventricular wall of the dog II. Am Heart J 1954,47:192-203

Ehring GR, Mover JW, Hondeghem LM: Quantitative structure activity studies of antiarrhythmic properties in a series of lidocaine and procainamide derivatives J Pharmacol Exp Ther 1988,244·479-492

Fozzard HA, January CT, Makielski J New studies of the excitatory sodium currents in heart muscle. Circ Res 1985;56.475-485

Fozzard HA Schoenberg M Strength-duration curves in cardiac Purkinje fibers· Effects of liminal length and charge distribution. J Physiol (London) 1976,260 593-618

Gallagher JJ, Kasell Jackie, Sealy WC, Pritchett ELC, Wallace AG Epicardial mapping in the Wolff-Parkinson-White syndrome Circulation 1978,58 Sup-866

Gang ES, Denton TA, Oseran DS, Mandel WJ, Peter T Rate-dependent effects of procainamide on His-Purkinje conduction in man Am J Cardiol 1985,55:1525-1529

Giardina EGV, Heissenbuttel RH, Bigger JT Jr Intermittent intravenous procaine amide to treat ventricular arrhythmias Ann Int Med 1980;46.453-462

Gintant GA, Hoffman BF, Naylor RE The influence of molecular form of local anesthetic-type antiarrhythmic agents on reduction of the maximum upstroke velocity of canine cardiac Purkinje fibers Circ Res 1983;52·735-746

Gliklich JI, Hoffman BF· Sites of action and active forms of lidocaine and some derivatives on cardiac Purkinje fibers Circ Res 1978,43 638-651

Grant AO, Starmer CF, Strauss HC Antiarrhythmic drug action Circ Res 1984;55:427-439

Grant AO, Strauss LJ, Wallace AG, Strauss HC The influence of pH on the electrophysiological effects of lidocaine in guinea pig ventricular myocardium Circ Res 1980;47 542-550

Grant AO, Trantham JL, Brown KK, Strauss HC: pH-dependent effects of quinidine on the kinetics of dV/dtmax in guinea pig ventricular myocardium. Circ Res 1982,50 210-217

Greenspan AM, Horowitz LN, Spielman SR, Josephson ME: Large dose procainamide therapy for ventricular tachyarrhythmia. Am J Cardiol 1980;46.453-462

Hille B, Schwarz W, Kischer P Mechanism of action of antifibrillatory drugs Naunyn-Schmiedebergs Arch Pharmacol 1971 269 199-212

Henderson R, Ritchie JM, Strichartz GR. The binding of labelled saxitoxin to the sodium channels in nerve membranes. J Physiol (London) 1973;235:783-804

Hille B Local anesthetics Hydrophilic and hydrophobic pathways for the drug-receptor reaction J Gen Physiol 1977,69:497-515

Hodgkin AL, Huxley AF A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (London) 1952,117 500-544

Hodgkin AL, Katz B The effect of sodium ions on the electrical activity of the giant axon of the squid J Physiol (London) 1949;108:37-77

Hodgkin AL, Rushton WAH The electrical constants of a crustacean nerve fiber Proc Soc 1946,133 444-479

Hohnloser S, Weirich J, Antoni H Effects of mexiletine on steady-state characteristics and recovery kinetics of $\dot{V}_{max}$ and conduction velocity in guinea pig myocardium J Cardiovasc Pharmacol 1982 4 232-239

Hondeghem LM, Katzung BG. Time and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels Biochim Biophys Acta 1977;472:373-398

Hondeghem LM, Katzung BG. Test of a model of antiarrhythmic drug action Circulation 1980,6·1217-1224

Hondeghem LM, Katzung BG. Antiarrhythmic agents the modulated receptor mechanism of action of sodium and calcium channel-blocking drugs Ann Rev Pharmacol Toxicol 1984,24 387-423

Hondeghem LM. Validity of $\dot{V}_{max}$ as a measure of the sodium current in cardiac and nervous tissue Biophys J 1978,23·147-152

Hondeghem LM: Comments on "Maximal upstroke velocity as an index of available sodium conductance comparison of maximal upstroke velocity and voltage clamp measurements of sodium current in rabbit Purkinje fibers" which appeared in Circ. Res 54.636-651, 1984 Circ Res 1985,57 636 6.1

Hondeghem LM: Antiarrhythmic agents· modulated receptor applications. Circulation 1987;75:514-520

Hunter PJ, McNaughton, Noble D Analytic models of propagation in excitable cells. Prog Biophys Molec Biol 1975,30 99-144

Jarillon P, Winkle RA Electrophysiologic comparative study of procainamide and N-acetylprocainamide in anesthetized dogs. concentration response relationships Circulation 1979;60:1385-1394

Jensen RA, Katzung BG Electrophysiological actions of diphenylhydantoin on rabbit atria Circ Res 1970;26 17-27

Johnson EA The effects of quinidine, procaine amide and pyrilamine on the membrane resting and action potential of guinea pig ventricular muscle fibers J Pharmacol Exp Ther 1956,117:237-244

Johnson EA, McKinnon MG The differential effect of quinidine and pyrilamine on the myocardial action potential at various rates of stimulation. J Pharmacol Exp Ther 1957,120 460-468

Josephson MA, Singh BN Hemodynamic effects of N-acetylprocainamide in man: comparison with other antiarrhythmic drugs Angiology 1986;37:950-958

Kadish AH Spear JF, Levine JH, Moore EN The effects of procainamide on conduction in anisotropic canine ventricular myocardium Circulation 1986,74 616-625

Kaiser GA, Waldo AL, Beach PM, Bowman FO, Hoffman BF, Malm JR: Specialized cardiac conduction system Arch Surg 1970;101:673-676

Kramer JB, Saffitz JE, Witkowski FX, Corr PB. Intramural re-entry as a mechanism of ventricular tachycardia during evolving canine myocardial infarction Circ Res 1985,56.736-754

Lee AG: Model for action of local anesthetics Nature 1976;262 545-548

Lapicque L: Recherches quantitative sur l'excitation electriques des nerfs traitee comme un popularisation. J Physiol (Paris) 1907,9:620-635

Lewis T, Rothschild MA: The excitatory process in the dog's heart II The ventricles. London: Philosophical transactions of the Royal Society 1915;206·181

McAllister RE, Noble D, Tsien RW Reconstruction of the electrical activity of cardiac Purkinje fibers J Physiol (London) 1975,251 1-59

Morady F, Dicarlo LA, Buitleir MD, Baerman JM, Krol RB Rate-dependent effects of intravenous lidocaine, procainamide and amiodarone on intraventricular conduction. J Am Coll Cardiol 1985,6:179-185

Morady F, Dicarlo LA, Buitleir MD, Krol RB, Baerman JM, Kou WH Effects of incremental doses of procainamide on ventricular tachycardia Circulation 1986;74:1355-1364

McNutt NS, Weinstein RS: Membrane ultrastructure at mammalian intercellular junctions. Prog Biophys 1973,26 45-101

Nattel S. Frequency-dependent effects of amitriptyline on ventricular conduction and cardiac rhythm in dogs Circulation 1985,72 898-906

Mattei S Interval-dependent effects of lidocaine on conduction in canine cardiac Purkinje fibers: experimental observations and theoretical analysis J Pharmacol Exp Ther 1987b;241.275-281

Mattei S Relationship between use-dependent effects of antiarrhythmic drugs on conduction and $\dot{V}_{max}$ in canine cardiac Purkinje fibers. J Pharmacol Exp Ther 1987a,241 282-288

Mattei S, Bailey JC: Time course of the electrophysiological effects of quinidine on canine cardiac Purkinje fibers: Concentration dependence and comparison with lidocaine and disopyramide J Pharmacol Exp Ther 1983,225 176-180

Mattei S, Elharrar V, Zipes DP, Bailey JC: pH-dependent electrophysiological effects of quinidine and lidocaine on canine cardiac Purkinje fibers Circ Res 1981,48 55-61

Noble D The initiation of the heartbeat 2nd edition. Clarendon Press, Oxford, 1979

Ohta S, Sada H, Kojima M, Ban T: Effects of tocainide and lidocaine on the transmembrane action potentials as related to external potassium and calcium concentration in guinea-pig papillary muscle. Naunyn-Schmiedeberg's Arch Pharmacol 1980;314:67-82

Prichon Y, Schmidtmayer J, Ulbricht Werner: Mutually exclusive blockage of sodium channels of myelinated frog nerve fibers by benzocaine and the indole alkaloid ervatamine Neuroscience Letters 1981;22:325-330

Postma SW, Catterall WA. Inhibition of binding of [^{3}H]Batrachotoxinin A 20-a-Benzoate to sodium channels by local anesthetics Mol Pharmacol 1984;25:219-227

Prinzmetal M, Ishikawa K, Oishi H, Ozkan E, Wakayama J, Barnes JM Effects of quinidine on electrical behavior in cardiac muscle J Pharmacol Exp Ther 1987;157:659-664

Puech P, Esclavissat M, Sodi-Pallares D, Cisneros F Normal auricular activation in the dog's heart. Am Heart J 1954,47 174-191

Reidenberg MM, Drayer DE, Levy M, Warner H Polymorphic acetylation of procainamide in man Clin Pharmacol Ther 1975,17 722

Rimmel C, Walle A, Keβler H, Ulbricht W. Rates of block by procaine and benzocaine and the procaine-benzocaine interaction at the node of Ranvier. PflÜgers Arch 1978,376.105-118

Ritchie JM, Rogart RB: The binding of saxitoxin and tetrodotoxin to excitable tissue. Rev Physiol Biochem Pharmacol 1977;79:1-50

Rosen MR Meaker C, Gelband H, Hoffman BF· Effects of procaine amide on the electrophysiologic properties of the canine ventricular conducting system. J Pharmacol Exp Ther 1973,185 438-446

Rosen MR, Meaker C, Pippenger CE. The effects of lidocaine on the canine ECG and electrophysiologic properties of Purkinje fibers. Am Heart J 1976,91:191-202

Rothberger CJ, Winterberg H: Studien über die bestimmung des ausgangspunktes ventrikulärer extrasystolen mit hilfe des elektrokardiogramms. Pflügers Arch Physiol 1913,154 571-598

Sachs L. Applied statistics. New York, 1984, Springer-Verlag

Sada H, Ban T Effects of various structurally related beta-adrenoceptor blocking agents on maximum upstroke velocity of action potential in guinea-pig papillary muscles Naunyn-Schmiedeberg's Arch Pharmacol 1981,317 245-251

Sada H, Kojima M, Ban T: Effect of procainamide on transmembrane action potentials in guinea-pig papillary muscles as affected by external potassium concentration. Naunyn-Schmiedeberg's Arch Pharmacol 1979,309:179-190

Sanchez-Chapula J, Tsuda Y, Josephson IR: Voltage- and use-dependent effects of lidocaine on sodium current in rat single ventricular cells. Circ Res 1983,52 557-565

Sano T, Takayama N, Shimamoto T: Directional difference of conduction velocity in the cardiac ventricular syncytium studies by microelectrodes. Circ Res 1959,7:262-267

Schmidtmayer J, Ulbricht W: Interaction of lidocaine and benzocaine in blocking sodium channels Pflügers Arch 1980;387:47-54

Schwarz W, Palade PT, Hille B: Local anesthetics effect of pH on use-dependent block of sodium channels in frog muscle Biophys J 1977,20 343-368

Seeman P: The membrane actions of anesthetics and tranquilizers Pharmacol Rev 1972;24:583-655

Sheldon RS, Cannon NJ, Duff HJ: Binding of [^{3}H]Batrachotoxinin A benzoate to specific sites on rat cardiac sodium channels Mol Pharmacol 1986,30:617-623

Sheldon RS, Cannon NJ, Duff HJ: A receptor for type I antiarrhythmic drug associated with rat cardiac sodium channels. Circ Res 1987,61 [illegible]

Singh BN, Feld G, Nademanee K: Arrhythmia control by selective lengthening of cardiac repolarization: Role of N-acetylprocainamide active metabolite of procainamide Angiology 1986;37:930-938

Singh BN, Vaughan Williams EM: Effect of altering potassium concentration on the action of lidocaine and diphenylhydantoin on rabbit atrial and ventricular muscle. Circ Res 1971;29 286-295

Spach MS, Dolber PC: Relating extracellular potential and their derivatives to anisotropic propagation at a microscopic level in human cardiac muscle Circ Res 1986;58:356-371

Spach MS, Barr RC, Johnson EA, Kootsey JM Cardiac extracellular potentials Circ Res 1973;33:465-473

Spach MS, Barr RC, Serwer G, Kootsey JM, Johnson EA: Extracellular potentials related to intracellular action potentials in the dog Purkinje system. Circ Res 1972;30 505-519

Spach MS, Dolber PC, Heidlage JF, Kootsey JM, Johnson EA: Propagating depolarization in anisotropic human and canine cardiac muscle: apparent directional differences in membrane capacitance Circ Res 1987;60:206-219

Spach MS, Dolber PC, Heidlage JF: Influence of the passive anisotropic properties on directional differences in propagation following modification of the sodium conductance in human atrial muscle. A model of re-entry based on anisotropic discontinuous propagation. Circ Res 1988,62 811-823

Spach MS, Miller WT, Miller-Jones III E, Warren RB, Barr RC. Extracellular potentials related to intracellular action potentials during impulse conduction in anisotropic canine cardiac muscle. Circ Res 1979;45:188-204

Sperelakis N, MacDonald RL: Ratio of transverse to longitudinal resistivities of isolated cardiac muscle fiber bundles. J Electrocardiology 19[illegible] 301-314

Starmer CF, Grant AO, Strauss HC Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics Biophys J 1984,46 15-27

Steiner C, Kovalik TW: A simple technique for production of chronic complete heart block in dogs. J Appl Physiol 1968,25 631-632

Strichartz GR: The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine J Gen Physiol 1973 62 37-5

Strichartz GR, Cohen I. $\dot{V}_{max}$ as a measure of G_{Na} in nerve and cardiac membranes Biophys J 1978,67 153-156

Talajic M, Nattel S: Frequency-dependent effects of calcium antagonists on atrioventricular conduction and refractoriness demonstration and characterization in anesthetized dogs Circulation 1986,74 111-116

Trautwein W· über die Veränderungen der elementaren daten der elektrischen erregungswelle des herzens bei der insuffizienz des myocards Pflugers Arch 1950;252:573-589

Tritthart H, Fleckenstein B, Fleckenstein A Some fundamental actions of antiarrhythmic drugs on the excitability and the contractility of single myocardial fibers Naunyn-Schmiedebergs Arch Pharmacol 1971 269 217-219

Uehara A, Hume JR. Interactions of organic calcium channel antagonists with calcium channels in single frog atrial cells J Gen Physiol 1985,85 621-647

Varro A, Elharrar V, Surawicz B· Frequency-dependent effects of several class I antiarrhythmic drugs on $\dot{V}_{max}$ of action potential upstroke in canine cardiac Purkinje fibers J Mol Cell Cardiol 1980,12 1273-1286

Vaughan Williams EM The mode of action of quinidine on isolated rabbit atria interpreted from intracellular potential records **Brit J Pharmacol** 1958,13 276-287

Wallace AG, Cline RE, Sealy WC, Young WG, Troyer WG Jr. Electrophysiogic effects of quinidine. **Circ Res** 1966;19:960-969

Watson M, Fozzard HA. The relation of $\dot{V}_{max}$ to I_{Na}, G_{Na}, and h_{∞} in a model of the cardiac Purkinje fiber **Biophys J** 1979;25:407-420

Watson M, Fozzard HA The conducted action potential. Models and comparison to experiments **Biophys J** 1983;44:9-26

Weidmann S The electrical constant of Purkinje fibers **J Physiolo** (London) 1952,118 348-360

Weidmann S The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. **J Physiol** (London) 1955,127,213-224

Weld FM, Coromilas J, Rottman JN, Bigger JT Jr. Mechanisms of oquinidine-induced depression of maximum upstroke velocity in ovine cardiac Purkinje fibers **Circ Res** 1982,50:369-376

Weld FM, Bigger JT Jr. Effect of lidocaine on the early inward transient current in sheep cardiac Purkinje fibers **Circ Res** 1975;37 630-639

Wellens HJJ, Janse MJ, van Dam RT, Dirrer D. Epicardial excitation of the atria in a patient with atrial flutter. Br Heart J 1971,33 233-237

West TC, Amory DW: Single fiber recording of the effects of quinidine at atrial and pacemaker sites in the isolated right atrium of the rabbit J Pharmacol Exp Ther 1960,130 183-193

Zipes DP Specific arrhythmias diagnosis and treatment In Braun et (ed): Heart disease, 2nd edition Saunders Press New York 19

APPENDIX

```
10  ************************************************************************
     THIS PROGRAM IS CALLED WUHUA-MODEL FIT.BAS
30  ************************************************************************
40 CLEAR
50 DIM F(200),COUP(200),E(200)
60 CLS
70 BEEP
80 LET SS=1000000!
90 S=0
100 PRINT 'TO CHANGE THE CURRENT STEP SIZE AND K, EDIT LINES 1070 AND 1080
110 PRINT WHAT IS THE EXPERIMENT NUMBER, DOSE NUMBER
120 INPUT EXPN,DOSEN
130 LPRINT
          EXPERIMENT EXPN',DOSE DOSEN
140 LPRINT 'MODEL CURVE FIT
150 LPRINT     CI ,'  CTr
160 LOCATE 10,1 INPUT 'WHAT IS THE LONGEST COUPLING INTERVAL    ',CIL
170 LOCATE 11,1 INPUT "WHAT IS THE SHORTEST COUPLING INTERVAL    ",CIR
180 LOCATE 12,1,INPUT 'WHAT IS THE ITERATION INTERVAL ',ITINT
190 CLS
200 LOCATE 10,1 INPUT 'WHAT IS THE LOWEST POSSIBLE VALUE OF CI    ',CIA
210 LOCATE 11,1 INPUT 'WHAT IS THE HIGHEST POSSIBLE VALUE FOR CI    ',CIB
220 LOCATE 15,1,INPUT 'WHAT IS THE LOWEST POSSIBLE VALUE OF K    ',KA
230 LOCATE 16,1:INPUT "WHAT IS THE HIGHEST POSSIBLE VALUE OF K    ",KB
240 CLS
250 N=0
260 PRINT 'THE NUMBERS OF OBSERVED EXPERIMENTAL VALUES ARE
270 INPUT Q
280 FOR N=1 TO Q
290 READ COUP(N),F(N)
300 PRINT COUP(N),F(N)
310 LPRINT COUP(N),F(N)
320 NEXT N
330 DATA
340 DATA
350 FOR CIR=CIRA TO CIRB STEP .002
360 FOR K=KA TO KB STEP .000025
370 LET S=0
380 LET M=1
390 LET CI=CIL
400 LET V=CIR
410 IF CI=COUP(M)  THEN 460
420 LET CI=CI-ITINT
430 LET DEL=ITINT*K*((V)^2+(V+1)*.5*(V-1))
440 LET V=V+DEL
450 GOTO 410
460 LET D=V-F(M)
470 PRINT 'M=',M,'INPUT CI=',COUP(M),'INPUT CTr=',F(M),'MODEL CTr=',V
480 LET S=S+D^2
490 PRINT  'THE CUMMULATIVE SUM OF SQUARES IS',S
500 PRINT 'M=',M,'Q=',Q
510 LET M=M+1
    IF M>Q THEN 540
530 GOTO 410
540 IF S<SS THEN 590
550 LET SS=S
560 LET R=CIR
```

```
      GET J
      PRINT  FOR CIR= CIR   AND K= K  THE SUM OF SQUARES= S
      PRINT  FOR A MIN CTr CIR  AND A K= K THE SUM OF SQUARES='S
      PRINT  FOR A MIN CTr CIR' AND A K='K'THE SUM OF SQUARES= S
      NEXT K
      NEXT CIR
      PRINT  THE BEST FIT IS FOLLOWING'
      PRINT  THE BEST FIT IS FOLLOWING'
      PRINT    ,R, K= ,U, SS=',SS
      PRINT    ,R, K= ,U, SS= ,SS
      END
```

```
10 '*****************************************************************
   ' THIS PROGRAM IS CALLED WUHUA-EXPONENTIAL FIT.BAS
30 '*****************************************************************
40 CLEAR
50 DIM F(200),COUP(200),E(200)
60 CLS
70 BEEP
80 LET SS=1000000!
90 S=0
100 PRINT'WHAT IS EXPERIMENT #, DOSE #"
110 INPUT EXPN,DOSEN
120 LPRINT"
                EXPERIMENT'EXPN", DOSE'DOSEN
130 LPRINT "EXPONENTIAL CURVE FIT'
140 PRINT "TO CHANGE THE CURRENT STEP SIZE ADN K,EDIT LINES 401) AND 4
150 LPRINT '    CI'."     CTr'
160 LOCATE 10,1:INPUT "WHAT IS THE LONGEST COUPLING INTERVAL ',CIL
170 LOCATE 11,1:INPUT "WHAT IS THE SHORTEST COUPLING INTERVAL ',CIMIN
180 LOCATE 12,1,INPUT 'WHAT IS THE ITERATION INTERVAL ',ITINT
190 CLS
200 LOCATE 10,1:INPUT "WHAT IS THE LOWEST POSSIBLE VALUE OF CIR ",CIRA
210 LOCATE 11,1:INPUT "WHAT IS THE HIGHEST POSSIBLE VALUE FOR CIR   ",CIRB
220 LOCATE 15,1:INPUT 'WHAT IS THE LOWEST POSCIBLE VALUE OF K ',KA
230 LOCATE 16,1:INPUT 'WHAT IS THE HIGHEST POSSIBLE VALUE OF K ',KB
240 CLS
250 N=0
260 PRINT 'THE NUMBERS OF OBSERVED EXPERIMENTAL VALUES ARE
270 INPUT Q
280 FOR N=1 TO Q
290 READ COUP(N),F(N)
300 LPRINT COUP(N),F(N)
310 PRINT COUP(N),F(N)
320 NEXT N
330 DATA
340 DATA
350 FOR CIR=CIRA TO CIRB STEP .002
360 FOR K=KA TO KB STEP .000025
370 LET S=0
380 LET M=1
390 LET CI=CIL
400 LET V=CIR
410 IF CI=COUP(M)  THEN 460
420 LET CI=CI-ITINT
430 LET DEL=(CIR-1)*(EXP(K*CIL))
440 LET V=DEL*(EXP((-K)*CI))+1
450 GOTO 410
460 LET D=V-F(M)
470 PRINT "M='M,'INPUT CI='COUP(M),"INPUT CTr=" F(M),'MODEL CTr=' V
480 LET S=S+D^2
490 PRINT 'THE CUMMULATIVE SUM OF SQUARES IS "S
500 PRINT "M="M,"Q="Q
510 LET M=M+1
520 IF M>Q THEN 540
530 GOTO 410
540 IF S<SS THEN 600
550 LET SS=S
560 LET R=CIR
```

```
570 LET U=F
580 LET W=DEL
590 PRINT  FOR CIR='CIR DEL='W' AND K='K"THE SUM OF  SQUARES= S
600 PRINT "FOR A MIN CTr CIR"DEL="DEL" AND A K='K"THE SUM OF SQUARES= 'S
610 LPRINT 'FOR A MIN CTr 'CIR"DEL='DEL" AND A  K='K"THE SUM OF SQUARES="S
620 NEXT F
630 NEXT CIR
640 PRINT  'THE BEST FIT IS FOLLOWING"
650 LPRINT  THE BEST FIT IS FOLLOWING
660 PRINT 'CIR=',R,'DEL=',W,"K=',U,"SS=";SS
670 LPRINT  CIR=",R,'DEL= ;W,'k=' ;U, SS=  ;SS
680 END
```