# ELECTROPHYSIOLOGICAL INTERACTIONS BETWEEN DISOPYRAMIDE AND ITS MAJOR METABOLITE, MONO-N-DEALKYLDISOPYRAMIDE, IN CANINE VENTRICULAR TISSUE

by

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This thesis is dedicated to

my parents

for their tremendous love and support.

## Organization of Thesis

The core of this thesis is composed of a general introduction, two manuscripts, and a general summary and conclusions. The first manuscript which constitutes chapter 2 of the thesis is in press in the Journal of Pharmacology and Experimental Therapeutics. However, in order to be appropriately incorporated into the thesis this article has been modified. The second manuscript presented in chapter 3, is in preparation for publication. For these two studies: the proposal; study design; experimentation; data analysis and interpretation; and dissertation of the two manuscripts and the thesis was principally the work of the author.

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## General Abstract

Mono-N-dealkyldisopyramide (MND), the major metabolite of disopyramide, reaches significant concentrations in patients; however, little is known about its electrophysiological effects. We therefore assessed its activity in canine ventricular tissue superfused *in vitro*. MND produced a concentration and rate dependent increase in the magnitude of $\dot{V}_{max}$ depression. MND shortened all phases of repolarization in Purkinje fibers but prolonged action potential duration and effective refractory period in ventricular muscle.

We then assessed the effects of the combination of disopyramide and MND at clinically relevant concentrations. The combination produced additive effects on depression of $\dot{V}_{max}$. MND accentuated the shortening of action potential duration in Purkinje fibers and the prolongation of refractory periods in both Purkinje fibers and ventricular muscle produced by disopyramide at normal heart rates.

In contrast, at slow stimulation rates and under predisposing electrolyte conditions, disopyramide produced a reverse use-dependent prolongation of action potential duration which led to the development of early afterdepolarizations and triggered activity in Purkinje fibers. Mexiletine and pacing abolished triggered activity, while MND shifted the incidence of triggered activity to longer cycle lengths.

The results suggest that MND, by depressing $\dot{V}_{max}$ and conduction velocity and prolonging refractory periods, enhances the effects of disopyramide and may have antiarrhythmic properties of its own. Its

effects on disopyramide-induced triggered activity suggest that it may also reduce the arrhythmogenic potential of disopyramide. Therefore MND may contribute to either the antiarrhythmic or toxic actions of disopyramide.

Résumé Général

Mono-N-déalkyldisopyramide (MND), le métabolite principal du disopyramide, atteint des concentrations significatives chez les patients; toutefois, ses effets électrophysiologiques sont peu connus. Nous avons par conséquent évalué ses effets dans les tissus ventriculaires canins superfusés *in vitro*. Le MND a provoqué une diminution de $\dot{V}_{max}$ dont l'intensité était fonction de la concentration et de la fréquence d'entrainement. Le MND a raccourci toutes les phases de la repolarisation dans des fibres de Purkinje mais a prolongé la durée du potentiel d'action et la période réfractaire effective du muscle ventriculaire.

Nous avons ensuite évalué les effets de la combinaison du disopyramide et du MND à des concentrations cliniquement pertinentes. La combinaison a provoqué des effets additifs sur la diminution de $\dot{V}_{max}$. Le MND a accentué le raccourcissement de la durée du potentiel d'action des fibres de Purkinje ainsi que la prolongation des périodes réfractaires des fibres de Purkinje et du muscle ventriculaire provoqués par le disopyramide à des fréquences cardiaques normales.

A l'opposé, à des basses fréquences de stimulation et sous des conditions d'électrolyte prédisposantes le disopyramide a provoqué une prolongation de la durée du potentiel d'action, en fonction inverse de la fréquence, qui a mené au développement de post-dépolarisations prématurées et d'activité déclenchée dans les fibres de Purkinje. La

mexilétine et l'entrainement ont aboli l'activité déclenchée, tandis que le MND a déplacé l'apparition de l'activité déclenchée à des durées de cycle plus longues.

Les résultats suggèrent que le MND, en diminuant la $\dot{V}_{max}$ et la vélocité de la conduction et en prolongeant les périodes réfractaires, accroit les effets du disopyramide et pourrait avoir ses propre propriétés antiarythmiques. Ses effets sur l'activité déclenchée induite par le disopyramide suggèrent qu'il pourrait aussi réduire le potentiel arythmogène du disopyramide. Par conséquent, le MND pourrait contribuer soit aux actions antiarythmiques ou toxiques du disopyramide.

# Chapter 1

# Introduction

## 1. Metabolites of Class I Antiarrhythmic Drugs

Many antiarrhythmic agents are extensively metabolized following administration to patients (Kates, 1984). These antiarrhythmic drugs, used in the treatment and prevention of life-threathening cardiac arrhythmias, also have low therapeutic indices. Therefore in those cases where significant metabolites are produced, it is essential to determine the pharmacological properties and disposition characteristics of both the parent drug and metabolite(s) for safe and optimal therapy. The presence of an active metabolite may, unpredictably, either potentiate the antiarrhythmic effects of the parent drug or alternatively increase the possibility of drug toxicity. These interactions complicate determination of the clinical efficacy of the parent antiarrhythmic agents.

Two important considerations which determine the significance of metabolites in the action of antiarrhythmic drugs are: 1) the amount of accumulation and 2) intrinsic pharmacodynamic activities of the metabolites. Metabolites must be present in sufficient plasma concentrations and be pharmacologically active in order to produce any significant effects. With Class I antiarrhythmic agents (sodium channel blockers), while the pharmacologic properties of most drugs have not been fully determined, the extent of metabolism and plasma concentrations of metabolites during therapy have been measured for most. The plasma metabolite levels of most of these agents can reach or even exceed those of the parent drugs (Kates, 1984 and 1986). In

addition, the concentration of metabolites may further increase in diseased states such as renal failure if renal excretion is a major elimination pathway. Such increases occur with the metabolites of procainamide (Drayer et al., 1977) and disopyramide (Aitio, 1981).

In this section, we review the pharmacological actions produced by the metabolites of various Class I antiarrhythmic agents. Most of these studies have evaluated the effects and relative potency of the metabolites compared to the parent drugs in *in vivo* and *in vitro* experimental models. The metabolism of the clinically available Class I agents produce compounds which vary in their pharmacological profiles and thus their clinical significance in the therapeutic or toxic effects of antiarrhythmic drug therapy. One possible outcome of metabolism is the production of inactive metabolites. Both mexiletine and tocainide are examples of drugs which have no active metabolites (Pentikainen et al., 1982; Ronfeld et al., 1982).

### 1.1 Lidocaine, Quinidine and Flecainide

Other antiarrhythmic agents are metabolized into compounds which are active and may therefore contribute to the clinical efficacy or toxicity produced by the parent drug. The antiarrhythmic agents in this group include lidocaine, quinidine and flecainide (Kates, 1986). Significant plasma concentrations of metabolites with these agents may occur in subgroups of patients (because of large interindividual variability) or under certain conditions such as renal failure (for drugs in which renal excretion is a major elimination pathway).

Lidocaine is metabolized to two important metabolites in man: monoethylglycylxylidide (MEGX) and glycylxylidide (GX) (Narang et al., 1978). MEGX has been shown to be equally or slightly less potent than lidocaine in suppressing experimentally-induced ventricular arrhythmias in mice and dogs (Smith and Duce, 1971; Strong et al., 1975; Freedman et al., 1982) and arrhythmias in isolated guinea-pig atrium (Burney et al., 1974). GX also produced antiarrhythmic effects but was much less potent than lidocaine in the same studies by Strong et al. (1975) and Burney et al. (1974).

The effects produced by GX *in vitro* in the study of Bennett et al. (1988), however, suggest a possible mechanism by which it may reduce the clinical efficacy of lidocaine. The metabolite competitively displaced lidocaine in isolated guinea-pig ventricular myocytes at potentials between -80 and -100 mV. This interaction could be explained by a modulated receptor model with two drugs competing for the same sodium channel receptor. In the presence of the combination, GX competitively displaced lidocaine from sodium channels due to its faster binding kinetics. Subsequent dissociation of GX from channels resulted in an increased in the sodium current compared to that with lidocaine alone. Thus under appropriate conditions, accumulation of the metabolite could prevent the sodium channel blocking effects (Class I action) of the parent drug.

Quinidine also has two important cardioactive metabolites in man: 3-hydroxy-quinidine (3-OH-Q) and 2'-oxo-quinidine (2'-oxo-Q) (Drayer et al., 1978). The electrophysiologic effects of both metabolites are

qualitatively similar to effects produced by quinidine (Thompson et al., 1987). All three compounds produced a use-dependent depression of $\dot{V}_{max}$ and a reverse use-dependent prolongation of action potential duration (ie. action potential prolongation was greater at slower stimulation rates) in canine Purkinje fibers. Like the parent drug, 3-OH-Q also induced early afterdepolarizations at very long cycle lengths. In experimental arrhythmia models, both metabolites were equipotent to quinidine in their antiarrhythmic efficacy against ventricular arrhythmias in mice, however, 3-OH-Q was less potent than quinidine or 2'-oxo-Q in rabbits (Drayer et al., 1978). Vozeh et al. (1987) also showed that 3-OH-Q was less potent than quinidine against ventricular arrhythmias in isolated rat heart, but determined that the interaction between quinidine and 3-OH-Q produced additive effects.

The two major metabolites of flecainide in man are meta-O-dealkylated flecainide and the meta-O-dealkylated lactam of flecainide (Conard and Ober, 1984). Meta-O-dealkylated flecainide produced electrophysiological effects on conduction, refractoriness and experimental arrhythmias in anesthetized dogs and was approximately one-half as potent as flecainide (Guehler et al., 1985). The meta-O-dealkylated lactam of flecainide exhibited qualitatively similar but much weaker electrophysiological actions.

## 1.2 Procainamide, Encainide and Propafenone

Lastly, there are examples of antiarrhythmics agents with which metabolites may be very important for clinical efficacy or toxicity during therapy. Procainamide is metabolized to the highly cardioactive compound N-acetyl procainamide (NAPA) (Drayer et al., 1977). NAPA was effective against chronic high frequency ventricular ectopic depolarizations in a subset of patients resistant to procainamide (Roden et al., 1980). Atkinson et al. (1983) also showed that NAPA effectively suppressed ventricular ectopy during long-term treatment. The pharmacological actions of the metabolites of encainide: O-demethyl encainide (ODE) and 3-methoxy-O-demethyl encainide (3MODE) (Kates et al., 1982), have also been evaluated directly in clinical trials. Both ODE and 3MODE suppressed chronic ventricular arrhythmias in patients (Barbey et al., 1988a).

The metabolism of procainamide to NAPA results in the loss of the Class I actions produced by procainamide while the Class III effects (action potential prolongation) are preserved (Singh et al., 1988). In canine Purkinje fibers and muscle, NAPA had no effects on $\dot{V}_{max}$ and action potential amplitude but lengthened the action potential duration and effective refractory period (Bagwell et al., 1976; Dangman and Hoffman, 1981). Consistent with the *in vitro* finding that NAPA does not depress $\dot{V}_{max}$, the QRS interval was not affected, while atrial and ventricular refractory periods and the $QT_c$ interval were increased *in vivo* in anesthetized dogs (Amlie et al., 1978; Jaillon and Winkle, 1979; Boucher et al., 1987).

The antiarrhythmic efficacy of NAPA is also demonstrated in animal arrhythmia models. NAPA provided protection against ventricular arrhythmias in mice (Drayer et al., 1974; Elson et al., 1975), dogs (Bagwell et al., 1976; Reynolds and Kamath, 1979) and rabbits (Minchin et al., 1978), and atrial arrhythmias in dogs (Drayer et al., 1974). The metabolism of procainamide to NAPA produces an effective antiarrhythmic agent with only Class III electrophysiological effects and a different clinical antiarrhythmic spectra compared to procainamide (Roden et al., 1980).

Several studies suggest that the metabolites of encainide may be responsible for the clinical efficacy or toxicity of encainide in most patients (Roden et al., 1982; Duff et al., 1983a; Barbey et al., 1988a). The electrophysiologic actions of ODE and 3MODE are qualitatively similar to those produced by encainide. ODE was more potent than encainide in depressing $\dot{V}_{max}$ (Elharrar and Zipes, 1982), slowing conduction velocity (Dresel, 1984; Davy et al., 1986) and suppressing experimental animal arrhythmias (Roden et al., 1982; Kerr et al., 1985). 3MODE was either of similar or greater potency than encainide in the same studies. In patients, during long term oral therapy with encainide, the levels of the metabolites greatly exceed that of the parent drug (Kates et al., 1982). In addition, arrhythmia suppression in patients correlate better with concentrations of ODE than encainide (Carey et al., 1981). Thus, the available data suggest that ODE and 3MODE are of clinical importance. The efficacy or toxicity of encainide may be due to or greatly modulated by its metabolites.

Finally, propafenone undergoes extensive metabolism (Siddoway et al., 1984) to produce two metabolites found in the plasma of patients during chronic therapy: 5-hydroxy- (5-OH-) propafenone and N-depropyl- (N-DP-) propafenone (Kates et al., 1985). The plasma ratio of propafenone : 5-OH-propafenone : N-DP-propafenone is approximately 10:2:2. However, Latini et al. (1987) showed that the myocardium/plasma partition ratio for 5-OH-propafenone is five time greater than for propafenone. Thus this metabolite is concentrated in the heart to a similar extent as the parent drug.

Like the parent drug, the primary electrophysiological effect of the metabolites *in vitro* is a rate-dependent depression of $\dot{V}_{max}$ (Thompson et al., 1988; Malfatto et al., 1988; Rouet et al., 1989). Both metabolites suppressed experimentally-induced ventricular arrhythmias *in vivo* in rats and dogs (Philipsborn et al., 1984; Malfatto et al., 1988; Oti-Amoako et al., 1990). In both *in vitro* and *in vivo* experimental models, 5-OH-propafenone has a similar potency compared to the parent drug while that of the N-DP metabolite is much smaller than propafenone. In addition to Class I activity, propafenone also displays Class II (beta-adrenoceptor blocking) and Class IV ($Ca^{2+}$ antagonist) antiarrhythmic actions at higher concentrations (Ledda et al., 1981; Dukes and Vaughan Williams, 1984). 5-OH-propafenone also displays beta-adrenoceptor blocking and $Ca^{2+}$ antagonist effects at higher concentrations (Philipsborn et al., 1984; Delgado et al., 1987). Thus the metabolites of propafenone also exhibit Class I, II and IV

antiarrhythmic actions and may be responsible for some of the antiarrhythmic or arrhythmogenic effects previously ascribed to propafenone.

### 1.3 Disopyramide

The Class Ia agent disopyramide is primarily metabolized in man by hepatic N-dealkylation to mono-N-dealkyldisopyramide (MND) (Karim, 1975). MND decreased the maximum atrial driving frequency suggesting that it lengthened refractory periods *in vitro* (Grant et al., 1978) and *in vivo* (Dubray et al., 1986), and prevented atrial arrhythmias in a canine model (Baines et al., 1976). However, more precise definition of the pharmacological activities of this metabolite have not been previously undertaken.

## 2. Class I Antiarrhythmic Drug Interactions

The electrophysiological interactions which occur between Class I antiarrhythmic drugs and cardiac cells or other Class I agents have been previously investigated in experimental and clinical studies. Some theoretical principles useful in understanding and predicting the outcome of these interactions have been presented by Hondeghem (1987). In this section, we review these principles, and experimental and clinical investigations within the framework of antiarrhythmic drug interactions.

## 2.1 Theoretical Considerations

### 2.1.1 *Therapeutic Synergism*

Class I antiarrhythmic drugs have characteristic time constants of recovery from depression of $\dot{V}_{max}$ or reduction of the sodium current (Courtney, 1980 and 1987). These time constants range from very fast for Class Ib agents (hundreds of msec) to very slow for Class Ic agents (tens of sec). Hondeghem and Katzung (1980) predicted that the combination of drugs with different kinetics of interaction with the sodium channel could provide a diastolic block that was not attainable by either agent alone. The combination of a slow with a fast drug could provide a more effective depression of early extrasystoles or tachycardias, while depression of impulses at normal heart rates would not be much greater than that produced by the slower agent alone. In the presence of the combination, both drugs would contribute to the block of sodium channels at the beginning of diastole. Block produced by the fast drug, however, would subsequently recover during diastole. Also, since such drug combinations may increase the magnitude of depression, lower concentrations of the individual agents would be required for antiarrhythmic efficacy thereby reducing the likelihood of potential adverse side effects. The combination of two drugs with very similar recovery kinetics, however, would not be of any great benefit beyond that of an increase in the total drug concentration. In fact, in the case of two slow agents, excessive depression of normal beats may

lead to drug toxicity. These concepts have provided a rational basis for the use of antiarrhythmic drug combinations to produce therapeutic synergy.

### 2.1.2 *Competitive Displacement Interactions*

Class I antiarrhythmic drugs have been postulated to bind to a specific receptor site on or very close to the transmembrane sodium channel (Hondeghem and Katzung, 1984). If the interaction of agents with the sodium channel is specific, then competition between two drugs should be possible at certain stimulation rates. The addition of a drug with a shorter time constant of recovery to a drug with a longer time constant could reduce the block of sodium channels and increase the sodium current through competitive displacement interactions. The slower agent must be present at a high enough concentration to block a significant fraction of sodium channels per action potential and a have a time contant short enough to result in significant unblocking during diastole. Under these conditions, addition of a faster drug may displace the slower agent from sodium channel receptors, recover more quickly and reduce the magnitude of block during diastole. Such interactions would argue against a nonspecific membrane disturbance model in which addition of a second drug would always increase the block of sodium channels.

If the recovery time constant of the slower agent is too long, however, then the fraction of blocked channels which could be competitively displaced with each action potential would be very small. In this situation, block would not be reduced.

State-dependent binding of the individual drugs to the sodium channel can also be an important consideration with these competitive interactions. Clinically useful Class I drugs have a low affinity for sodium channels in the resting state and a high affinity for those in the activated and/or inactivated states (Hondeghem and Katzung, 1984). Consequently, block develops with each action potential and recovery from block occurs during diastole. Since the activated state occurs before the inactivated state, open channel blockers will sequester channels first and have an advantage over inactivated channel blockers. Similarly, for two drugs which have a large affinity for the same channel state, the faster blocker will have an advantage over the slower one.

### 2.1.3 *Action Potential Duration*

The effects of Class I antiarrhythmic drugs on action potential duration may also have an influence on their effectiveness in blocking sodium channels. Drugs which preferentially block open channels will not be affected by changes in action potential duration. However, since changes in repolarization alters the amount of time the sodium channel remains in the inactivated state, the action of inactivation blockers may be modified by drug effects on action potential repolarization.

## 2.2 Experimental Investigations

### 2.2.1 $\dot{V}_{max}$

The mechanism by which Class I antiarrhythmic drug combinations produce therapeutic synergy has been demonstrated *in vitro*. Hondeghem and Katzung (1980), using $\dot{V}_{max}$ as an index of sodium channel block, demonstrated that the combination of a faster and a slower agent (lidocaine and quinidine, respectively) produced a large depression of early extrasystoles while smaller effects were observed on impulses at a normal steady-state rate of 1 Hz. Other combinations of kinetically different drugs investigated *in vitro* include: quinidine plus tocainide (Valois and Sasyniuk, 1987) or mexiletine (Roden et al., 1987), and propafenone plus mexiletine (Valenzuela et al., 1989). All these studies demonstrate that such drug combinations can produce a more effective depression of early premature impulses or those elicited at rapid heart rates compared to effects with single agents, while depression of $\dot{V}_{max}$ at normal heart rates is smaller.

Frequency dependent interactions have also been observed on steady-state conduction *in vivo* in the presence of the combinations of mexiletine plus quinidine (Bajaj et al., 1987) or lidocaine plus quinidine or flecainide (Pallandi and Campbell, 1988). Myocardial conduction with the combinations at normal heart rates were similar to effects with the slower agents alone, but very rapid pacing further slowed conduction. Enhanced antiarrhythmic efficacy has also been demonstrated experimentally with drug combinations *in vivo* in dogs

following ischemic injury. The combination of quinidine and mexiletine was significantly more effective than monotherapy against induction of ventricular tachycardia (Duff, 1989).

Although the combination of drugs with different kinetics of interaction with the sodium channel can produce a therapeutic synergy (eg. selective depression of premature beats or tachycardias and/or reduced drug toxicity), the pharmacological interactions between these agents when present in low to moderate concentrations generally produced additive effects. That is, when the cycle length dependencies of individual drug actions are considered, the effects of the combinations on depression of $\dot{V}_{max}$ *in vitro* or slowing of conduction *in vivo* can be accounted for by the sum of the individual drug effects (Fransen et al., 1984; Kohlhardt and Seifert, 1985; Valois and Sasyniuk, 1987; Roden et al., 1987; Bajaj et al., 1987).

Kohlhardt and Seifert (1985) investigated the interaction between the kinetically similar drugs quinidine and propafenone *in vitro*. The combination of these two agents produced a rate independent increase in the magnitude of both tonic and use-dependent $\dot{V}_{max}$ depression. The effects produced with this combination resembled effects expected from a rise in the concentration of either drug.

Pharmacological synergism was reported with the combination of quinidine plus mexiletine on $\dot{V}_{max}$ (Burke et al., 1986) and ventricular refractory period (Duff et al., 1986). These conclusions were based on the observation that the addition of a low ineffective concentrations of mexiletine increased the depression of $\dot{V}_{max}$ or prolongation of

ventricular refractory period produced by quinidine alone. However, these results are limited in that drug action was examined only at a fairly slow stimulation rate which did not take into account the cycle length dependencies of the individual agents (Burke et al., 1986), nor that with the combination there is an increase in total drug concentration. Synergism did not occur at higher drug concentrations in both studies.

Competitive displacement interactions with sodium channel blockers have also been demonstrated with various drug combinations. The addition of lidocaine to bupivacaine (Clarkson and Hondeghem, 1985a) or glycylxylidide (GX) (Bennett et al., 1988) has been shown to reduce the magnitude of sodium channel block and therefore increase steady-state $\dot{V}_{max}$ or the sodium current. Competitive interactions have also been demonstrated through a decrease in the magnitude of the drug-induced slow component of $\dot{V}_{max}$ recovery in the presence of the combination compared to effects with single agents. For example, the magnitude of the slow component of reactivation induced by quinidine alone was significantly diminished when lidocaine was also present (Clarkson and Hondeghem, 1985a; Sanchez-Chapula 1985). Similar results were observed with mixtures of mexiletine plus quinidine or ropitoin (Valenzuela and Sanchez-Chapula, 1989). All these interactions, however, required high drug concentrations in order that each drug bind a large fraction of sodium channels and interfere with each other's binding. At lower concentrations, mixing two agents would result in more block than

produced by either drug alone since the probability that two drugs interact simultaneously with the same receptor would be relatively smaller.

Effects with the combination of lidocaine and bupivacaine may be explained by the competitive displacement interactions described above. Bupivacaine binds avidly to inactivated sodium channels but has a relatively low affinity for resting and activated channels (Clarkson and Hondeghem, 1985b). Lidocaine binds to both activated and inactivated channels (Hondeghem and Katzung, 1984; Clarkson et al., 1988) and could therefore sequester a large fraction of channels before block by bupivacaine can become appreciable. Subsequently, faster recovery from block produced by lidocaine (Clarkson and Hondeghem, 1985a) in the presence of the combination would increase the sodium current during diastole compared to effects with bupivacaine alone. Similar events can explain effects with the combination of lidocaine and GX. In addition, the interaction between lidocaine and GX can be modulated by membrane voltage. GX-blocked channels recovered faster and more completely than lidocaine-block channels at normal membrane potentials, while the reverse was true at more negative membrane potentials (Bennett et al., 1988). Therefore, addition of GX to lidocaine can increase the sodium current only at normal potentials, while addition of lidocaine to GX can increase the sodium current only at more hyperpolarized potentials.

Sanchez-Chapula (1985), but not Clarkson and Hondeghem (1985a), found an increased steady-state $\dot{V}_{max}$ in the presence of the combination of lidocaine plus quinidine compared to effects produced by quinidine

alone. Quinidine binds avidly to activated channels (Weld at al., 1982; Kodama et al., 1986 and 1987; Hondeghem and Matsubara, 1988) and dissociated very slowly. Since there would be little cycling of occupied channels between quinidine-bound and drug-free states at normal heart rates, it would be more difficult for lidocaine to displace quinidine than bupivacaine or GX. Nevertheless, both studies did find a decrease in the magnitude of the slow component of $\dot{V}_{max}$ recovery due to quinidine with the combination suggesting that lidocaine interfered with its binding. Competitive drug interactions and displacement support the concept of a common receptor site associated with sodium channel block by these agents.

### 2.2.2 *Action Potential Duration*

The effects of the combinations of quinidine plus tocainide (Valois and Sasyniuk, 1987) or mexiletine (Roden et al., 1987) on action potential duration have been investigated *in vitro*. Quinidine alone produced a prolongation of the action potential while both tocainide and mexiletine markedly shortened repolarization in Purkinje fibers. The combination of quinidine with either Ib agent produced changes in action potential duration reflecting the sum of the opposing actions of the individual drugs. There was either a net change of zero or only a slight shortening of action potential duration. Similar results were observed with the combination of quinidine and mexiletine *in vivo* in canine myocardium (Costard-Jaeckle et al., 1989). Mexiletine attenuated the prolongation of repolarization produced by quinidine.

At higher drug concentrations, however, Burke et al. (1986) found that the combination of quinidine and mexiletine *in vitro* did not produce simple additive effects. In this study, both quinidine and mexiletine singly shortened action potential duration in canine Purkinje fibers. Mexiletine produced a greater shortening than quinidine, but the combination did not abbreviate repolarization beyond that observed with quinidine alone. Net effects on action potential duration may have resulted from changes in several membrane currents during repolarization. More insight into these results will require further investigation of the currents (including tissue specificity) and drug effects during repolarization as well as the frequency and concentration dependence of such interactions.

## 2.3 Clinical Investigations

The benefits of combining two Class I agents with different kinetics of interaction with the sodium channel have been demonstrated in clinical studies. In this setting, the combination of antiarrhythmic drugs have been used when single agent therapy was ineffective, only partially effective or poorly tolerated (due to adverse side effects). Duff et al. (1983b) showed that the combination of well-tolerated doses of the Class Ia drug quinidine with the Ib agent mexiletine markedly suppressed ventricular tachycardias compared to single agent therapy with either drug alone. Since administration of the two drugs in combination produced a greater depression of early extrasystoles, lower doses of the individual drugs were used which resulted in fewer side

effects. Similar findings with this drug combination have been observed by Greenspan et al. (1985), Duff et al. (1987) and Whitford et al. (1988). The coadministration of other drug combinations of kinetically different agents have also produced enhanced antiarrhythmic responses. The combinations of mexiletine plus procainamide (Greenspan et al., 1985) or disopyramide (Kim et al., 1989), and tocainide plus quinidine (Kim et al., 1987; Barbey et al., 1988b) significantly increased suppression of premature ventricular complexes and/or ventricular tachycardias compared to effects produced with monotherapy.

The combinations of two kinetically slow agents have also been shown to produce enhanced antiarrhythmic responses. Coadministration of procainamide with quinidine (Kim et al., 1985), and propafenone with procainamide or quinidine (Klein et al., 1987) increased the suppression of premature ventricular complexes and/or ventricular tachycardias compared to effects with monotherapy. However, Duffy et al. (1983) did not find any additional benefits with the coadministration of procainamide with quinidine or disopyramide against ventricular tachycardias. The recovery time constants of $\dot{V}_{max}$ depression with propafenone and quinidine *in vitro* are similar (Grant et al., 1982; Weld et al., 1982; Kohlhardt and Seifert, 1983), while that with procainamide is slightly shorter (Courtney, 1980; Ehring et al., 1988) and that with disopyramide is longer (Campbell, 1983a; Flemming and Sasyniuk, 1989; Gruber and Carmeliet, 1989). The recovery kinetics of all these agents, however, are so slow that there is minimal recovery of $\dot{V}_{max}$ during clinical diastole. With these antiarrhythmic drugs, although enhanced

antiarrhythmic efficacy may have resulted from effects similar to that of an increase in drug concentration, their combinations would not be useful in discriminating between premature and normal impulses.

Clinical studies of the combination of quinidine plus mexiletine (Duff et al., 1983b) or tocainide (Barbey et al., 1988b) on action potential duration produced similar effects to results obtained *in vitro*. Mexiletine or tocainide in combination with quinidine limited the $QT_c$ prolongation produced by quindine alone. With these combinations, the effectiveness of quinidine is probably not reduced following shortening of the action potential because quinidine is predominantly an open channel blocker (Weld et al., 1982; Kodama et al., 1986 and 1987; Hondeghem and Matsubara, 1988). In fact, shortening of the $QT_c$ interval with the combination may even be beneficial against long QT syndrome arrhythmias with quinidine (Bauman et al., 1984; Roden et al., 1986a and 1986b). On the other hand, prolongation of $QT_c$ interval with the combination relative to effects with mexiletine alone may increase the effectiveness of mexiletine since it has a greater affinity for inactivated sodium channels (Kodama et al., 1986 and 1987).

Finally, the competitive displacement interactions observed between GX and lidocaine *in vitro* (Bennett et al., 1988) may explain why some patients become refractory to lidocaine antiarrhythmic therapy after initial success (Lie et al., 1974; Chopra et al., 1971). In the study of Bennett et al. (1988), this phenomena was observed in two patients who initally responded to treatment with lidocaine for recurrent ventricular tachycardias. Failure of lidocaine therapy was accompanied

by the appearance of GX in the plasma while plasma lidocaine concentrations were similar throughout. The competitive interactions demonstrated *in vitro* with these compounds raise the possibility that similar interactions *in vivo* may be responsible for the loss of responsiveness to lidocaine.

## 2.4 Interactions with Metabolites

A special case of drug combinations in which interactions must be considered are those between antiarrhythmic drugs and their active metabolites. However, very few studies have investigated such interactions directly. The presence of a metabolite might beneficially increase the block of sodium channels and slowing of conduction produced by the parent drug. The metabolites of both quinidine and amiodarone have been shown to produce additive antiarrhythmic effects in combination with their parent drugs against experimentally-induced ventricular arrhythmias (Vozeh et al., 1987; Nattel et al., 1988). Conversely, the presence of a metabolite with faster kinetics of recovery from block might competitively antagonize the parent drug under appropriate conditions. Competitive displacement of lidocaine by its metabolite GX has been demonstrated *in vitro* (Bennett et al., 1988).

## 2.5 Proarrhythmic Antiarrhythmic Drug Interactions

### 2.5.1 *Ventricular Tachycardia and Slow Conduction*

Although the aim of antiarrhythmic drug therapy is suppression or control of life-threatening cardiac arrhythmias, paradoxical provocation or aggravation of arrhythmias can be a side effect of these agents (Bigger and Sahar, 1987; Roden, 1990). One characteristic type of proarrhythmic response is clinically worsening ventricular tachycardia attended by widening of the QRS complex. Therapy with Class Ic antiarrhythmic agents such as flecainide and encainide have been most commonly implicated with such events (Roden, 1990). This subclass of sodium channel blockers have characteristic long time constants of recovery (Campbell, 1983b) which do not allow relief of block even at long clinical diastolic intervals. Therefore, marked slowing of conduction at normal heart rates is a manifestion of therapy with these agents. Reentry caused by slow conduction with minimal prolongation of the refractory period may be the underlying mechanism. Recent data in a subset of patients have provided further evidence for the proarrhythmic potential of these drugs (Supression Trial (CAST) Investigators, 1989).

### 2.5.2 *Torsade de Pointes and Triggered Arrhythmias*

#### 2.5.2.1 *Torsade de Pointes Arrhythmias in Patients*

A second characteristic proarrhythmic response to antiarrhythmic agents is torsade de pointes multiform ventricular tachycardias accompanied by prolongation of the QT interval (Bigger and Sahar, 1987; Roden, 1990). The term torsade de pointes is used to describe

polymorphic ventricular tachycardia in the setting of marked QT interval prolongation (long QT syndrome) (Tzivoni et al., 1983). Torsade de pointes almost invariably occurs following a pause or abrupt deceleration in the ventricular rhythm and the first tachycardia complex is late coupled to the preceding normal complex, emerging from a large postpause U wave (Jackman et al., 1988).

The etiology of torsade de pointes is frequently associated with the administration of action potential prolonging antiarrhythmic drugs amongst which are the Class Ia agents, sotalol and NAPA (Stratmann and Kennedy, 1987; Jackman et al., 1988; Roden, 1990). In particular, the Class Ia drug quinidine is most often cited as the culprit. Furthermore, with the Class Ia agents, proarrhythmic events usually occur within the normal range of therapeutic drug concentrations. Predisposing factors for torsade de pointes arrhythmias include electrolyte abnormalities (hypokalemia and hypomagnesemia) and bradycardia (Smith and Gallagher, 1980; Kay et al., 1983; Roden et al., 1986a and 1986b). Conversely, removing the drug, correcting the electrolyte abnormality or pacing is effective in preventing the recurrence of torsade de pointes.

#### 2.5.2.2 *Early Afterdepolarizations and Triggered Activity In Vitro*

Similarities between events *in vitro* and *in vivo* have led to the hypothesis that the mechanism for induction of torsade de pointes may be triggered activity due to early afterdepolarizations (Brachmann et al., 1983; Sasyniuk et al., 1989). Early afterdepolarizations are

depolarizing afterpotentials which interupt or delay normal repolarization of the cardiac action potential and can induce triggered activity (Cranefield, 1975; Damiano and Rosen, 1984). A triggered action potential is a second nondriven upstroke induced by an early afterdepolarization.

The characteristics of drug-induced early afterdepolarizations and triggered activity *in vitro* have been recently reviewed (Wit and Rosen, 1986; Cranefield and Aronson, 1988; Sasyniuk et al., 1989). Consistent with clinical findings, triggered activity due to early afterdepolarizations: 1) requires marked drug-induced prolongation of action potential duration in association with bradycardia, 2) is facilitated by reduced external potassium concentrations and 3) is abolished or prevented by the various intervention that are effective clinically. Early afterdepolarizations have been induced in isolated cardiac tissue by numerous conditions or drugs that either increase inward depolarizing currents or reduce outward repolarizing currents to prolong action potential duration (Cranefield and Aronson, 1988). Antiarrhythmic agents associated with torsade de pointes arrhythmias in patients can induce early afterdepolarizations *in vitro* and include quinidine (Roden and Hoffman, 1985; Valois and Sasyniuk, 1987; Roden et al., 1987; Nattel and Quantz, 1988; Davidenko et al., 1989), sotalol (Lathrop and Varro, 1989) and NAPA (Dangman and Hoffman, 1981). Disopyramide has also been associated with torsade de pointes

arrhythmias in patients (Stratmann and Kennedy, 1987; Jackman et al., 1988), however, its arrhythmogenic potential has not been previously investigated *in vitro*.

In addition, several issues still remain to be resolved before the precise role of early afterdepolarizations and triggered activity in drug-induced torsade de pointes can be established. Both the heart rate for induction and intrinsic frequency of sustained triggered activity *in vitro* are generally much slower than the physiological range of correlate events in the long QT syndrome in patients (Sasyniuk et al., 1989).

## 3. Objectives

The major metabolite of disopyramide in man, MND, occurs in significant plasma concentrations during disopyramide therapy (Karim, 1975). Electrophysiological studies of MND have demonstrated that it is an active compound in cardiac muscle. MND decreased the maximum atrial driving frequency both *in vitro* (Grant et al., 1978) and *in vivo* (Dubray et al., 1986) suggesting that it prolonged refractory periods. The parent compound, disopyramide, produces its antiarrhythmic effect by binding to a specific site within the sodium channel (Sheldon et al., 1987). MND may also have sodium channel blocking properties and depending on its kinetics of interaction may produce additive or antagonistic effects to disopyramide. Also, the effects of MND on action potential duration may be similar or opposite to disopyramide depending on its effects on other ion channels. Furthermore, the effects of the combination of disopyramide and MND when both are present simultaneously in the myocardium will be of clinical relevance and must be investigated directly.

Torsade de pointes arrhythmias in patients have been reported during disopyramide therapy (Stratmann and Kennedy, 1987; Jackman et al., 1988). Although disopyramide produces variable effects on action potential duration, it can prolong repolarization which is accentuated in the presence of external hypokalemia (Kus and Sasyniuk, 1978; Winslow et al., 1986). Similar effects have been observed with the Class Ia drug quinidine (Stratmann and Kennedy, 1987; Jackman et al., 1988). In

addition, quinidine induces early afterdepolarizations and triggered activity *in vitro* which have been implicated in the initiation of torsade de pointes *in vivo* (Sasyniuk et al., 1989). Similar effects by disopyramide and quinidine suggest that disopyramide may also have such an arrhythmogenic potential *in vitro*.

We therefore assessed: 1) the effects of MND singly on $\dot{V}_{max}$, action potential duration and effective refractory period of the propagated transmembrane action potential in canine ventricular tissue superfused *in vitro*; 2) the interaction between MND and disopyramide on these characteristics; 3) the ability of disopyramide to induce early afterdepolarizations and triggered activity in Purkinje fibers; and 4) the effects of MND on such triggered rhythms.

Chapter 2

# FREQUENCY AND VOLTAGE DEPENDENT EFFECTS OF MONO-N-DEALKYLDISOPYRAMIDE, THE MAJOR METABOLITE OF DISOPYRAMIDE, IN CANINE VENTRICULAR TISSUE

(In press in the Journal of Pharmacology and Experimental Therapeutics.)

## 1. Abstract

Mono-N-dealkyldisopyramide (MND), the major metabolite of disopyramide, reaches significant concentrations in patients; however, the contribution of MND to the antiarrhythmic or toxic effects of disopyramide is not known. We assessed the kinetics and magnitude of interaction of MND with the sodium channel in canine ventricular tissue superfused *in vitro* using $\dot{V}_{max}$ as an index of sodium channel block. At a basic cycle length of 1000 msec, MND (4 - 32 ug/ml) produced a concentration dependent depression of both $\dot{V}_{max}$ and amplitude of the action potential and accelerated all phases of repolarization in Purkinje fibers. To assess rate-dependent block, Purkinje fibers were stimulated with pulse trains at interstimulus intervals of 400 to 2000 msec. MND produced a concentration and rate dependent increase in the magnitude of rate-dependent block. There was also a concentration dependent increase in the kinetics of onset of block (decrease in rate constant). The rate constant increased with faster stimulation rates. Minimal tonic block occurred at clinically relevant concentrations. Recovery from rate-dependent block followed a single exponential time course with time constants of 5.23 ± 0.90 and 4.88 ± 0.94 sec for $\dot{V}_{max}$ and activation time, respectively. There was no shift of the normalized $\dot{V}_{max}$-membrane potential relationship except at the highest concentration, 32 ug/ml.

At cycle lengths of 250 to 1000 msec MND, 4 ug/ml, shortened all phases of repolarization in Purkinje fibers, the greatest shortening occurring at the longest cycle length. Prolongation of effective refractory period occurred only at rapid heart rates. Both action potential duration and effective refractory period were prolonged in ventricular muscle which was independent of rate. Differential effects on action potential duration in Purkinje fibers and ventricular muscle decreased the disparity in durations between them.

The results suggest that MND, by depressing $\dot{V}_{max}$ and conduction velocity and prolonging effective refractory period may have antiarrhythmic properties of its own. Therefore it may contribute to either the antiarrhythmic or toxic actions of disopyramide. Opposite effects to that of disopyramide on action potential duration in Purkinje fibers may be beneficial in preventing bradycardia dependent prolonged QT syndrome arrhythmias.

## 2. Introduction

In man, disopyramide is metabolized primarily by hepatic N-dealkylation to MND and both compounds are excreted in the urine (Ranney et al., 1971; Karim et al., 1972; Karim, 1975). As much as 25 to 50 % of an administered dose can be found in the urine as MND; yet, its contribution to the antiarrhythmic and toxic effects of disopyramide is not known. Recent studies suggest that in patients with normal renal function on chronic oral therapy, MND reaches a concentration of approximately 30 % of that of the parent compound (Aitio, 1981). Since the average protein binding of disopyramide is approximately 80 % while that of MND is only 22 to 35 %, the free drug fraction of both compounds would be similar in patients with normal renal function. In patients with depressed renal function, the free drug concentration of MND would be even higher. In patients on simultaneous therapy with microsomal enzyme inducing drugs the mean levels of MND are frequently higher than those of disopyramide (Aitio, 1981). Furthermore, there is pronounced interindividual variability with plasma MND:disopyramide ratios as high as 4 in patient populations (Bredesen et al., 1982).

Electrophysiological studies of MND have demonstrated that it is an active compound in cardiac muscle. The metabolite was effective in reversing experimentally-induced atrial fibrillation in dogs (Baines et al., 1976). In vitro, Grant et al. (1978) demonstrated that MND decreased the maximum driving frequency of guinea pig atria which

suggested that MND prolongs refractory periods. Dubray et al. (1986) also demonstrated *in vivo* that levels of MND which are achieved during therapy diminished the maximal pacing frequency of canine atrial muscle.

Disopyramide, like all Class I antiarrhythmic drugs, produces its antiarrhythmic effect by binding to a specific site within the sodium channel (Sheldon et al., 1987). MND may also have sodium channel blocking properties. Depending on its kinetics of interaction with the sodium channel, the electrophysiological actions of MND may be additive or antagonistic to that of the parent compound. Furthermore, the effects of MND on action potential duration may be similar or opposite to that of disopyramide depending on its effects on other ion channels.

Therefore, the present study was undertaken to assess the kinetics and magnitude of interaction of MND with the sodium channel using $\dot{V}_{max}$ as an index of sodium channel block and to determine its effects on action potential duration and effective refractory period. Some of these results have been published previously in abstract form (Toy and Sasyniuk, 1988).

## 3. Methods

### 3.1 *Experimental preparation*

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and their hearts were removed via a left thoracotomy. Both ventricles were thoroughly flushed with chilled,

oxygenated Tyrode's solution. Purkinje fibers with small pieces of attached ventricular muscle were quickly excised and pinned to a Sylgard block at the bottom of a tissue bath (4 ml capacity). Fibers were continuously perfused with modified Tyrode's solution aerated with 95 % $O_2$ - 5 % $CO_2$ at a rate of 10 ± 0.5 ml/min. Our standard Tyrode's solution contained (in mM): NaCl, 119.0; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHPO_4$, 0.9; dextrose, 5.5; and $NaHCO_3$, 25. pH of the solution was 7.33 ± 0.03. The temperature was maintained at 37.0 ± 0.2° C.

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

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

Transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl and coupled to the input of a high-impedance, capacitance-neutralized amplifier (Model KS-700, WPI instruments). The

output of the KS-700 was displayed on a Tektronix 5113 dual-beam storage oscilloscope and simultaneously displayed in digital form on a Data 6000 waveform analyzer (Data Precision, Inc.).

During control measurements and equilibration with drug, the preparations were stimulated at a basic cycle length of 1000 msec. Action potential variables were recorded on-line every 15 sec until steady-state effects were observed.

### 3.2 *Protocols*

Drug effects on action potential parameters as a function of steady-state changes in stimulation frequency was examined by varying the basic cycle length from 250 to 1000 msec. Sufficient time (3 min or more) was allowed at each basic cycle length to permit changes in action potential configuration to reach steady-state.

To determine effective refractory period, test pulses were introduced after a stimulus train of 20 beats had produced steady-state changes in action potential duration. Test pulses of twice the diastolic threshold voltage, determined under control conditions, were initially introduced at the end of the action potential and then progressively earlier into phase 3 by steps of 10 msec until a response was no longer elicited. The test pulse was then reintroduced from the last responding test interval to progressively earlier intervals by steps of 2 msec until the response was no longer elicited. The

effective refractory period was defined as the shortest $R_1$-$R_2$ interval in which the response to the test pulse propagated to a distal recording site.

To study the rate of development of $\dot{V}_{max}$ depression with drive, the preparations were driven by 44 beat trains at interstimulus intervals of 400, 600, 1000, and 2000 msec. Rest periods between trains of stimuli were long enough to ensure full recovery from rate-dependent block. Preparations were stimulated at a basic cycle length of 600 msec prior to each rest period in order to maintain membrane potential at similar levels prior to each action potential train.

To determine recovery from $\dot{V}_{max}$ depression, test pulses were introduced at varying diastolic intervals after a stimulus train had produced a steady-state level of $\dot{V}_{max}$ depression. The onset of diastole was defined as that time when repolarization had returned to the maximum diastolic potential. Test pulses were initially introduced during phase 3 of the action potential and then progressively later into diastole. The test intervals were initially increased in small increments and then progressively in larger steps with steps ranging from 50 to 5000 msec. Each recovery curve was comprised of a minimum of 30 points.

To study drug effect on the steady-state relationship between $\dot{V}_{max}$, activation time and membrane potential, preparations were stimulated at cycle lengths of 1 to 5 sec under control conditions and 10 or 15 sec with drug. Activation time was defined as the interval between the stimulus artifact and the maximum upstroke of the action potential. Purkinje fibers were exposed to progressively higher potassium

concentrations ranging from 2.7 to 12 mM until the preparations stopped responding. The preparations were then returned to a 2.7 mM potassium Tyrode's solution. During alterations of potassium concentration $\dot{V}_{max}$, activation time and membrane potential were recorded on-line every 5 sec.

$\dot{V}_{max}$ was used as an indirect method to evaluate $I_{Na}$. Although $\dot{V}_{max}$ is a monotonic but not a linear index of $I_{Na}$ (Sheets et al., 1988; Fozzard et al., 1989), $\dot{V}_{max}$ does reflect net maximal inward current during the action potential and can provide a convenient, albeit nonquantitative index of changes in $I_{Na}$.

It was necessary to expose preparations to MND for at least 25 min before steady-state effects on action potential characteristics were obtained. In the majority of experiments it was not possible to maintain cell impalements long enough to study the entire concentration range in one cell. Thus, onset of and recovery from block at different drug concentrations were studied in different experiments and the normalized data were pooled. Results from all other protocols under control and drug conditions were obtained from the same impalement.

### 3.3 *Drug*

MND used was generously supplied by Roussel Laboratories. The drug was added to Tyrode's solution made up from refrigerated stock solutions. Concentrations refer to that of the base.

3.4 *Data analysis*

All action potential variables were determined using a Data 6000 waveform analyzer as previously described (Sasyniuk and Jhamandas, 1984). Action potential variables measured were: take-off potential; maximum diastolic potential; action potential amplitude; $\dot{V}_{max}$; and APD at the 50, 75, and 95% levels of repolarization (APD 50%, APD 75%, and APD 95% respectively).

Where applicable, data are expressed as mean ± S.D.. Paired Student's t test was used to make statistical comparisons between control and drug effects on steady-state action potential characteristics and action potential duration. One- or two-way analysis of variance (anova) was used where appropriate to make comparisons between control and drug effects on the kinetics of onset and magnitude of rate-dependent block. $p<0.05$ was considered significant. The time course of onset or recovery from block was defined with the use of a least squares error nonlinear exponential fitting program (Hewlett-Packard Statistical Library). The exponential onset or recovery from $\dot{V}_{max}$ depression contained only one component. Least squares analysis of the data indicated that this component could be approximated by a single exponential function of the form $Y = Aexp(-t/\tau) + B$. No attempt was made to fit the data with more complex functions. However, when appropriate, it was determined whether a double exponential function fit the data better than a single exponential. A

double exponential was considered a better fit if the residual sum of squares for the double exponential was one-third or less than that for the single exponential (Clarkson and Hondeghem, 1985a).

## 4. Results

### 4.1 *Concentration dependent effects on action potential characteristics in Purkinje fibers*

Table 1 summarizes the concentration-response relationship of MND on action potential characteristics in Purkinje fibers stimulated at a basic cycle length of 1000 msec. MND did not alter the take-off potential but produced a concentration dependent depression of both $\dot{V}_{max}$ and action potential amplitude and shortened all phases of the action potential.

### 4.2 *Effects of MND on action potential duration and effective refractory period during steady-state changes in basic cycle length*

#### 4.2.1 *Purkinje fibers*

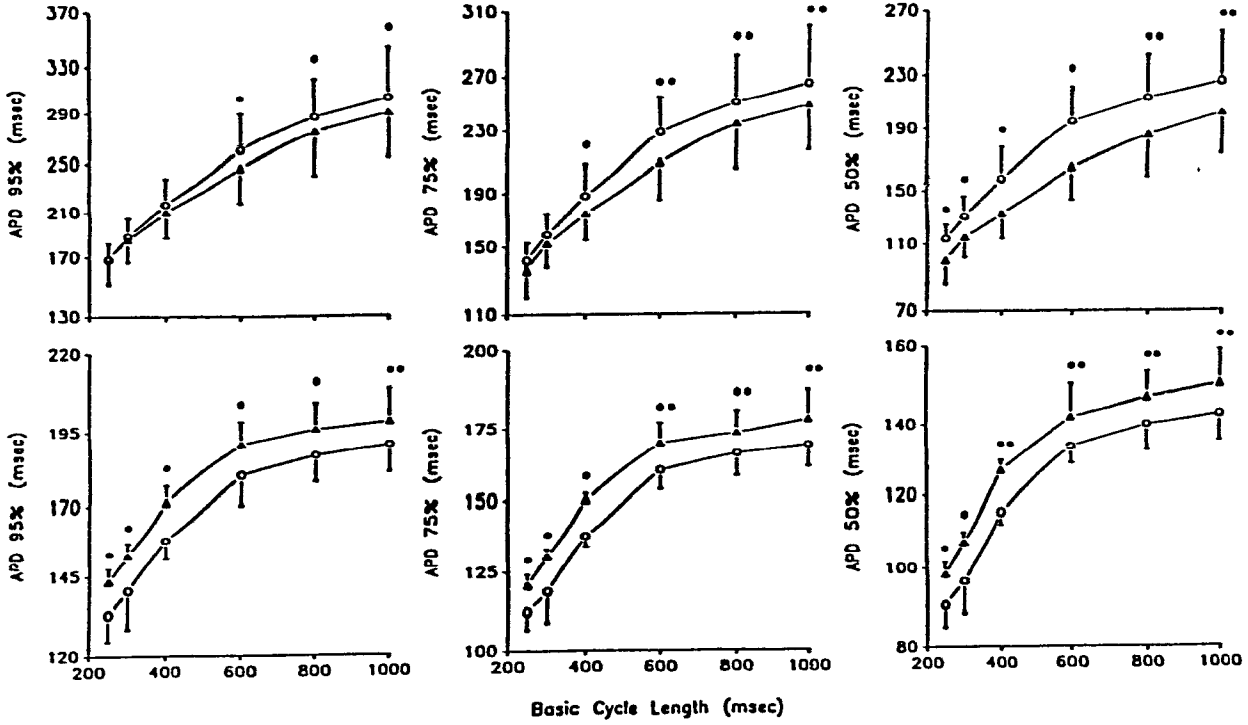
Figure 1 summarizes the effects of 4 ug/ml MND on action potential duration when basic cycle length was decreased from 1000 to 250 msec. MND shortened all phases of repolarization at all cycle lengths. Shortening of action potential duration was greatest at the longest basic cycle length and most pronounced at the level of the plateau (APD

TABLE 1. Concentration dependent effects of MND on action potential characteristics in canine Purkinje fibers

| | TOP[a] (mV) | AMP[b] (mV) | $\dot{V}_{max}$ (V/sec) | APD 50%[c] (msec) | APD 75%[c] (msec) | APD 95%[c] (msec) |
|---|---|---|---|---|---|---|
| Control | -88.9 ± 2.0 | 123.0 ± 4.1 | 588 ± 97 | 206 ± 28 | 245 ± 28 | 286 ± 28 |
| 4 µg/ml (n = 7) | -88.7 ± 1.4 | 121.2 ± 4.2* | 554 ± 90** | 182 ± 27*** | 228 ± 24** | 269 ± 22** |
| Control | -89.3 ± 1.1 | 121.9 ± 3.7 | 555 ± 108 | 225 ± 21 | 264 ± 26 | 307 ± 27 |
| 8 µg/ml (n = 6) | -88.3 ± 1.3 | 118.9 ± 3.2** | 507 ± 94** | 176 ± 23*** | 229 ± 25*** | 276 ± 29*** |
| Control | -89.5 ± 1.3 | 121.7 ± 2.7 | 557 ± 112 | 229 ± 20 | 269 ± 21 | 312 ± 19 |
| 16 µg/ml (n = 5) | -87.1 ± 1.7 | 114.8 ± 3.1*** | 488 ± 89** | 135 ± 25*** | 203 ± 21*** | 259 ± 24*** |
| Control | -87.9 ± 2.4 | 121.8 ± 4.1 | 556 ± 98 | 217 ± 36 | 257 ± 36 | 300 ± 38 |
| 32 µg/ml (n = 5) | -87.7 ± 1.1 | 110.0 ± 5.5*** | 430 ± 82*** | 102 ± 20** | 176 ± 21** | 239 ± 27** |

Values are expressed as mean ± SD. Fibers were stimulated at a BCL of 1000 msec.
[a]TOP = take-off potential; [b]AMP = amplitude; [c]APD 50%, APD 75%, APD 95% = action potential duration at 50, 75, and 95 % levels of repolarization.
*P<.05, **P<.01 and ***P<.001.

Figure 1 - Effects of 4 ug/ml MND on rate dependent shortening of action potential duration in Purkinje fibers and ventricular muscle. APD 50%, 75% and 95% are plotted against steady-state cycle lengths in Purkinje fibers (top) and ventricular muscle (bottom) under control conditions (circles) and following 4 ug/ml MND (triangles). Data points represent mean ± S.D. of 6 experiments in Purkinje fibers and 5 experiments in ventricular muscle. *Significantly different from control, $P<.05$; **$P<.01$.


APD 95% (msec)
APD 75% (msec)
APD 50% (msec)
Basic Cycle Length (msec)

50%). Despite shortening of action potential duration, effective refractory period was either unchanged or prolonged, particularly at shorter cycle lengths (Figure 2).

4.2.2 *Ventricular muscle*

MND produced opposite effects on repolarization in ventricular muscle. Action potential duration was significantly prolonged at all levels of repolarization and at all cycle lengths (Figure 1). This reduced the disparity in durations between muscle and Purkinje fibers (Figure 2). Unlike the effect in Purkinje fibers, absolute changes in action potential duration were similar at all cycle lengths. Effective refractory period was prolonged similarly at all cycle lengths which also reduced the disparity between muscle and Purkinje fibers even at normal rates (Figure 2).

4.3 *Effects on the kinetics of onset and magnitude of rate-dependent* $\dot{V}_{max}$ *depression*

Figure 3 illustrates a typical example of the rate-dependent effects of MND. Rate-dependent effects on $\dot{V}_{max}$ were minimal under control conditions and may be attributed to a small decline in the take-off potential during the stimulus train. In the presence of MND, $\dot{V}_{max}$ decreased with each beat following an exponential decline to a steady-state. With the exception of a small depolarization prior to the first beat in the stimulus train, there were minimal changes in take-off potential throughout the train with 4 ug/ml of drug. Higher concentrations produced a slightly greater depolarization.

Figure 2 - Cycle-length dependent effects of MND on action potential duration and effective refractory period (ERP) in Purkinje fibers and ventricular muscle. The left panels shows a plot of APD 95% (open symbols) and effective refractory period (solid symbols) against steady-state basic cycle length in Purkinje fibers (top panel) and ventricular muscle (bottom panel) under control (circles) and following 4 ug/ml MND (triangles). The right panels illustrate the cycle length dependent reduction of disparity in APD 95% (top panel) and effective refractory period (bottom panel) between Purkinje fibers (diamonds) and ventricular muscle (squares) both under control conditions (open symbols) and following 4 ug/ml MND (solid symbols). Data points represent the means of 3 experiments.

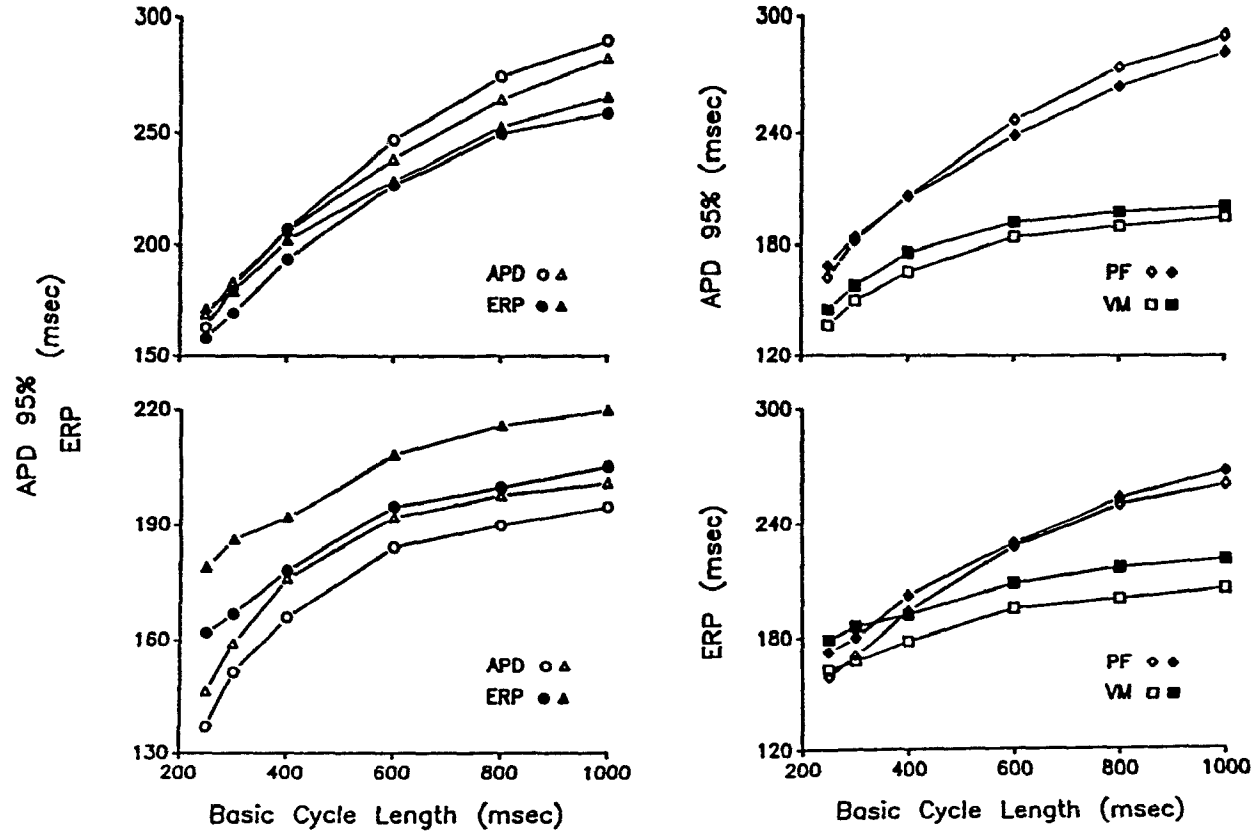
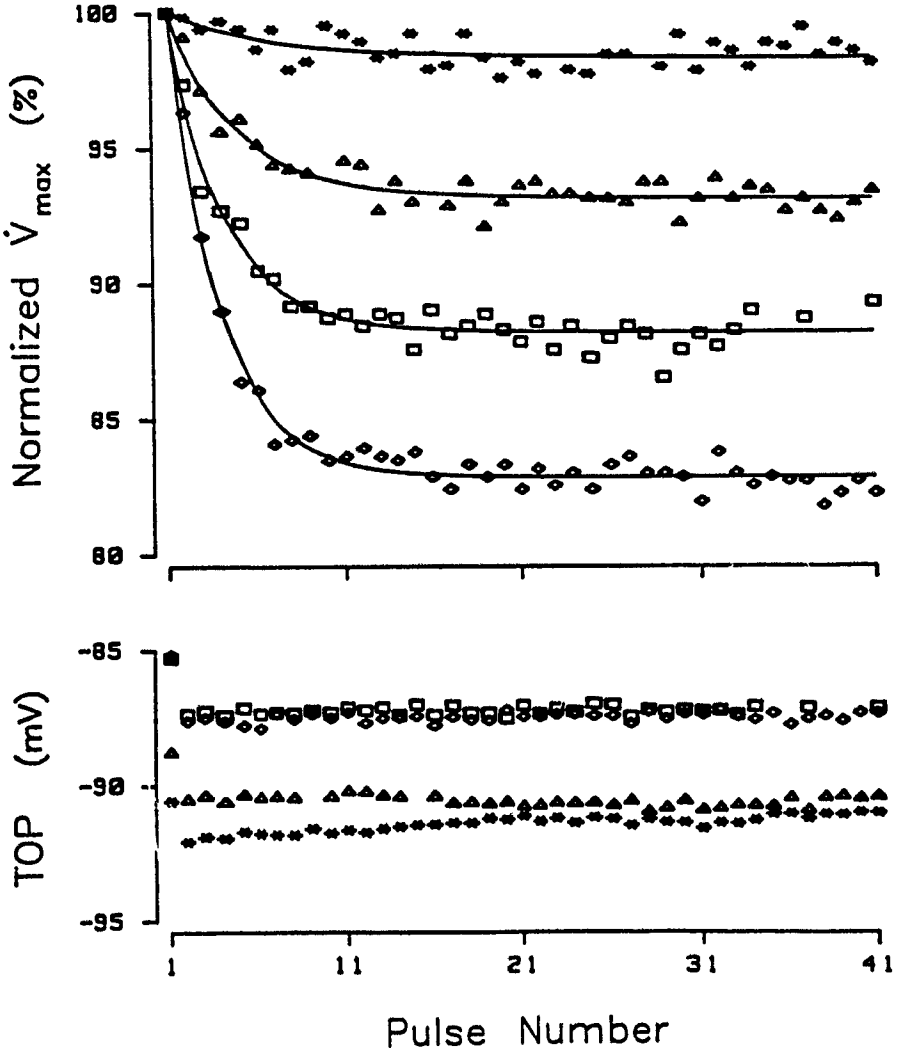

APD 95% (msec)
ERP
300
250
200
150
APD ○ △
ERP ● ▲
220
190
160
130
200
400
600
800
1000
Basic Cycle Length (msec)
APD 95% (msec)
300
240
180
120
PF ◇ ◆
VM □ ■
ERP (msec)
Basic Cycle Length (msec)

Figure 3 - Typical example of the concentration dependent effects of MND on the kinetics of onset and magnitude of rate-dependent block. $\dot{V}_{max}$ (normalized to the $\dot{V}_{max}$ of the first action potential of the stimulus train) and take-off potential (TOP) are plotted for each action potential during the stimulus train under control conditions (asterisks), and following 4 (triangles), 8 (squares), and 16 (diamonds) ug/ml of MND. Each stimulus train was preceded by a quiescent interval of 20 sec. Rate constants were 4.00, 3.05, and 2.76 beats and rate-dependent block was 6.8, 11.6, and 17.7 % following 4, 8, and 16 ug/ml of MND, respectively. Take-off potential of the first action potential of the train was depolarized relative to the steady-state take-off potential. Steady-state take-off potential was more depolarized at higher concentrations.


100
95
90
85
80
Normalized $\dot{V}_{max}$ (%)
-85
-90
-95
TOP (mV)
1
11
21
31
41
Pulse Number

MND produced a concentration dependent decrease in the rate constant (faster kinetics) and an increase in the magnitude of rate-dependent $\dot{V}_{max}$ depression. The results are summarized in Figure 4. Tonic block, which represents block of sodium channels that is independent of rate, was minimal in the presence of 4 - 16 ug/ml MND. A significant increase in tonic block only occurred after 32 ug/ml of drug. Thus, total $\dot{V}_{max}$ depression was due to rate dependent effects at clinically relevant concentrations. MND produced a small but significant concentration dependent depolarization during the quiescent interval preceding onset of the stimulus train.

The effects of MND on rate-dependent block were also dependent on the interstimulus interval of the action potential train. Effects of interstimulus interval on the kinetics of onset and magnitude of rate-dependent block produced by MND are summarized in Table 2. The rate constant and magnitude of rate-dependent block were greatest at the shortest interstimulus interval.

### 4.4 *Characteristics of recovery from rate-dependent block*

Figure 5 shows a typical example of the recovery of $\dot{V}_{max}$ and activation time from rate-dependent block in the presence of MND. Minimal recovery (5 %) occurred during the first two seconds of the diastolic interval. $\dot{V}_{max}$ recovered over a longer diastolic interval following a single exponential time course of 5.72 sec. The large depression of $\dot{V}_{max}$ early during diastole was consistently accompanied by a corresponding increase in activation time suggesting that depression

Figure 4 - Concentration-dependent changes in rate constant (upper panel), depression of $\dot{V}_{max}$ (middle panel) and take-off potential (TOP; bottom panel) produced by MND. Data points represent mean ± S.D. of 6 to 8 preparations. Interstimulus interval was 600 msec. Circles represent tonic block; squares, rate-dependent block and triangles, total block. Total block is the sum of tonic and rate-dependent block. There was a significant concentration dependent effect on all parameters (anova).

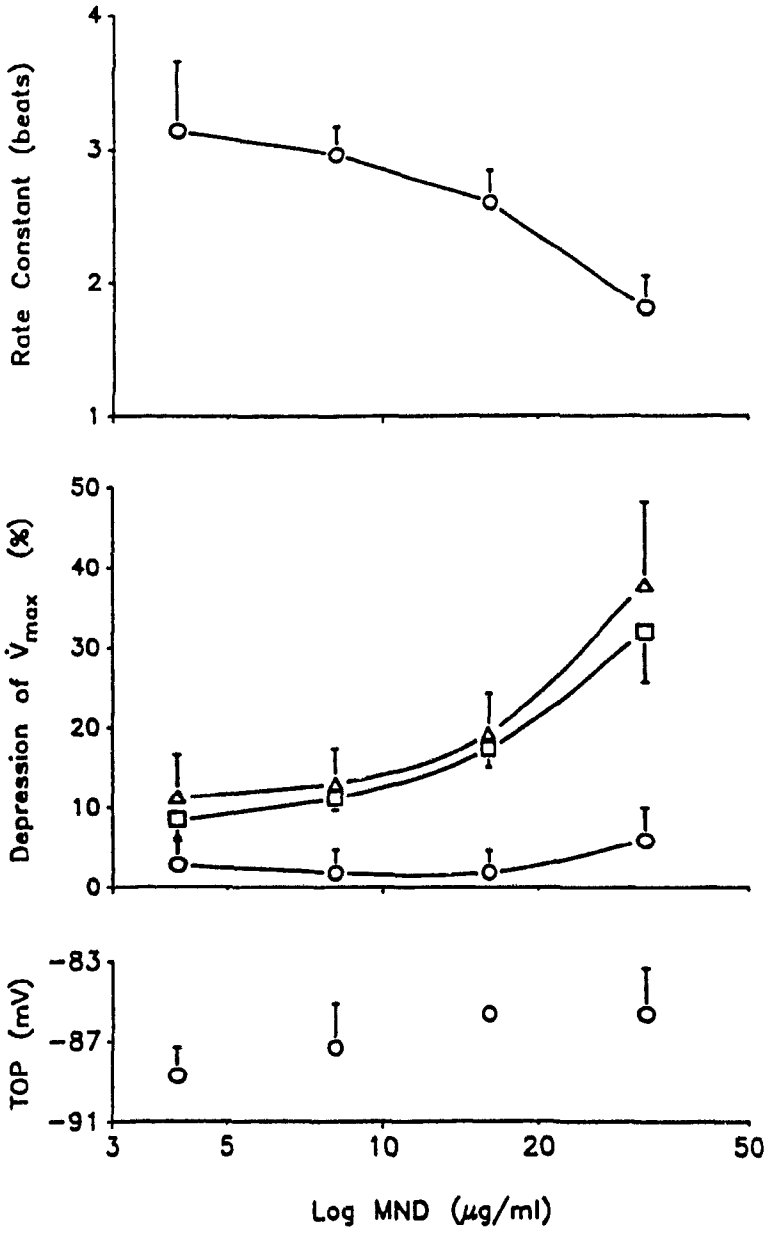

Rate Constant (beats)
4
3
2
1
Depression of $\dot{V}_{max}$ (%)
50
40
30
20
10
0
TOP (mV)
-83
-87
-91
3
5
10
20
50
Log MND (μg/ml)

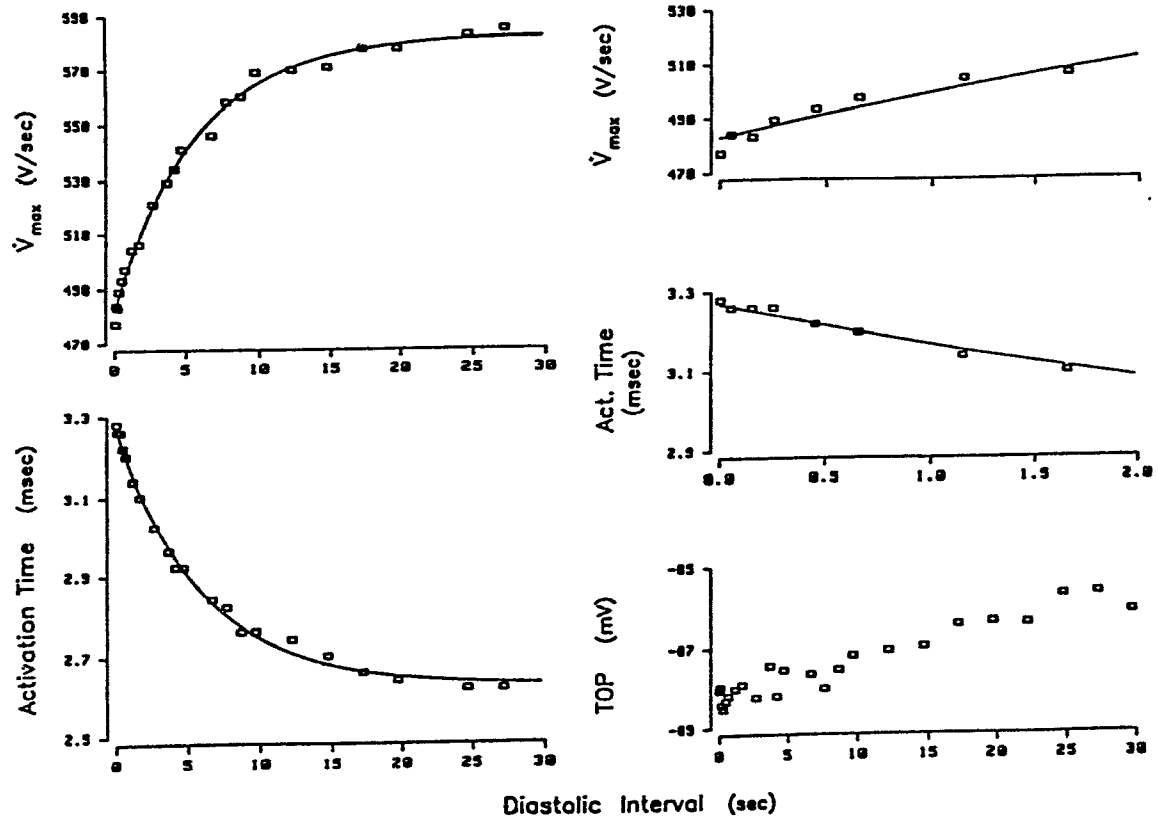
TABLE 2. Effects of interstimulus interval on the kinetics of onset and magnitude of rate-dependent $\dot{V}_{max}$ depression produced by MND

| | | EFFECTS ON RATE CONSTANT (beats) | | | |
|---|---|---|---|---|---|
| | | ISI (msec) | | | |
| MND (ug/ml) | n | 400 | 600 | 1000 | 2000 |
| 4 | 6 | 3.98 ± 1.02 | 3.14 ± 0.52 | 2.89 ± 0.45 | 2.05 ± 0.61 |
| 8 | 6 | 3.48 ± 0.44 | 2.96 ± 0.21 | 2.85 ± 0.65 | 2.11 ± 0.78 |
| 16 | 7 | 2.98 ± 0.41 | 2.60 ± 0.24 | 2.09 ± 0.48 | 2.10 ± 0.77 |
| 32 | 8 | 1.83 ± 0.51 | 1.81 ± 0.24 | 1.60 ± 0.26 | 1.50 ± 0.40 |

| | | EFFECTS ON RDB (%) | | | |
|---|---|---|---|---|---|
| | | ISI (msec) | | | |
| MND (ug/ml) | n | 400 | 600 | 1000 | 2000 |
| 4 | 6 | 10.3 ± 1.9 | 8.5 ± 2.3 | 7.8 ± 1.9 | 5.6 ± 1.9 |
| 8 | 6 | 12.2 ± 1.1 | 11.2 ± 1.5 | 8.8 ± 1.4 | 5.3 ± 1.2 |
| 16 | 7 | 20.5 ± 3.9 | 17.4 ± 2.3 | 15.0 ± 2.6 | 11.0 ± 2.0 |
| 32 | 8 | 37.2 ± 5.1 | 32.0 ± 6.3 | 29.2 ± 3.5 | 21.1 ± 2.5 |

Values are expressed as mean ± S.D.
ISI = interstimulus interval; RDB = rate-dependent block.
Onset of block was best fitted by a single exponential function.
Interaction between drug concentration and ISI were significant for both rate constant and RDB (two-way anova).

Figure 5 - Typical example of the time course of recovery of $\dot{V}_{max}$ and activation time with 16 ug/ml of MND. The right upper and middle panels show the first 2 sec of recovery on an expanded scale. Early diastolic block, defined as the magnitude of $\dot{V}_{max}$ depression or prolongation of activation time (act. time) at zero diastolic interval relative to full recovery, was 17.2 % for $\dot{V}_{max}$ and 19.6 % for activation time. Changes in take-off potential (TOP) during the 30 sec recovery interval are plotted in the bottom right panel. Take-off potential depolarized slightly from -88.06 to -85.67 mV.


$\dot{V}_{max}$ (V/sec)
Activation Time (msec)
$\dot{V}_{max}$ (V/sec)
Act. Time (msec)
TOP (mV)
Diastolic Interval (sec)

of $\dot{V}_{max}$ of early responses reflected a slowing of impulse conduction. Activation time recovered with a single exponential time constant of 5.83 sec. There were never more than 2 to 3 millivolts of depolarization during the 30 sec interval. If there were more than a few millivolts of depolarization the results were not used.

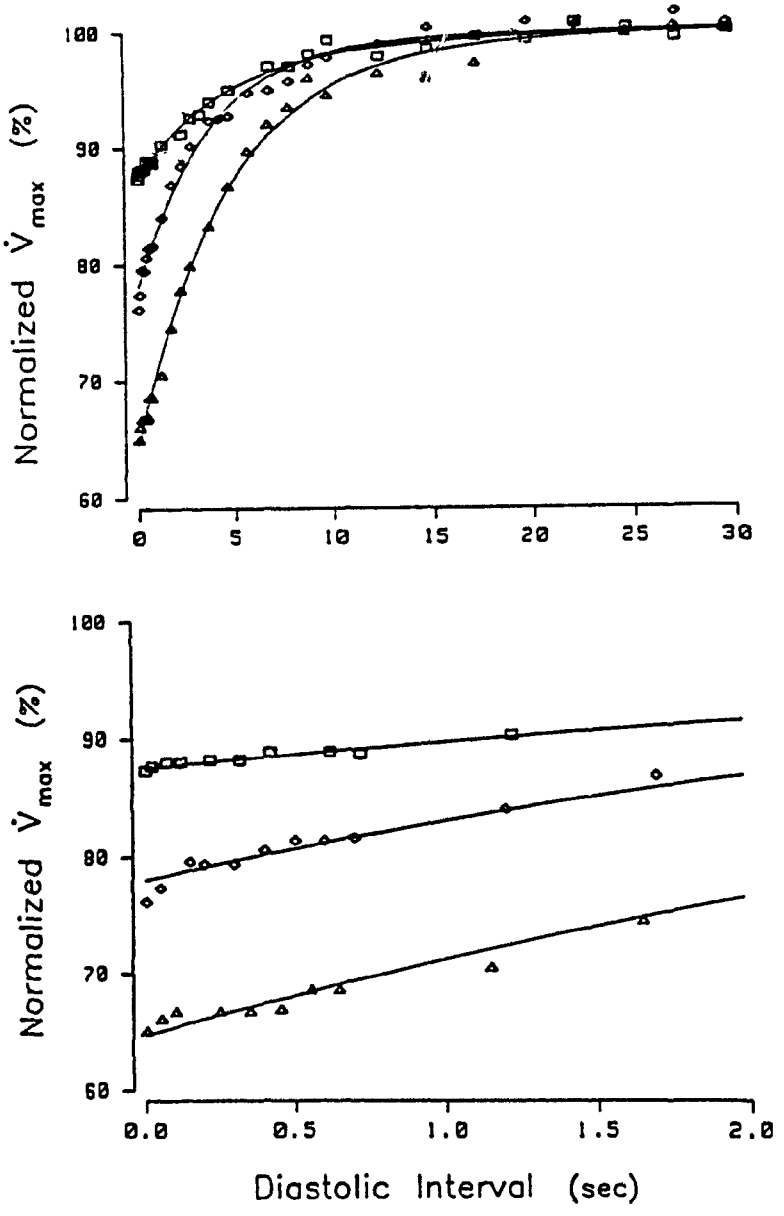
A recovery period of 30 sec was sufficient for $\dot{V}_{max}$ to recover completely in the presence of MND. We could not accurately determine recovery time constants under control because $\dot{V}_{max}$ had already attained its maximum value when repolarization was complete. In 6 experiments, the recovery of $\dot{V}_{max}$ and activation time in the presence of MND had time constants of 5.23 ± 0.9 and 4.88 ± 0.94 sec, respectively. Thus, during the first two seconds of the diastolic interval, recovery from block averaged only 8.1 ± 2.9 %.

$\dot{V}_{max}$ recovery was independent of drug concentration. Figure 6 illustrates a typical example of the recovery of $\dot{V}_{max}$ in the presence of 8, 16, and 32 ug/ml MND. Time constants for $\dot{V}_{max}$ recovery were similar although the initial level of block was greater with higher concentrations of drug.

### 4.5 *Voltage-dependent effects*

In a total of 6 experiments we assessed the effects of MND on the relationship between $\dot{V}_{max}$, activation time and membrane potential. It was not possible to study all concentrations in the same experiment. Thus, in 2 preparations we studied concentrations ranging from 4 to 16 ug/ml. In 3 experiments only 32 ug/ml was used. In the example

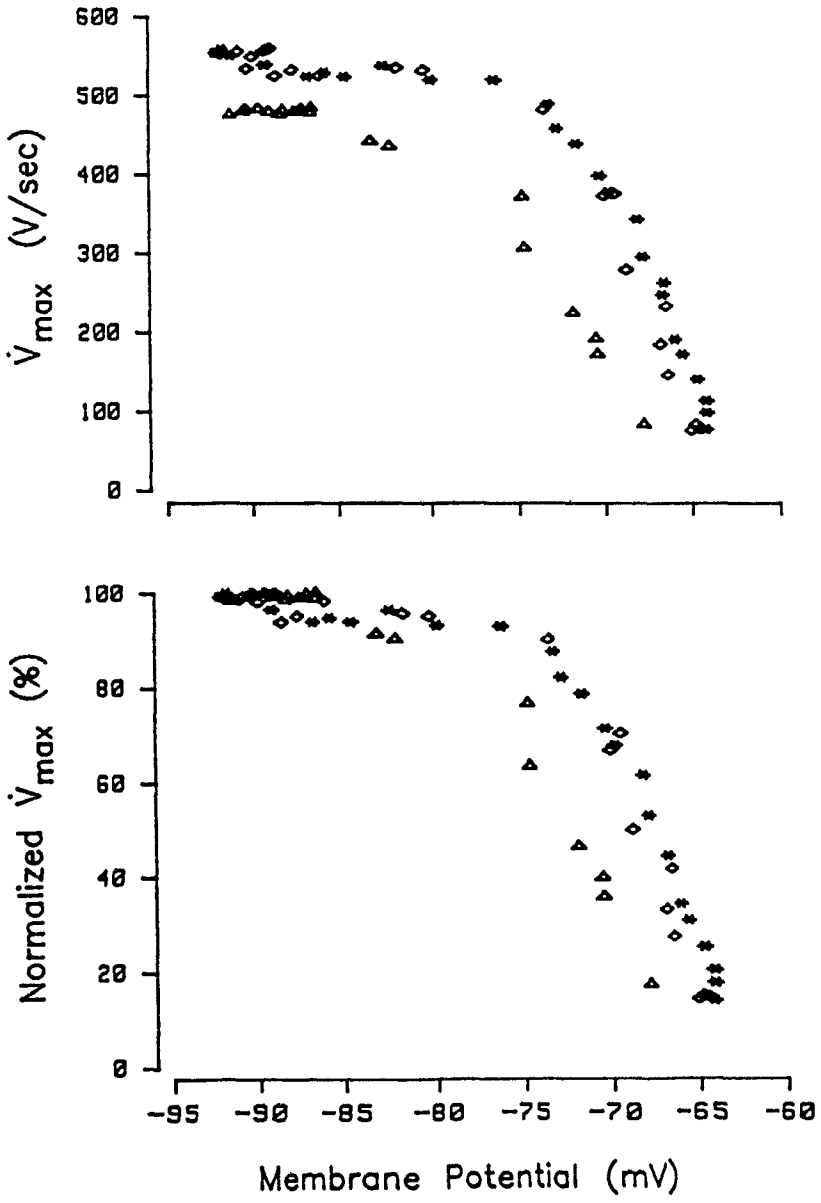
Figure 6 - Concentration dependent effects on the magnitude and time course of recovery of $\dot{V}_{max}$. The curves have been normalized to their respective $\dot{V}_{max}$ value at 30 sec. All curves were fitted by a single exponential function. Early diastolic block increased from 12.4 for 8 ug/ml (squares) to 22.0 and 35.3 % for 16 (diamonds) and 32 (triangles) ug/ml of MND respectively. Time constants were 5.18, 4.01 and 4.83 sec for the three concentrations, respectively. The curves in the bottom panel show the first 2 sec of the recovery interval.


Normalized $\dot{V}_{max}$ (%)
100
90
80
70
60
0
5
10
15
20
25
30
Normalized $\dot{V}_{max}$ (%)
100
90
80
70
60
0.0
0.5
1.0
1.5
2.0
Diastolic Interval (sec)

illustrated in Figure 7, 8 to 32 ug/ml were used but only the effects of 16 and 32 ug/ml are shown. MND, 4 to 16 ug/ml, did not produce a shift in the normalized $\dot{V}_{max}$-membrane potential curve. At the highest concentration, 32 ug/ml, MND produced a 1 to 4 mV shift of the curve at $E_h$ (membrane potential at which $\dot{V}_{max}$ is reduced to half of its maximum value) to more negative potentials and decreased its maximum.

The shift due to 32 ug/ml may have a small rate-dependent component as complete recovery from rate-dependent block would not have occurred at a stimulation rate of 15 sec. Voltage dependent effects on activation time were similar.

Figure 7 - Typical example of the effects of MND on the relationship between $\dot{V}_{max}$ and membrane potential. $\dot{V}_{max}$ is plotted for control conditions (asterisks), following 16 (diamonds) and 32 (triangles) ug/ml of MND in the same experiment. Stimulation rate was 15 sec under drug and 1 sec for control.


600
500
400
300
200
100
0
$\dot{V}_{max}$ (V/sec)
100
80
60
40
20
0
Normalized $\dot{V}_{max}$ (%)
-95
-90
-85
-80
-75
-70
-65
-60
Membrane Potential (mV)

## 5. Discussion

Our results show that MND depresses $\dot{V}_{max}$ of the action potential suggesting that this compound blocks cardiac sodium channels. Depression of $\dot{V}_{max}$ was rate dependent and developed following a single exponential process, characteristic of many Class I antiarrhythmic agents (Campbell, 1983b). Furthermore, $\dot{V}_{max}$ depression occurred at clinically relevant concentrations and stimulation rates. The lowest concentration of MND, 4 ug/ml, was within the clinical range of concentrations measured in patients during chronic therapy with disopyramide (Aitio, 1981; Bredesen et al., 1982). Increasing both the concentration and stimulation rate increased the magnitude of $\dot{V}_{max}$ depression. As an event dependent process, the kinetics of onset of block were faster at higher drug concentrations and slower stimulation rates. However, as a time dependent process the kinetics were faster at both higher concentrations and faster frequencies.

The relative potency of MND was less than that of the parent compound. The amount of rate-dependent block produced by MND was approximately one-half to two-thirds of that observed with disopyramide by Flemming and Sasyniuk (1989) under identical experimental conditions. In contrast to disopyramide which produced a significant amount of tonic block even at therapeutic concentrations (Flemming and Sasyniuk, 1989), MND produced no significant tonic block at concentrations less than 32 ug/ml. Thus, with MND only rate-dependent block was significant at

therapeutic concentrations. The onset of rate-dependent block was approximately three times faster than that seen previously with disopyramide.

Clinically useful Class I antiarrhythmic agents all delay recovery of the sodium channel from inactivation by direct interaction with a receptor site within the sodium channel (Hondeghem and Katzung, 1984). This delay in repriming kinetics was also observed with MND. The time course of recovery from rate-dependent block followed a single exponential process with a time constant of 5.23 ± 0.90 sec. This single recovery process contrasts with the effects of the parent compound which has been reported to recover following at least 2 exponential processes (Kojima et al., 1982; Flemming and Sasyniuk, 1989). The first component had a time constant of hundreds of msec and increased with drug concentration. Such a component of recovery was not observed with MND. Recovery from block with MND was independent of drug concentration. The rate of recovery from block at normal diastolic potentials was slow enough so that block would not fully dissipate between beats throughout the clinical range of heart rates. The rate of recovery was approximately 3 times faster than that attributed to dissociation of disopyramide from the sodium channel by Flemming and Sasyniuk (1989) and was more similar to that of the Class Ia drug quinidine (Grant et al., 1982; Clarkson and Hondeghem, 1985a; Valois and Sasyniuk, 1987; Thompson et al., 1987) or the Class Ic drug propafenone (Kohlhardt and Seifert, 1983; Thompson et al., 1988; Rouet et al., 1989).

Recovery of activation time (used as an estimate of effects on conduction) was similar to that of $\dot{V}_{max}$ and indicates that changes in $\dot{V}_{max}$ of the action potential reflect changes in conduction in the whole heart.

MND produced depolarization at higher concentrations. Drug-induced depolarization of membrane potential has been observed previously with many Class I agents. Significant concentration dependent depolarization has been observed with disopyramide (Flemming and Sasyniuk, 1989), quinidine (Weld et al., 1982) and NAPA (Dangman and Hoffman, 1981) in canine Purkinje fibers. In guinea-pig atrial and ventricular muscle fibers, high concentrations of propafenone produced a significant depolarization (Delgado et al., 1985). Although $\dot{V}_{max}$ studies were generally conducted at membrane potentials over the flat well polarized region of the $\dot{V}_{max}$-membrane potential curve, slight depolarization would have resulted in an overestimation of tonic block and an underestimation of rate-dependent block particularly at higher drug concentrations. More precise determination of drug effects on tonic and rate-dependent block may require voltage clamp experiments in order to minimize the effects of depolarization on $\dot{V}_{max}$.

Shifts in the $\dot{V}_{max}$-membrane potential relationship to more negative potentials is characteristic of many sodium channel blocking agents (Hondeghem and Katzung, 1984). Such a shift was not observed with levels of MND that may be achieved during disopyramide therapy suggesting that additional block is not produced by such a mechanism at

clinically relevant concentrations. Thus, effects of MND on sodium channels at clinically relevant concentrations reside mainly in the rate dependent depression of $\dot{V}_{max}$.

Several studies have shown that disopyramide blocks the sodium channel mainly during activation (Kodama et al., 1986 and 1987; Gruber and Carmeliet, 1989). The parent compound also fails to shift the $\dot{V}_{max}$-membrane potential relationship (Gruber and Carmeliet, 1989). Thus MND, like the parent compound, may also interact with the sodium channel during activation.

MND consistently shortens action potential duration in Purkinje fibers at clinically relevant heart rates and concentrations. This action is different from that of the parent compound which produces variable effects on action potential duration depending on drug concentration, stimulation rate and extracellular potassium concentration (Kus and Sasyniuk, 1975 and 1978; Fiemming and Sasyniuk, 1989). The effects of MND resembles more closely the disopyramide derivative, penticainide (Gautier et al., 1987) or the Class Ic agents flecainide (Yabek et al., 1987) and encainide (Elharrar and Zipes, 1982) which also shorten action potential duration in canine Purkinje fibers but prolong it in ventricular muscle.

Shortening of action potential duration by MND in Purkinje fibers probably results from block of the TTX-sensitive sodium "window" current involved in maintaining the action potential plateau (Attwell et al., 1979; Coraboeuf et al., 1979). This is also the mechanism by which

penticainide (Gautier et al., 1987), flecainide (Smallwood et al., 1989) and encainide (Elharrar and Zipes, 1982) have been suggested to shorten action potential duration.

Lengthening of the action potential duration in ventricular muscle was uniform at all cycle lengths suggesting inhibition of the inward rectifying time-independent potassium current ($i_{k1}$). A parallel shift of the action potential duration-rate relationship in canine endocardium following 4-aminopyridine was attributed to inhibition of $i_{k1}$ by Litovsky and Antzelevitch (1988). A similar rate independent prolongation of action potential duration was produced by quinidine *in vivo* in dog epicardium (Franz and Costard, 1988). In isolated canine ventricular myocytes quinidine decreased $i_{k1}$ independently of frequency (Salata and Wasserstrom, 1988).

The inward rectifying outward current is responsible for a considerable fraction of the outward potassium current between -40 mV and the resting potential and reduction leads to the slowing of final (phase 3) repolarization (Van Bogaert and Snyders, 1982; Tseng et al., 1987). Since the TTX-sensitive "window" current plays a much smaller role in determining plateau durations in ventricular muscle than in Purkinje fibers (Coraboeuf et al., 1979; Bhattacharyya and Vassalle, 1982), block of $i_{k1}$ may exceed reduction of the TTX-sensitive "window" current thereby prolonging action potential duration. Prolongation of action potential duration was not reversible following washout of the

drug for up to 90 min (Toy and Sasyniuk, unpublished observations). Similarly, block of $i_{k1}$ was irreversible with quinidine (Hiraoka et al., 1986; Salata and Wasserstrom, 1988).

Prolongation of action potential duration in ventricular muscle has also been observed with the parent compound (Kus and Sasyniuk, 1975 and 1978; Kojima, 1981; Flemming and Sasyniuk, 1989) although rate dependent effects have not been investigated. Differential effects of MND may be beneficial in reducing disparity of action potential duration between Purkinje fibers and ventricular muscle and thus provide protection against re-entrant types of arrhythmias. The metabolite prolonged effective refractory period uniformly in ventricular muscle. However in Purkinje fibers effective refractory period was prolonged only at fast heart rates indicating that frequency-dependent block of sodium channels by MND probably contributed to an increase in effective refractory period. Prolongation of effective refractory period may account for the findings of Grant et al. (1978) and Dubray et al. (1986) who reported decreases in the maximum pacing frequency in the presence of MND.

The present *in vitro* study of the major metabolite of disopyramide demonstrates that it has significant electrophysiological effects on $\dot{V}_{max}$, action potential duration and effective refractory period. Further study will be required to evaluate these effects when MND is present in combination with the parent drug. Such investigation will

provide information concerning the interaction of the two compounds and the potential clinical efficacy of their combined antiarrhythmic effects.

Chapter 3

# ELECTROPHYSIOLOGICAL INTERACTIONS BETWEEN DISOPYRAMIDE AND ITS MAJOR METABOLITE, MONO-N-DEALKYLDISOPYRAMIDE, IN CANINE VENTRICULAR TISSUE

(Manuscript in preparation for publication.)

## 1. Abstract

Mono-N-dealkyldisopyramide (MND), the major metabolite of disopyramide in man, has been shown to have Class I antiarrhythmic actions. To predict the potential clinical effects of its presence during disopyramide therapy, we investigated the interaction between disopyramide and MND with the sodium channel in canine ventricular tissue superfused *in vitro*. $\dot{V}_{max}$ was used as an indirect index of sodium channel block. Pulse trains at an interstimulus interval of 600 msec were used to assess the kinetics and magnitude of rate-dependent block in Purkinje fibers. With disopyramide (10 uM) alone, the rate constant and magnitude of rate-dependent block were 7.04 ± 0.96 beats and 11.1 ± 2.0 %; with MND (10 uM) the respective values were 4.39 ± 1.58 beats and 6.3 ± 1.1 %. The combination produced an additive increase in the magnitude of rate-dependent block (15.1 ± 3.9 %), while the rate constant (4.56 ± 1.94 beats) was similar to that with MND alone. Tonic block was 6.3 ± 3.6 % for disopyramide alone; 2.5 ± 2.9 % for MND alone; and 9.1 ± 6.0 % for the combination. Recovery from rate-dependent block with the combination followed a single exponential time course with a time constant of 9.10 ± 2.17 sec.

Disopyramide produced a rate-dependent prolongation of the effective refractory period of 3 tightly coupled sequential extrasystoles but not the basic beat in Purkinje fibers. In ventricular

muscle the effective refractory period of the basic beat in addition to the 3 extrasystoles were prolonged. Effects in both tissues were accentuated in the presence of the combination.

The combination produced an additive shortening of action potential duration at normal heart rates. However, disopyramide alone produced a reverse use-dependent prolongation of action potential duration following a long diastolic interval. In the presence of external hypokalemia, acidosis and bradycardia, disopyramide significantly increased the slope of the action potential duration-rate relationship which led to the induction of early afterdepolarizations and triggered activity in Purkinje fibers at cycle lengths of 2 sec or greater. MND shifted the incidence of triggered activity to longer cycle lengths. Addition of mexiletine (8 - 11 uM) or pacing at 1 Hz abolished triggered activity.

Additive interactions between disopyramide and MND suggest that MND may contribute to the clinical efficacy of disopyramide by enhancing $\dot{V}_{max}$ depression, thereby further slowing conduction, and prolonging refractory periods. Reverse use-dependent effects of disopyramide on action potential duration suggest it may be potentially proarrhythmic. The effects of MND may be beneficial against triggered arrhythmias by shifting the incidence of triggered activity to longer cycle lengths.

## 2. Introduction

Interactions between antiarrhythmic drugs and their metabolites must be carefully considered in the clinical efficacy of these agents. The Class Ia drug disopyramide is primarily metabolized in man by hepatic N-dealkylation to MND (Karim, 1975). Plasma concentrations of the metabolite generally reach approximately 30 % of disopyramide levels during chronic oral therapy in patients, but may exceed this due to renal impairment or with interindividual variability (Aitio, 1981; Bredesen, 1982).

The primary electrophysiological effect of disopyramide *in vitro* is a depression of $\dot{V}_{max}$ (Class I effect), while prolongation of action potential duration (Class III effect) has also been observed (Kojima, 1981; Campbell, 1983b; Flemming and Sasyniuk, 1989). MND also depressed $\dot{V}_{max}$ in cardiac Purkinje fibers (Toy and Sasyniuk, 1990a). Its kinetics of interaction with the sodium channel are faster than with disopyramide. Unlike the parent compound, MND consistently shortened action potential duration in Purkinje fibers, but like the former prolonged it in ventricular muscle. At normal heart rates, the combination of disopyramide and MND should increase depression of $\dot{V}_{max}$ and prolongation of refractoriness. However, opposite effects on action potential duration by MND in Purkinje fibers may antagonize the Class III antiarrhythmic actions of disopyramide.

Conversely, the Class III actions of disopyramide may also render it proarrhythmic. Torsade de pointes arrhythmias associated with prolongation of the $QT_c$ interval have been reported in patients during disopyramide therapy (Jackman et al., 1988). Studies with the Class Ia drug, quinidine, have implicated drug-induced early afterdepolarizations and triggered activity observed *in vitro* in the genesis of torsades de pointes (Roden et al., 1987; Davidenko et al., 1989; Sasyniuk et al., 1989). Similar effects of disopyramide and quinidine on action potential prolongation *in vitro* (Kus and Sasyniuk, 1978; Winslow et al., 1988) suggest that disopyramide may also induce early afterdepolarizations and triggered activity. Abbreviation of action potential duration by MND in this situation, however, may oppose abnormal lenghtening of repolarization and be antiarrhythmic.

Therefore, the present study was undertaken to examine the interactions between disopyramide and MND with the sodium channel, using $\dot{V}_{max}$ as an index of sodium channel block, and determine effects on effective refractory period and action potential duration. Effects on action potential duration were also investigated under conditions which facilitated induction of triggered activity. Some of these results have been published previously in abstract form (Toy and Sasyniuk, 1990b).

## 3. Methods

### 3.1 *Experimental preparation*

Mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg) and their hearts were removed via a left thoracotomy. Both ventricles were thoroughly flushed with chilled, oxygenated Tyrode's solution. Purkinje fibers with small pieces of attached ventricular muscle were quickly excised and pinned to a Sylgard block at the bottom of a tissue bath (4 ml capacity). Fibers were continuously perfused with modified Tyrode's solution aerated with 95 % $O_2$ - 5 % $CO_2$ at a rate of 10 ± 0.5 ml/min. Our standard Tyrode's solution contained (in mM): NaCl, 119.0; KCl, 4.0; $CaCl_2$, 1.8; $MgCl_2$, 0.5; $NaHPO_4$, 0.9; dextrose, 5.5; and $NaHCO_3$, 25. pH of the solution was 7.33 ±0.03. The temperature was maintained at 37.0 ± 0.2° C. To study drug-induced early afterdepolarizations and triggered activity the following changes were made with the superfusing Tyrode's solution (in mM): KCl, 2.7; and $NaHCO_3$, 12. pH of the solution was reduced to 7.09 ± 0.04.

We used small, free-running intertrabecular Purkinje fiber bundles (length < 5 mm) with attached ventricular muscle or ventricular papillary muscle preparations mainly from the right ventricle to study steady-state and rate-dependent drug effects. Free running false tendons with small pieces of attached ventricular muscle predominantly from the left ventricular were used to study drug-induced early

afterdepolarizations and triggered activity. Techniques for stimulating and recording have been described in detail previously (Valois and Sasyniuk, 1987; Toy and Sasyniuk, 1990a).

During control measurements and equilibration with drug, the preparations were stimulated at a basic cycle length of 1000 msec. Action potential variables were recorded on-line every 15 sec until steady-state effects were observed.

### 3.2 *Protocols*

To study the rate of development of $\dot{V}_{max}$ depression with drive, the preparations were driven by 44 beat trains at an interstimulus interval of 600 msec. Rest periods between trains of stimuli were long enough to ensure full recovery from rate-dependent block.

To determine recovery from $\dot{V}_{max}$ depression, test pulses were introduced at varying diastolic intervals after a stimulus train had produced a steady-state level of $\dot{V}_{max}$ depression. The onset of diastole was defined as that time when repolarization had returned to the maximum diastolic potential. Test pulses were initially introduced during phase 3 of the action potential and then progressively later into diastole. The test intervals were initially increased in small increments and then progressively in larger steps with steps ranging from 50 to 10000 msec. Each recovery curve was comprised of a minimum of 30 points. The results were not used if there were more than 2 to 3 mV of depolarization during the diastoic interval.

To determine the effects on effective refractory period of the basic beat (S1) and sequential extrastimuli (S2-S4), test pulses of twice the diastolic threshold were introduced following steady-state changes in action potential parameters. Test pulses were initially introduced at the basic cycle length and then at progressively shorter intervals until they failed to elicit a response. The effective refractory period was defined as the shortest S1-S2 interval which elicited a propagated response. S2 was set at 10 msec past the effective refractory period and S3 was used to determined the effective refractory period of the response to S2. Similarly, S3 was set at 10 msec past the effective refractory period and S4 was used to determine the effective refractory period of the response to S3. Preparations were stimulated at steady-state cycle lengths of 400 and 1000 msec.

All comparisons between control and drug conditions were made during the same impalement. $\dot{V}_{max}$ was used as an indirect method to evaluate $I_{Na}$. Although $\dot{V}_{max}$ is a monotonic but not a linear index of $I_{Na}$ (Sheets et al., 1988; Fozzard et al., 1989), $\dot{V}_{max}$ does reflect net maximal inward current during the action potential and can provide a convenient, albeit nonquantitative index of changes in $I_{Na}$.

To investigate the ability of disopyramide to induced early afterdepolarizations and triggered activity and to characterize its frequency dependence, preparations were perfused in the hypokalemic-acidotic Tyrode's solution described above. A low concentration of cesium chloride (0.5 - 1 mM) was also added to the

perfusate to reduce intrinsic automaticity by reducing the pacemaker current, $I_f$ (Di Francesco, 1981). Early afterdepolarizations and triggered activity were induced by disopyramide in approximately 50 % of preparations prior to addition of cesium (unpublished observations). Although both extreme acidosis or high concentrations of cesium alone have been used to induce triggered activity (Coraboeuf et al., 1980; Brachmann et al., 1983; Damiano and Rosen, 1984), the milder conditions of acidosis and lower concentrations of cesium we used do not induce triggered activity in Purkinje fibers stimulated at slow rates in the absence of drug (Valois and Sasyniuk, 1990, submitted). Purkinje fibers were exposed to a low therapeutic concentration of disopyramide (10 uM) for at least 1 hr before data recording began.

The preparations were stimulated over a wide range of cycle lengths (1 - 6 sec). If early afterdepolarizations or triggered activity were observed, the pattern and range of cycle lengths over which they occurred were determined. At each frequency data was collected only after steady-state changes in action potential duration were achieved (minimum of 2 minutes). When triggered activity was sustained and stable, a test drug (MND, mexiletine) was added to the disopyramide containing solution. Mexiletine was added during stimulation at slow rates. However, since effects with MND require at least 25 min to reach steady-state (Toy and Sasyniuk, 1990a), stimulation at slow rates was begun only after equilibration.

### 3.3 *Drugs*

Disopyramide used was disopyramide phosphate for injection (Rythmodan). MND used was generously supplied by Roussel Laboratories. The concentration of disopyramide or MND used was 10 uM unless specified otherwise. Plasma concentrations of disopyramide in patients during chronic therapy range between 9 - 20 uM, while MND levels can reach up to 15 uM (Aitio, 1981; Bredesen, 1982; Brogden and Todd, 1987). Since the protein binding of disopyramide and MND in this concentration range vary from 50 - 80 and 20 - 50 %, respectively (Aitio, 1981; Bredesen, 1982), concentrations used reflect therapeutic plasma levels of the free fraction of these drugs in patients. Mexiletine used was mexiletine hydrochloride generously supplied by Boehringer-Ingelheim. Drugs were added to Tyrode's solution made up from refrigerated stock solutions. Concentrations used refer to the concentration of the base.

### 3.4 *Data analysis*

All action potential variables were determined with a Data 6000 waveform analyzer as previously described (Sasyniuk and Jhamandas, 1984). Action potential variables measured were: take-off potential; maximum diastolic potential; action potential amplitude; $\dot{V}_{max}$; and APD at the 50, 75, and 95% levels of repolarization (APD 50%, APD 75%, and APD 95% respectively).

Where applicable, data are expressed as mean ± S.D.. Paired Student's t test was used to make statistical comparisons between control and drug effects on: steady-state action potential

characteristics, tonic block and the slope of the action potential duration-rate relationship. One- or two-way analysis of variance and Newman-Keuls tests for multiple comparisons were used to compare drug effects on: rate-dependent block, recovery of $\dot{V}_{max}$ and effective refractory period. $p<0.05$ was considered significant. Standard linear regression techniques were used to analyze the relationship between action potential duration and basic cycle length (Roden and Hoffman, 1985). The time course of onset or recovery of $\dot{V}_{max}$ depression was defined with the use of a least square error nonlinear exponential fitting program (Hewlett-Packard Statistical Library). The time constants of onset or recovery of $\dot{V}_{max}$ block contained only one component. Least squares analysis of the data indicated that this component could be approximated by a single exponential function of the form $Y = Aexp(-t/\tau) + B$. No attempt was made to fit the data with more complex functions. However, when appropriate, it was determined whether a double exponential function fit the data better than a single exponential. A double exponential was considered a better fit if the residual sum of squares for the double exponential was one-third or less than that for the single exponential (Clarkson and Hondeghem, 1985a).

### 3.5 *Definitions*

Early afterdepolarizations (EADs) and triggered activity were defined according to Cranefield (1975) and Damiano and Rosen (1984). An EAD was considered to be an afterpotential which interrupts or delays normal repolarization of the action potential. EADs and the triggered

action potentials they induced were classified into two categories based on the membrane potential of the inflection point at which the repolarization process was interrupted. High- or low-membrane potential EADs were defined as depolarizing afterpotentials which delayed the terminal repolarization phase at membrane potentials more negative than -45 mV or less negative than -30 mV, respectively. Low membrane potential EADs occurred too infrequently to permit analysis and will not be considered further. Thus, all references to EADs in this study refer to those at high membrane potentials.

These EADs either caused a delay of repolarization or gave rise to a second nondriven large amplitude upstroke. Characteristics of these triggered action potentials and their preceding diastolic intervals were measured off line on stored digitized traces. Activation voltage was defined as the membrane potential just prior to initiation of the depolarization phase of the triggered action potential. The coupling interval of the triggered response was defined as the time from the phase 0 upstroke of the driven action potential to phase 0 of the upstroke of the triggered action potential. For multilple triggered responses, the coupling intervals of the second and subsequent triggered action potentials were calculated from the upstroke of the preceding triggered response to the upstroke of the subsequent one.

# 4. Results

## 4.1 Effects at rapid stimulation rates

### 4.1.1 *Effects on action potential characteristics in Purkinje fibers*

Table 1 summarizes the effects of disopyramide, MND and their combination on action potential characteristics in Purkinje fibers stimulated at a basic cycle length of 1000 msec. Disopyramide and MND administered singly did not alter the maximum diastolic potential but depressed $\dot{V}_{max}$ and action potential amplitude, and shortened action potential duration. Addition of MND to disopyramide increased depression of $\dot{V}_{max}$ and amplitude and shortening of all phases of repolarization. Similarly, addition of disopyramide to MND increased depression of $\dot{V}_{max}$ and amplitude and significantly shortened APD 50 and 75%. Table 2 shows that the combination of disopyramide and MND produced effects consistent with the algebraic sum of the individual drug actions on all parameters with the exception of APD 95%.

### 4.1.2 *Drug-induced tonic and rate-dependent block*

To investigate drug-induced depression of $\dot{V}_{max}$ we used clinically relevant concentrations. Tonic block following a long rest period was produced by both disopyramide (6.3 ± 3.6 %; n = 5) and MND (2.5 ± 2.9 %; n = 4) administered singly. The combination of disopyramide and MND consistently increased the magnitude of tonic block regardless of the order of drug administration, although results were not statistically

TABLE 1. Effects of disopyramide, MND and their combination on action potential characteristics in canine Purkinje fibers

| | MDP (mV) | AMP (mV) | $\dot{V}_{max}$ (V/sec) | APD 50% (msec) | APD 75% (msec) | APD 95% (msec) |
|---|---|---|---|---|---|---|
| Control | -91.0 ± 2.8 | 124.1 ± 4.9 | 565 ± 65 | 241 ± 32 | 278 ± 38 | 316 ± 40 |
| DISO[a] 10 uM | -90.5 ± 3.7 | 120.6 ± 5.0** | 502 ± 45** | 206 ± 39** | 264 ± 43* | 312 ± 43 |
| Combination[b] | -89.9 ± 3.3 | 117.0 ± 5.4[c] | 470 ± 52[c] | 184 ± 40[c] | 251 ± 43[c] | 306 ± 43[c] |
| (n = 6) | | | | | | |
| Control | -93.9 ± 2.1 | 126.4 ± 3.1 | 509 ± 15 | 224 ± 28 | 265 ± 65 | 304 ± 26 |
| MND 10 uM | -92.3 ± 2.5 | 123.7 ± 2.6* | 495 ± 32* | 197 ± 7* | 250 ± 17* | 294 ± 22* |
| Combination[b] | -92.7 ± 1.1 | 120.6 ± 1.8[d] | 447 ± 43[d] | 143 ± 15[d] | 238 ± 20[d] | 296 ± 22 |
| (n = 4) | | | | | | |

Values are expressed as mean ± S.D. Fibers were stimulated at a BCL of 1000 msec. MDP = maximum diastolic potential; AMP = amplitude; APD 50%, APD 75%, APD 95% = action potential duration at 50, 75, and 95 % levels of repolarization; [a]DISO = disopyramide; [b]combination = disopyramide 10 uM + MND 10 uM.
*Significantly different from control, $p<0.05$; **$p<0.01$.
[c]Significantly different from disopyramide alone ($p<0.05$).
[d]Significantly different from MND alone ($p<0.05$).

TABLE 2. Effects of the interaction between disopyramide and MND on action potential characteristics in canine Purkinje fibers

| | % DEPRESSION | | |
|---|---|---|---|
| | Disopyramide 10 uM (n = 6) | MND 10 uM (n = 4) | Combination[a] (n = 10) |
| AMP | 2.9 ± 1.4 | 2.1 ± 1.2 | 5.1 ± 2.0 |
| $\dot{V}_{max}$ | 10.9 ± 4.5 | 3.7 ± 3.3 | 15.0 ± 5.2 |

| | % SHORTENING | | |
|---|---|---|---|
| | Disopyramide 10 uM (n = 6) | MND 10 uM (n = 4) | Combination[a] (n = 10) |
| APD 50% | 14.9 ± 6.3 | 11.6 ± 8.6 | 28.6 ± 11.4 |
| APD 75% | 5.4 ± 3.6 | 5.2 ± 4.1 | 10.1 ± 5.1 |
| APD 95% | 1.4 ± 3.4 | 3.6 ± 2.5 | 3.1 ± 3.4 |

Values are expressed as mean ± S.D.
AMP = amplitude; APD 50%, APD 75%, APD 95% = action potential duration at 50, 75, and 95 % levels of repolarization.
[a]Combination = disopyramide 10 uM + MND 10 uM.

significant. The increase in tonic block with the combination (9.1 ± 6.0 %; n = 9), nevertheless, was consistent with effects predicted by an additive interaction of the two drugs (8.8 %).

Both disopyramide and MND alone produced rate-dependent block at an interstimulus interval of 600 msec. The results are summarized in Table 3. The rate constant and magnitude of rate-dependent block in the presence of disopyramide was greater than with MND. The magnitude of rate-dependent block with the combination was equivalent to the sum of effects produced by both drugs while the rate constant was not significantly different from that with MND alone Figure 1 illustrates a typical example of tonic and rate-dependent block produced by disopyramide versus the combination of disopyramide and MND.

4.1.3 *Effects on action potential duration during abrupt decreases in cycle length*

Drug-induced changes of action potential duration reflect their effects on ion currents during action potential repolarization. Figure 2 shows a typical example of drug-induced changes in action potential duration during onset of a rapid pulse train following a long rest period. Under control conditions and in the presence of drug, APD 95% of the first action potential of the trains were prolonged Prolongation of APD 95% in the presence of disopyramide was tremendous compared to control conditions, suggesting block of potassium channels in well polarized tissue. APD 50% of the first action potential and steady-steady APD 50 and 95% in the presence of disopyramide, however,

TABLE 3 Effects of disopyramide, MND and their combination on the rate constant and magnitude of rate-dependent block

| Drug | Rate constant (pulses) | RDB (%) |
|---|---|---|
| Disopyramide 10 uM (n = 6) | 7.04 ± 0.96 | 11.1 ± 2.0 |
| MND 10 uM (n = 4) | 4.39 ± 1.58* | 6.3 ± 1.1* |
| Combination[a,b] (n = 10) | 4.56 ± 1.94* | 15.1 ± 3.9*+ |

Values are expressed as mean ± S.D. Fibers were stimulated at an interstimulus interval of 600 msec. RDB = rate-dependent block.
[a]Combination = disopyramide 10 uM + MND 10 uM.
[b]Onset of block in the presence of the combination was best fitted by a single exponential
*Significantly different from disopyramide alone, $p<0.05$;
+significantly different from MND alone, $p<0.05$ (anova).

Figure 1 - Typical example of the effects of disopyramide and the combination of disopyramide and MND on tonic and rate-dependent block in Purkinje fibers. $\dot{V}_{max}$ (top panel) and take-off potential (TOP; bottom panel) are plotted for each action potential during the stimulus train under control conditions (diamond), and following disopyramide 10 uM (square), and the combination of disopyramide 10 uM and MND 10 uM (triangle). Each stimulus train was preceded by a quiescent interval of 60 sec. Rate-dependent depression of $\dot{V}_{max}$ was minimal under control conditions and may be attributed to a small decline in take-off potential during the stimulus train. Rate constants were 6.44 and 4.47 pulses, tonic block was 5.2 and 8.6 % and rate-dependent block was 7.6 and 9.2 % following disopyramide and the combination of disopyramide and MND, respectively. The interstimulus interval was 600 msec

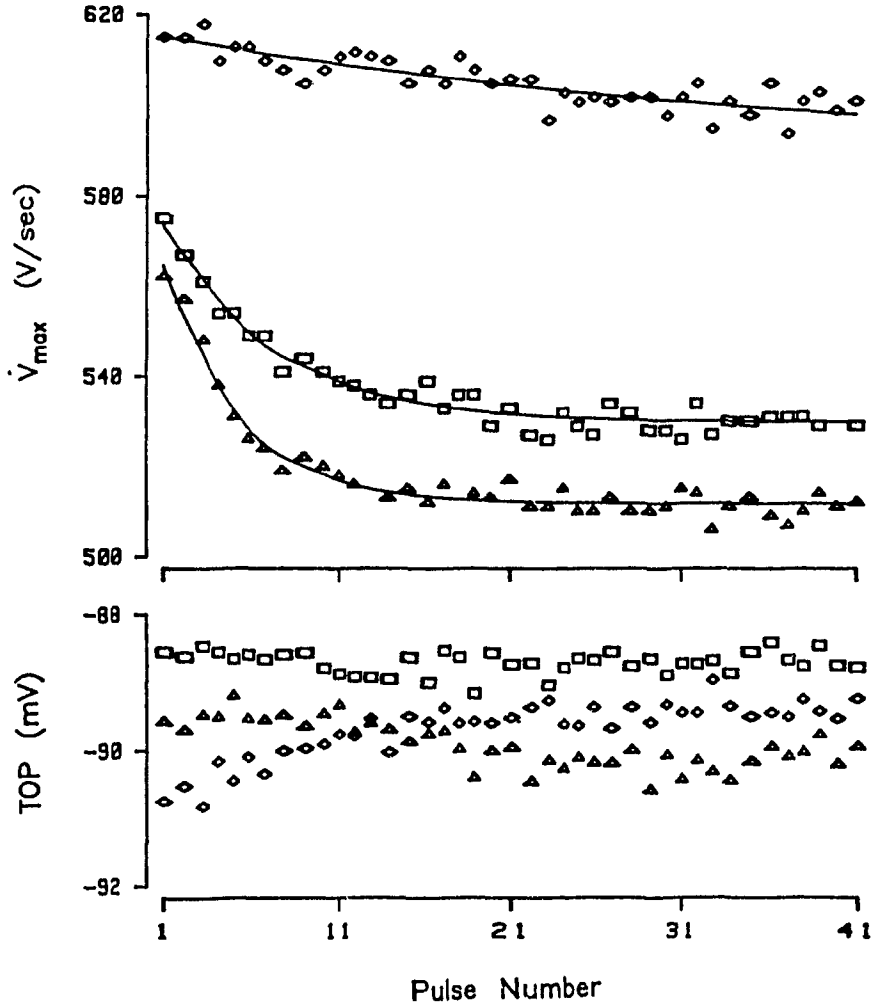
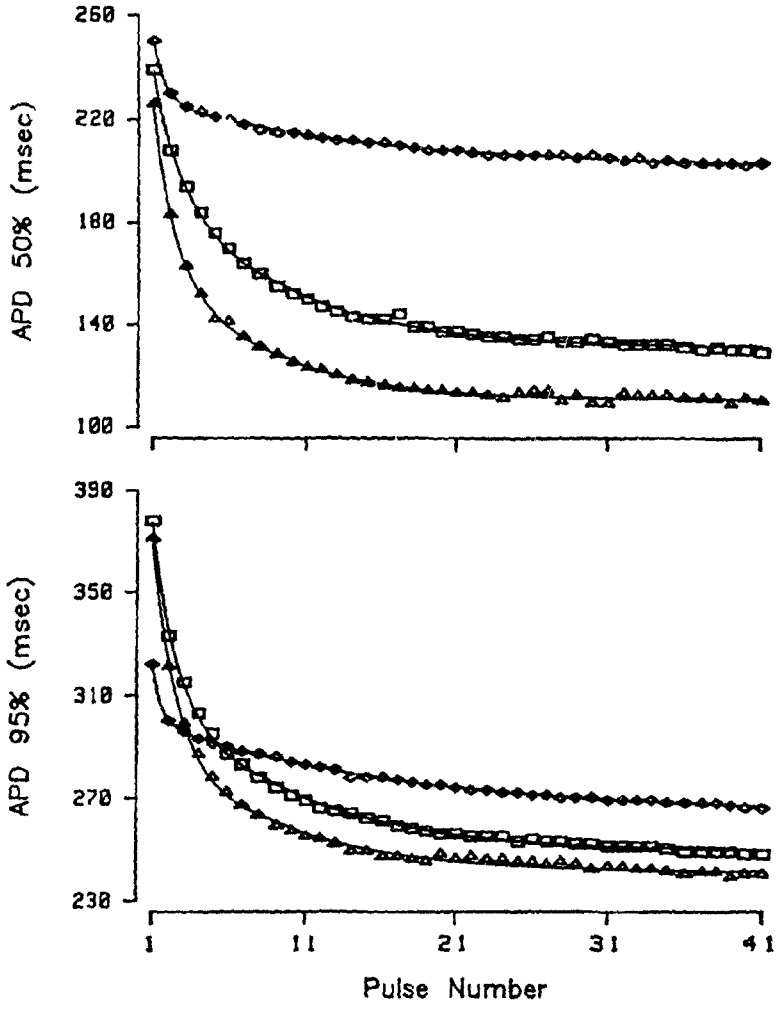

620
580
540
500
$\dot{V}_{max}$ (V/sec)
-88
-90
-92
TOP (mV)
1
11
21
31
41
Pulse Number

Figure 2 - Typical example of the effects of disopyramide and the combination of disopyramide and MND on rate dependent changes in action potential duration during onset of a stimulus train in Purkinje fibers. APD 50% (top panel) and APD 95% (bottom panel) are plotted for each action potential during the stimulus train under control conditions (diamond), following disopyramide 10 uM (square), and the combination of disopyramide 10 uM and MND 10 uM (triangle). The stimulus train was preceded by a quiescent interval of 60 sec. The interstimulus interval was 600 msec


260
220
180
140
100
APD 50% (msec)
390
350
310
270
230
APD 95% (msec)
1
11
21
31
41
Pulse Number

were abbreviated compared to control conditions. The combination of disopyramide and MND increased the shortening produced by disopyramide alone but did not alter APD 95% of the first action potential compared to disopyramide alone. Qualitatively similar results were observed in 5 other experiments.

4.1.4 *Characteristics of recovery from rate-dependent block*

The time course of $\dot{V}_{max}$ recovery in the presence of the combination of disopyramide and MND was best approximated by a single exponential function with a time constant of 9.10 ± 2.17 sec (n = 4). The magnitude of early diastolic block was 14.8 ± 4.1 %. Recovery time constants under control conditions could not be determined since $\dot{V}_{max}$ has already attained its maximum value following action potential repolarization.

Figure 3 summarizes the effects of disopyramide versus the combination of disopyramide and MND on recovery of $\dot{V}_{max}$ over a clinically relevant diastolic interval. Disopyramide produced a significant depression of $\dot{V}_{max}$ at zero diastolic interval which remained throughout the 2 sec interval. The combination of disopyramide and MND, 10 and 20 uM, produced a further depression of $\dot{V}_{max}$ at zero diastolic interval which also persisted throughout the 2 sec diastolic interval.

4.1.5 *Effects on effective refractory period*

The ability of disopyramide versus the combination of disopyramide and MND to prolong the effective refractory period of basic beats versus tightly coupled sequential extrastimuli is summarized in Figure 4.

Figure 3 - Effects of disopyramide and the combination of disopyramide and MND on recovery of $\dot{V}_{max}$ over a clinically relevant diastolic interval $\dot{V}_{max}$ is plotted over a distolic interval of 2 sec under control conditions (diamond), following disopyramide 10 uM (square), and the combination of disopyramide 10 uM and MND 10 uM (triangle) or MND 20 uM (circle). Data points represent mean ± S D of 6 experiments in Purkinje fibers $^{*}$Significantly different from control, $p<0.01$; $^{+}$significantly different from disopyramide 10 uM, $p<0.05$, $\phi$significantly different from disopyramide 10 uM + MND 10 uM, $p<0.01$ (anova)

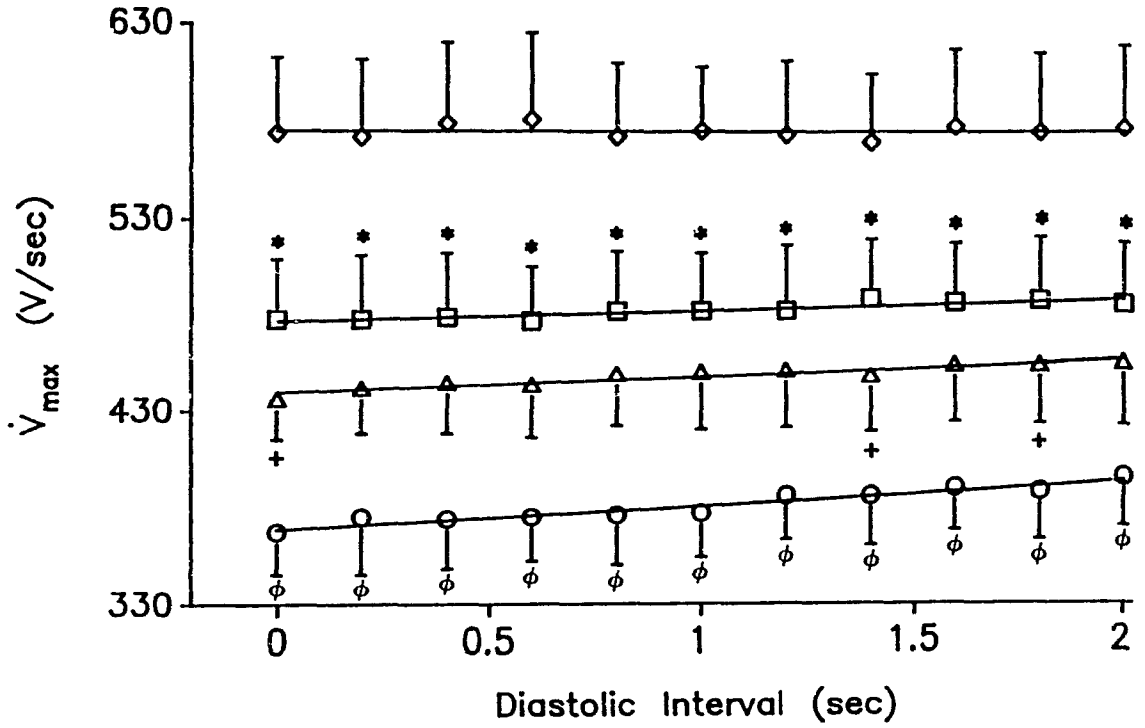
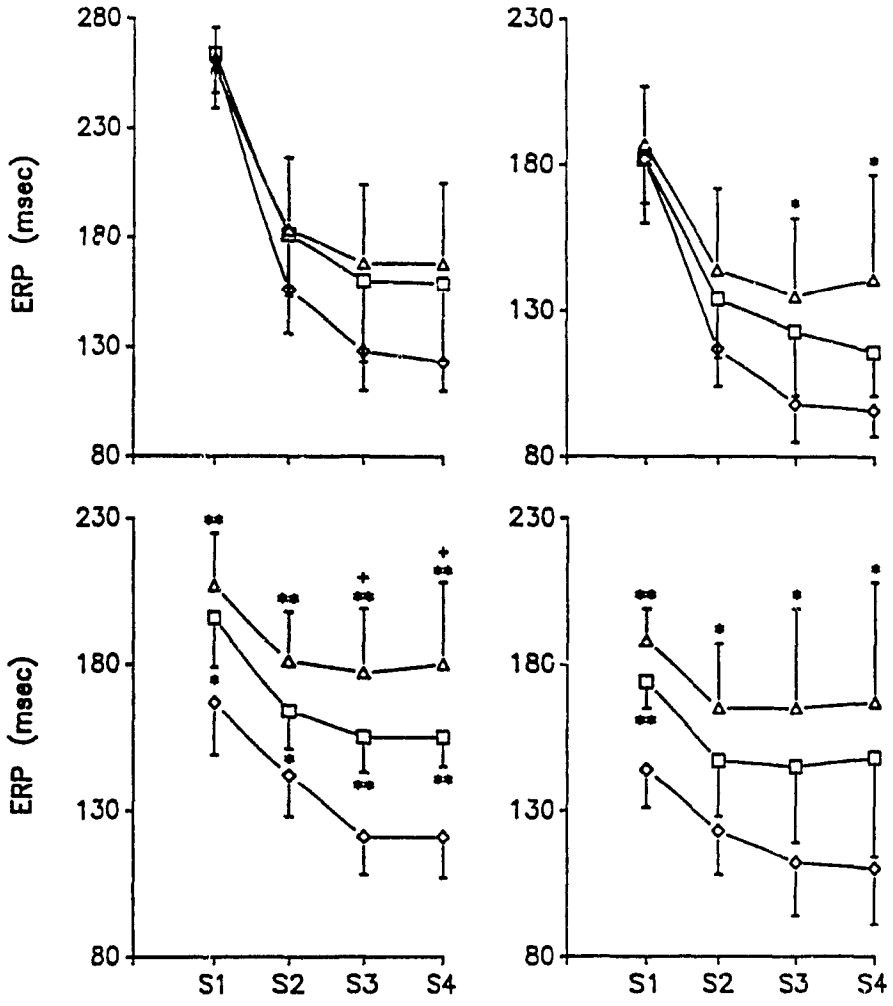

630
530
430
330
$\dot{V}_{max}$ (V/sec)
0
0.5
1
1.5
2
Diastolic Interval (sec)

Figure 4 - Effects of disopyramide and the combination of disopyramide and MND on the effective refractory period in Purkinje fibers versus ventricular muscle. Effective refractory period of the basic beat (S1) and 3 subsequential extrastimuli (S2, S3 and S4) in Purkinje fibers (top panels) and ventricular muscle (bottom panels) are plotted under control conditions (diamond), following disopyramide 10 uM (square) and the combination of disopyramide 10 uM and MND 10 uM (triangle). Data points represent mean ± S.D. of 6 and 5 experiments in Purkinje fibers and ventricular muscle, respectively. The cycle lengths were 1000 (left panels) and 400 msec (right panels). *Significantly different from control, $p<0.05$, **$p<0.01$; †significantly different from disopyramide 10 uM, $p<0.05$ (anova).


ERP (msec)
280
230
180
130
80
230
180
130
80
ERP (msec)
230
180
130
80
230
180
130
80
S1
S2
S3
S4
S1
S2
S3
S4

Disopyramide consistently but nonsignificantly increased the effective refractory period of the 3 sequential extrastimuli but not the basic beat in Purkinje fibers This effect was enhanced by the combination of disopyramide and MND and was rate-dependent, there was a greater increase in effective refractory period with each subsequent extrastimulus.

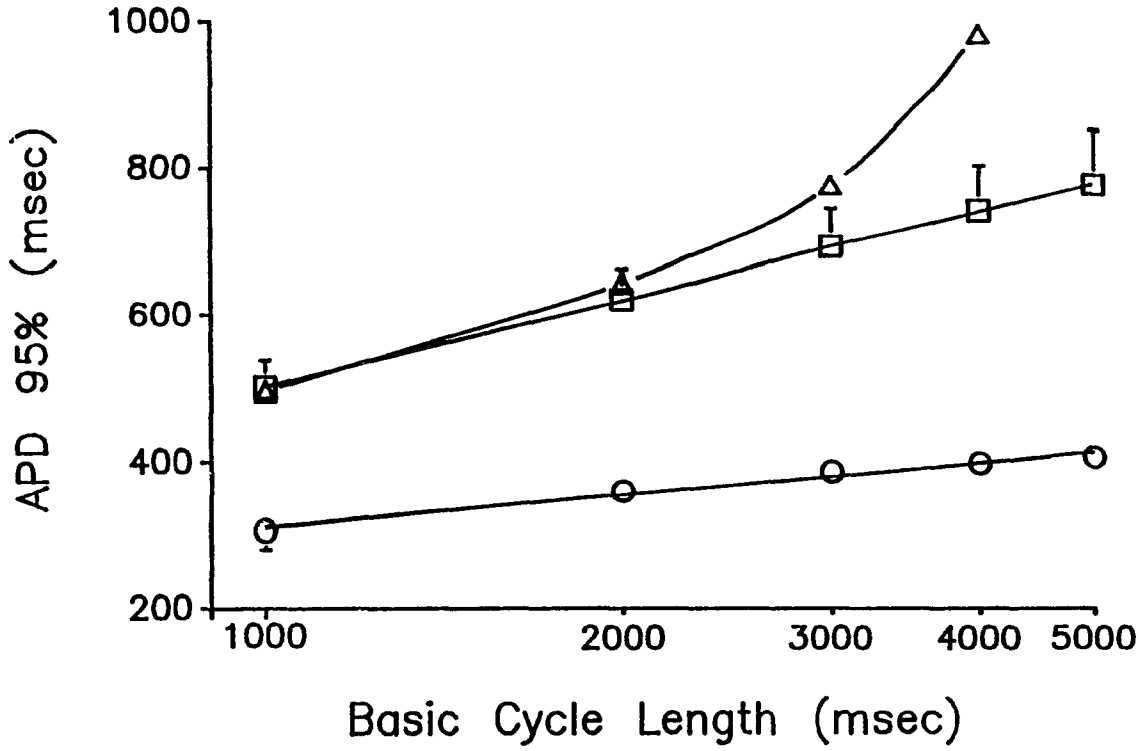
Under control conditions, the effective refractory period shortened minimally in ventricular muscle compared to Purkinje fibers when the basic cycle length was decreased from 1000 to 400 msec. Disopyramide consistently increased the effective refractory period of both the basic beat and the 3 sequential extrastimuli. This effect was further enhanced by the combination of disopyramide and MND.

## 4.2 Effects at slow stimulation rates

### 4.2.1 *Action Potential Duration in the Absence of Triggered Activity*

We investigated the ability of disopyramide to induce triggered activity in a total of 17 preparations. In 4 preparations, we were able to define the relationship between action potential duration and stimulation frequency. Figure 5 summarizes the effects of disopyramide (10 uM) on the steady-state action potential duration-rate relationship at cycle lengths from 1 to 5 sec. There was a rate-related increase in action potential duration under control conditions which was linear in the log cycle length versus action potential duration plot ($r = .992$).

Figure 5 - Effects of disopyramide on the action potential duration-rate relationship in Purkinje fibers. Action potential duration is plotted over the range of cycle lengths of 1000 to 5000 msec under control conditions (circle), and following disopyramide 10 uM in the presence of hypokalemia, acidosis and cesium 0 5 mM (square) The slope was 0 147 and 0.400 under control conditions and following disopyramide, respectively Data points represent mean ± S.D. of 4 experiments. Error bars located within symbols were not plotted. Action potential duration is also plotted for 2 experiments in which disopyramide, under identical conditions, induced EADs and triggered activity (triangle). Deviation of the action potential duration-rate relation from linearity was due to the appearance of EADs Action potential duration was not plotted for stimulated beats accompanied by triggered action potentials.


1000
800
600
400
200
APD 95% (msec)
1000
2000
3000
4000
5000
Basic Cycle Length (msec)

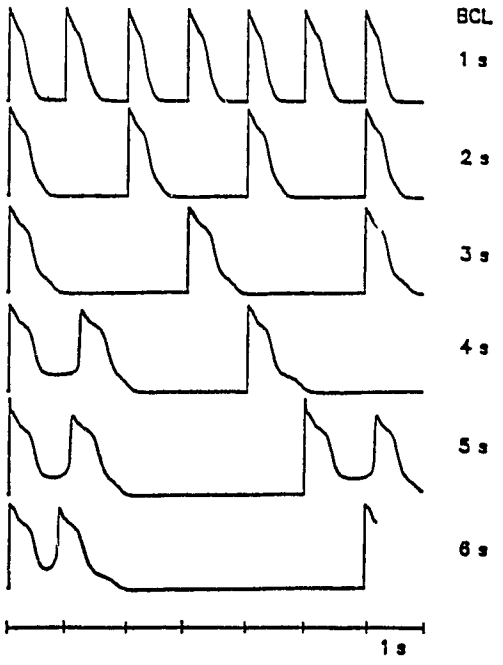
Disopyramide in a hypokalemic-acidotic Tyrode's solution produced a reverse use-dependent prolongation of action potential duration and increased the slope of the relationship ($p<0.05$) This relationship could not be determined for the hypokalemic-acidotic Tyrode's solution in the absence of drug due to automaticity. When disopyramide did induce EADs and triggered activity, the action potential duration-rate relationship deviated from linearity at longer cycle lengths (Figure 5).

4 2.2 *Early Afterdepolarizations (EADs) and Triggered Activity*

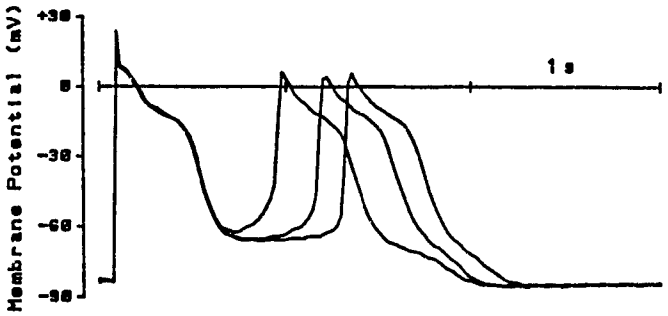
In 13 preparations superfused with the hypokalemic-acidotic Tyrode's solution, disopyramide (10 uM) induced EADs and triggered activity during bradycardia. This activity attended by marked prolongation of repolarization generally appeared 60 - 90 min after administration of disopyramide. In any given preparation, there was a characteristic relationship between the steady-state pattern of activity and basic cycle length. Figure 6a shows a typical example. EADs delayed repolarization of each action potential at a basic cycle length of 2 sec and became more prominent at 3 sec. At 4 sec, a triggered action potential followed the EAD of every third action potential. As the stimulation rate was further slowed, there was an increase in the incidence of triggered action potentials. At a cycle length of 5 sec, 2 of every 3 action potentials were followed by a triggered action potential. Finally, at 6 sec, every action potential was followed by a triggered response.

Figure 6 - (a) Typical example of the effects of cycle length on EADs and triggered activity induced by disopyramide 10 uM in the presence of hypokalemia, acidosis and cesium 0.5 mM in Purkinje fibers. Transmembrane action potentials at cycle lengths ranging from 1 to 6 sec are plotted. (b) Transmembrane action potentials at cycle lengths of 4 to 6 sec are superimposed.

(a)




(b)

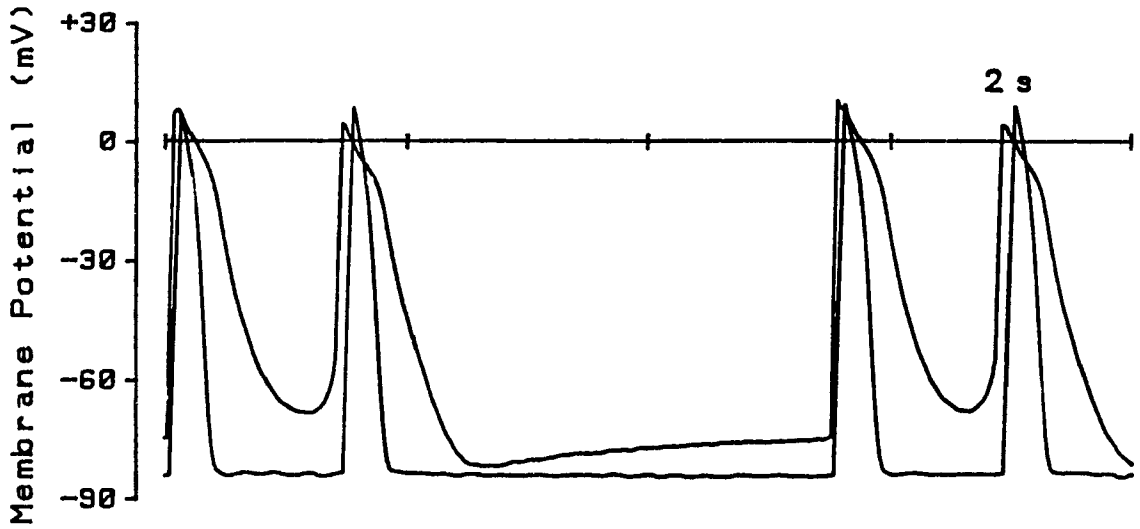


In all preparations, there was a similar progression from action potential prolongation to the appearance and increase in the size of EADs to induction and increase in the number of action potentials followed by triggered responses. Although the shortest cycle length at which triggered activity first occurred varied in different fibers, it was clearly bradycardia-dependent. Of the 11 Purkinje fibers studied, only 1 showed triggered activity at a cycle length of 2 sec, 2 at 3 sec, 11 at 4 sec and all 13 at 5 sec.

In each preparation, consistent changes in triggered action potential characteristics were observed with changes in cycle length. Slower stimulation rates shortened the coupling interval and decreased the activation voltage to more positive potentials (Figure 6b). Multiple triggered action potentials were observed only in a few fibers. In these examples, multiple responses occurred at longer cycle lengths than required for single responses. Each subsequent upstroke occurred at a more negative activation voltage until the last upstroke was followed by full repolarization. All triggered activity developed in the potential range in which the fast sodium current could be partially activated and thus exhibited fast upstrokes and prominent overshoots.

Disopyramide-induced EADs and triggered activity were never observed to occur in ventricular muscle. Triggered activity occurring in Purkinje fibers propagated to muscle to produce prolonged but otherwise normal action potentials. Figure 7 shows a typical example.

Figure 7 - Typical example of the differential effects by disopyramide in the presence of hypokalemia, acidosis and cesium 0.5 mM on induction of triggered activity in Purkinje fibers versus ventricular muscle. Transmembrane action potentials from a false tendon and a distal muscle cell from the left ventricle at a spontaneous cycle length of 5.5 sec are plotted and superimposed.


Membrane Potential (mV)
+30
0
-30
-60
-90
2 s

4.2 3 *Prevention and Abolition of Triggered Activity*

4 2.3.1 *MND*

In 2 of 4 preparations with sustained and stable triggered activity, addition of MND (10 uM) to the superfusate shifted the incidence of triggered activity to longer cycle lengths Figure 8 shows one example. Disopyramide induced prominent EADs at a cycle length of 3 sec and triggered action potentials at 4 and 5 sec Superfusion with the combination of disopyramide and MND prevented EADs and triggered activity at cycle lengths of 3 and 4 sec, respectively Although triggered activity was still induced at 5 sec, triggered action potentials occurred relatively later during repolarization and at more negative membrane potentials. Also, triggered responses did not follow every action potential as before. Washout of MND reversed these effects. In the other 2 experiments, MND had no effects on the cycle length dependence of triggered activity.

4.2.3.2 *Mexiletine*

In 4 preparations, therapeutic concentrations of mexiletine (8 - 11 uM) was added to the superfusate. In all experiments, mexiletine abolished triggered activity and eliminated EADs within minutes of administration. Following exposure to mexiletine, there was a prolongation of the coupling interval and an increase in the activation voltage (more negative) of the triggered action potential until it was abolished (Figure 9a). Subsequently, there was a progressive decrease in the magnitude and duration of EADs thereby shortening action potential duration (Figure 9b). Thus, abolition of triggered action

Figure 8 - Example of the effects of MND on EADs and triggered activity induced by disopyramide in Purkinje fibers Transmembrane action potentials at cycle lengths ranging from 1 to 5 sec are plotted following disopyramide 10 uM (left panels), the combination of disopyramide 10 uM plus MND 10 uM (middle panels), and disopyramide 10 uM after washout of MND (right panels) in the presence of hypokalemia, acidosis and cesium 0.5 mM

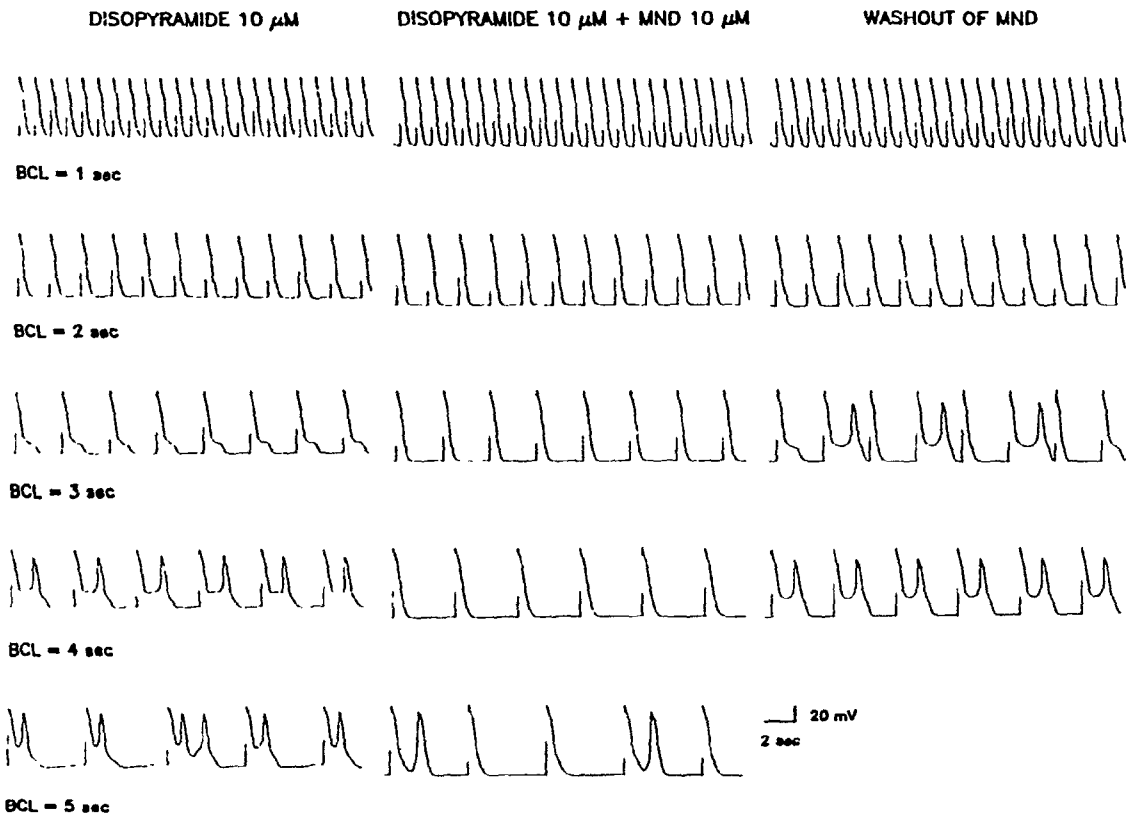
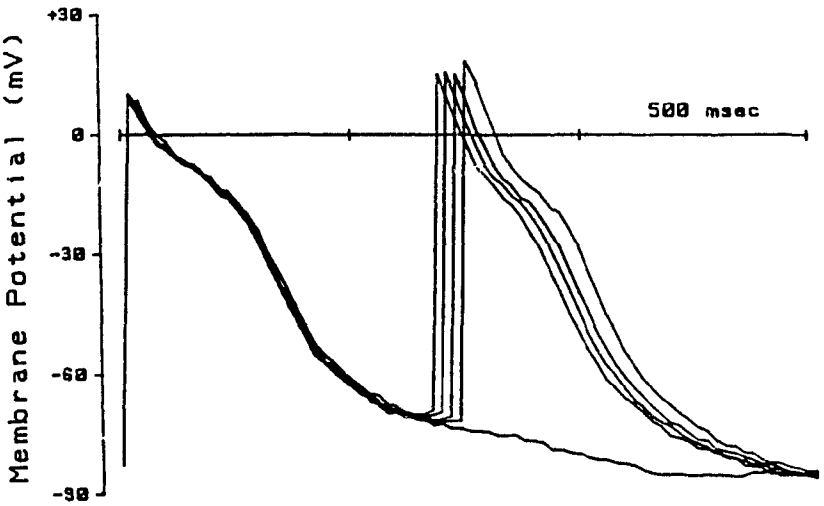
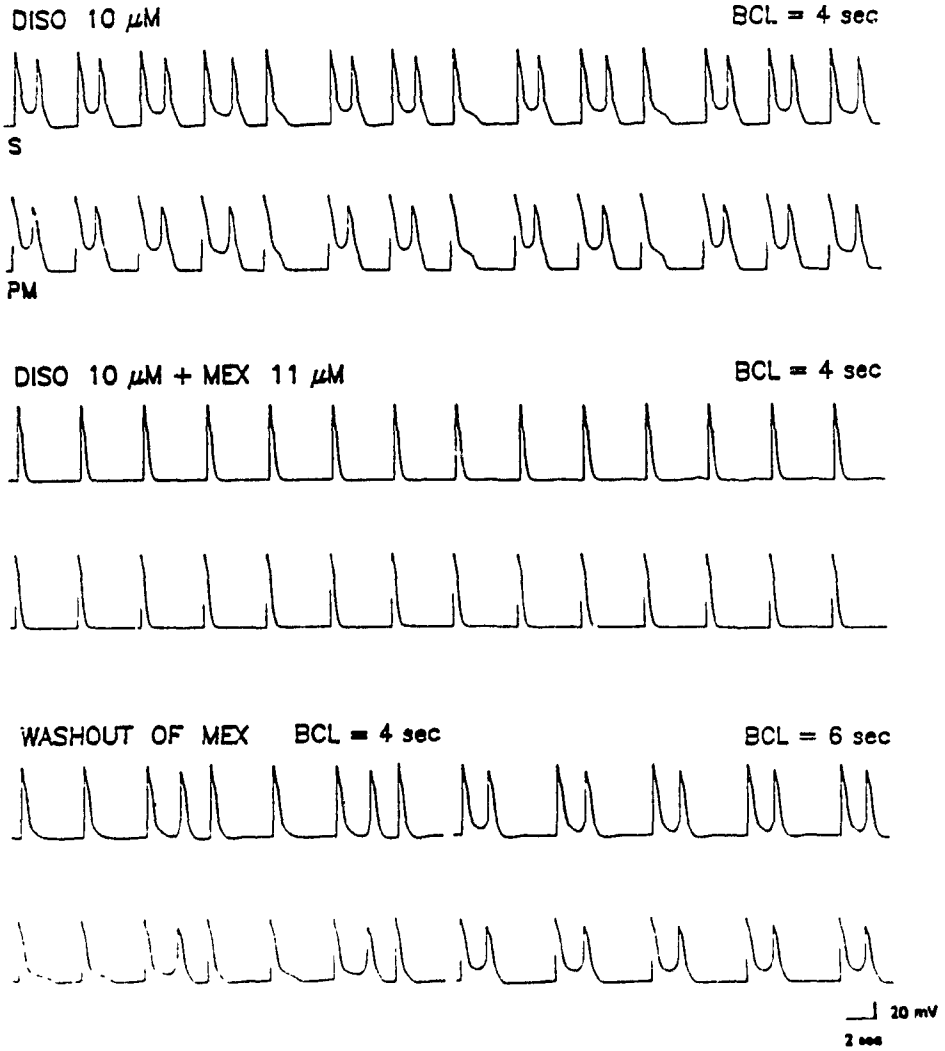

DISOPYRAMIDE 10 μM
DISOPYRAMIDE 10 μM + MND 10 μM
WASHOUT OF MND
BCL = 1 sec
BCL = 2 sec
BCL = 3 sec
BCL = 4 sec
BCL = 5 sec
20 mV
2 sec

Figure 9 - Typical examples of the effects of mexiletine on EADs and triggered activity induced by disopyramide 10 uM in the presence of hypokalemia, acidosis and cesium 0.5 mM in Purkinje fibers. (a) Transmembrane action potentials showing the abolition of triggered action potentials following addition of mexiletine 8 uM at a cycle length of 4 sec are plotted and superimposed. (b) From a different preparation, transmembrane action potentials recorded from both the septal (S) and papillary muscle (PM) insertion regions of the false tendon are plotted following steady-state changes in the presence of disopyramide 10 uM (top panels), the combination of disopyramide 10 uM plus mexiletine 11 uM (middle panels), and disopyramide 10 uM after washout of mexiletine (bottom panels).

(a)


+30
0
-30
-60
-90
Membrane Potential (mV)
500 msec

(b)


DISO 10 μM
BCL = 4 sec
S
PM
DISO 10 μM + MEX 11 μM
BCL = 4 sec
WASHOUT OF MEX
BCL = 4 sec
BCL = 6 sec
20 mV
2 sec

potentials occurred prior to shortenting of action potential duration. Removal of mexiletine from the superfusate led to the reappearance of EADs and triggered action potentials.

*4.2.3.3 Pacing*

In all Purkinje fibers, EADs and triggered activity occurring during bradycardia was immediately abolished by an abrupt increase in the driving rate to 1 Hz. This effect was accompanied by shortening of action potential duration

## 5 Discussion

Our results show that the interaction between disopyramide and MND at clinically relevant concentrations and heart rates produced additive effects on the magnitude of tonic and rate-dependent $\dot{V}_{max}$ depression. The combination produced net effects on shortening of the plateau phase of the action potential which were also additive. These effects are most likely related to block of sodium channels by both drugs. Additive effects were not observed on phase 3 repolarization presumably due to the effects of disopyramide in blocking potassium channels.

Both disopyramide and MND produces a depression of $\dot{V}_{max}$ which recovers minimally over the clinical range of diastolic intervals. Both drugs also produced a prolongation of effective refractory periods. The effects of the combination resemble that expected from an increase in drug concentration of either drug. Additive increases in the magnitude of tonic and rate-dependent block have been previously seen with the

combination of quinidine and propafenone due to similar kinetics of onset and recovery for both drugs (Kohlhardt and Seifert, 1985). MND has a recovery time constant which is approximately three time faster than disopyramide (Flemming and Sasyniuk, 1989; Toy and Sasyniuk, 1990a), however, at heart rates of 1 Hz or faster recovery from $\dot{V}_{max}$ depression with both drugs is slow enough to appear indistinquishable.

The recovery kinetics of rate-dependent block produced by the combination of disopyramide and MND was best described by a single exponential process with a time constant intermediate to values for each drug singly: smaller than disopyramide alone (Campbell, 1983a; Flemming and Sasyniuk, 1989; Gruber and Carmeliet, 1989; Hiraoka et al., 1989) and greater than MND alone (Toy and Sasyniuk, 1990a). The rate constants of onset and magnitude of rate-dependent block with the individual drugs were within the range of values previously observed (Flemming and Sasyniuk, 1989; Toy and Sasyniuk, 1990a). Onset kinetics with the combination treatment was also characterized as a single exponential but was similar to the faster kinetics (smaller rate constant) with MND alone. This may be accounted for by a higher total concentration of drug in the presence of the combination. Both disopyramide and MND have been shown to produce a concentration-dependent increase in the kinetics of onset (Flemming and Sasyniuk, 1989; Toy and Sasyniuk, 1990a).

Monoexponential kinetics have been observed with the combination of quinidine and propafenone (Kohlhardt and Seifert, 1985), while two components described the exponential onset or recovery produced by the

combinations of propafenone plus lidocaine or prajmalium (Kohlhardt and Seifert, 1985) or mexiletine (Valenzuela et al., 1989), and quinidine plus tocainide (Valois and Sasyiuk, 1987) or mexiletine (Roden et al., 1987). Biexponential kinetics with combinations of drugs result with one component being attributable to each drug and have been distinquished only when the difference between the two components were approximately one order of magnitude or greater. Single exponential processes with the combination of disopyramide and MND are most likely observed because the components of each drug cannot be resolved with our protocol and are not due to any physiological changes in onset or recovery processes of $\dot{V}_{max}$ depression.

The effective refractory period is a function of both action potential duration and rate-dependent block of rapid sodium channels. The effects of the latter component is consequentially related to the kinetics of onset for a particular drug (Campbell, 1983b). In Purkinje fibers, shortening of action potential duration by both disopyramide and MND may have offset any increase in effective refractory period of the basic beat produced by block of sodium channels. However, since both disopyramide and MND have relatively slow onset kinetics and require several beats to produce significant effects, prolongation of refractoriness with each subsequent extrasystoles may be due to a rate related increase in the block of sodium channels. Similar effects by disopyramide on effective refractory period have been shown previously by Flemming and Sasyniuk (1989) in Purkinje fibers.

Unlike in Purkinje fibers, both disopyramide and MND prolonged action potential duration in ventricular muscle (Kojima, 1981; Campbell, 1983b; Toy and Sasyniuk, 1990a), and in the present study prolonged the effective refractory period of the basic beat. Although block of sodium channels may have contributed to increases in the effective refractory period, rate-independent prolongation of the effective refractory period of the extrasystoles suggest that the predominant effect was due to prolongation of action potential duration.

At therapeutic concentrations and normal heart rates, both shortening and prolongation of action potential duration (APD 95%) was observed with disopyramide in Purkinje fibers. Variable effects on action potential duration have been previously noted and are likely due to concommitant effects by disopyramide on several membrane conductances during repolarization (Coraboeuf et al., 1988; Flemming and Sasyniuk, 1989). Disopyramide depresses both the TTX-sensitive sodium "window" and slow calcium currents which help to sustain the plateau of the action potential, as well as outward potassium currents involved in repolarization (Coraboeuf et al., 1988; Hiraoka et al., 1989). Although disopyramide predominantly shortened action potential duration in the present study, prolongation of action potential duration was also observed. MND abbreviated repolarization regardless of the effects of disopyramide. However, despite statistically significant effects, the overall changes in action potential duration with either drug or their combination were minor ($\leq$ 10 msec) at clinically relevant heart rates.

In contrast, after a long rest interval, disopyramide consistently produced a pronounced prolongation of action potential duration. These observations reaffirm results obtained previously by Flemming and Sasyniuk (1989) with disopyramide. Furthermore, the most striking finding in our study was that in the presence of bradycardia, hypokalemia and acidosis, prolongation of action potential duration led to the development of EADs and triggered activity in Purkinje fibers. To our knowledge, this is the first report of triggered activity induced by disopyramide *in vitro*. A preliminary report has been previously presented (Sasyniuk et al., 1989). Triggered activity has been induced in Purkinje fibers by many antiarrhythmic drugs which prolong repolarization such as quinidine (Roden and Hoffman, 1985; Valois and Sasyniuk, 1987), NAPA (Dangman and Hoffman, 1981) and sotalol (Lathrop and Varro, 1990). Experimental investigations suggest that prolonged QT syndrome arrhythmias such as torsade de pointes *in vivo* may originate from drug-induced EADs and triggered activity in cardiac Purkinje fibers (Brachmann et al., 1983; Sasyniuk et al., 1989). Therapeutic doses of disopyramide have been associated with the occurrence of these arrhythmias in patients (Casedevant and Sabaut, 1975; Tzivoni et al., 1981; Schweitzer and Mark, 1982; Riccioni et al., 1983).

Abrupt increases in cycle length and hypokalemia are common clinical features of antiarrhythmic drug-induced torsade de pointes (Jackman et al., 1988). Therefore, studies of the electrophysiological effects of antiarrhythmic drugs under these conditions are relevant. In

addition, we used mild acidosis and bradycardia to set up an environment *in vitro* in which disopyramide would readily evoke triggered activity for systematic observation. Valois and Sasyniuk (1990, submitted) have investigated the role of acidosis on the characteristics of triggered activity. They found that acidosis prolongs action potential duration and slows the spontaneous cycle length which permits characterization of triggered activity during bradycardia.

The generation of triggered activity with disopyramide may occur by a mechanism similar to that with the Class Ia drug quinidine. Both drugs induce triggered activity at low therapeutic doses associated with torsade de pointes arrhythmias *in vivo* (Valois and Sasyniuk, 1987; Stratmann and Kennedy, 1987). Quinidine blocks the time-dependent outward potassium current ($i_K$) at negative resting membrane potentials (Roden et al., 1988). This effect combined with further reductions in outward repolarizing current due to low rates and hypokalemia (Gadsby, 1980; Falk and Cohen, 1984; Scamps and Carmeliet, 1989) may be sufficient to allow normal inward currents during repolarization to precipitate triggered activity (Davidenko et al., 1989). In addition, block of sodium channels by quinidine may be attenuated at slower rates (Roden and Hoffman, 1985). Disopyramide has also been shown to reduce $i_K$ in Purkinje fibers (Coraboeuf et al., 1988). Although the time- and voltage-dependent reduction of this current by disopyramide remains to be elucidated, a pronounced resting block of these channels may account for reverse use-dependent effects on action potential duration and contribute to induction of triggered activity at slow stimulation rates

As in previous experimental models (Sasyniuk et al., 1989), a marked prolongation of action potential duration in association with bradycardia was required for the induction of triggered activity with disopyramide. Once induced, there was a greater incidence of triggered action potentials at longer cycle lengths which occurred at shorter coupling intervals and more positive membrane potentials. These characteristics are similar to effects observed with the high membrane potential EADs and triggered activity induced by cesium (Damiano and Rosen, 1984), and indicate that there is an increase in the net inward current during repolarization with longer cycle lengths.

Addition of the Class Ib drug mexiletine readily eliminated triggered action potentials. Roden et al. (1987) attributed the abolition of qunidine-induced triggered activity by mexiletine to shortening of action potential duration and\or blocking of the inward current generating triggered activity. Our results show that mexiletine is also effective in abolishing triggered activity induced by disopyramide and suggest that there is first a reduction of excitability so that EADs are unable to produce triggered action potentials. This is then followed by a shortening of action potential duration and elimination of EADs.

MND was effective in prolonging the cycle length at which triggered activity first appeared. In contrast to shortening of action potential duration at normal heart rates, in 2 experiments MND prolonged repolarization during bradycardia under the conditions used to induce triggered activity with disopyramide (unpublished observations).

Prolongation with MND was much smaller than with disopyramide and did not induce triggered activity. Therefore, its effects on disopyramide-induced triggered activity was most likely mediated by a reduction of an inward current involved in the generation of the triggered impulse. MND may possibly reduce the TTX-sensitive sodium "window" current (Attwell et al., 1979; Coraboeuf et al., 1979), which would account for shortening of action potential duration at normal heart rates. However, reduction of this current was not sufficient to prevent triggered activity at longer cycle lengths at which the net inward current would be greater. Also, since MND has been suggested to block activated sodium channels (Toy and Sasyniuk, 1990a), its effectiveness to reduce sodium currents may be attenuated due to voltage-dependent as well as time-dependent recovery at longer cycle lengths.

Nevertheless, this is the first time that a metabolite has been shown to reduce such arrhythmogenic effects of an antiarrhythmic drug. The metabolites of quinidine may enhance its arrhythmogenic potential since several metabolites can also produce EADs (Thompson et al., 1987). In the case of procainamide, only the metabolite NAPA has been shown to induce triggered activity (Dangman and Hoffman, 1981).

In summary, the combination of disopyramide and MND produced additive effects on $\dot{V}_{max}$ and action potential duration at clinically relevant concentrations and heart rates. Our results suggest that MND may contribute to the clinical efficacy of disopyramide by enhancing $\dot{V}_{max}$ depression, thereby further slowing conduction, and prolongation of

refractoriness. However, disopyramide displays reverse use-dependent effects on action potential duration in Purkinje fibers which under predisposing electrolyte conditions and bradycardia induced EADs and triggered activity. Reverse use-dependent effects on action potential duration may reduces the Class III antiarrhythmic actions of disopyramide during tachycardias as well as rendering it proarrhythmic at slow heart rates. The effects of MND, however, suggest that it may be beneficial against triggered arrhythmias.

# Chapter 4

# General Summary and Conclusions

## General Summary and Conclusions

Metabolites can play a role in the clinical efficacy or toxicity of antiarrhythmic drug therapy (Roden et al., 1980; Barbey et al., 1988a). With the exception of decreasing the maximum pacing frequency and reversing experimentally-induced fibrillation in atrial muscle (Baines et al., 1976; Grant et al., 1978; Dubray et al., 1986), little is known concerning the electrophysiological actions of MND, the major metabolite of disopyramide in man.

We have shown that MND depresses $\dot{V}_{max}$ of the action potential in a concentration and rate dependent manner in canine Purkinje fibers suggesting that it blocks cardiac sodium channels. At clinically relevant concentrations, depression of $\dot{V}_{max}$ resides mainly from rate dependent actions with minimal voltage dependent effects. The kinetics of onset and recovery with MND are faster than with disopyramide. However, the potency of rate-dependent $\dot{V}_{max}$ depression by MND ranges from only one-half to two-thirds compared to disopyramide. MND shortens action potential duration in Purkinje fibers, but lengthens repolarization and refractory periods in ventricular muscle. Differential effects on action potential duration in Purkinje fibers versus ventricular muscle decreases the disparity in durations between them The prolongation of refractory periods in muscle may explain the decrease in maximal pacing frequency in atrial muscle observed previously with MND (Grant et al., 1978; Dubray et al., 1986).

The combination of disopyramide and MND produces an additive depression of $\dot{V}_{max}$ at clinically relevant concentrations. Additive interactions are produced by the combination on tonic and rate-dependent $\dot{V}_{max}$ depression at normal to fast heart rates. In our study, both compounds shortened action potential duration at normal stimulation rates, and in the presence of the combination a net additive shortening of action potential plateau was also observed. Disopyramide produces a rate dependent prolongation of the effective refractory period of three sequential extrasystoles but not the basic beat in Purkinje fibers. In ventricular muscle, disopyramide produces an increase in effective refractory period of the basic beat in addition to the three extrasystoles. Effects in both tissues are accentuated in the presence of the combination. Our results demonstrate *in vitro* that the electrophysiological actions of MND enhance and contribute to those produced by disopyramide in the presence of the combination.

Only additive interactions are observed with the combination of disopyramide and MND at clinically relevant concentrations and heart rates. Kohlhardt and Seifert (1985) found additive effects when they investigated the combination of quinidine and propafenone. In their study, additive tonic and rate-dependent block with the combination occurred because the two drugs had similar onset and recovery kinetics for rate-dependent block. Therefore, additive effects at normal heart rates in our study with the combination of disopyramide and MND are not surprising since both compounds produce a depression of $\dot{V}_{max}$ which

recovers minimally over the clinical range of heart rates. The effects of the combination resembles that of an increase in the concentration of either drug.

Therapeutic synergy as described by Hondeghem and Katzung (1980) has been shown with combinations of Class Ia and Ib agents such as quinidine plus tocainide (Valois and Sasyniuk, 1987) or mexiletine (Roden et al., 1987). Due to different kinetics of unblocking, these combinations allow more effective depression of premature beats than either drug alone, but at normal heart rates depression produced by the faster Ib drug is reduced during diastole. This type of interaction, however, is not possible with the combination of disopyramide and MND (at least at clinically relevant heart rates). Despite different kinetics of unblocking, these drugs recover so slowly that recovery from rate-dependent block for both is minimal during clinical diastole.

Antagonistic interactions resulting from competitive displacement such as with the combination of lidocaine and its metabolite (Bennett et al., 1988) is also not possible. Although competitive displacement can occur between disopyramide and MND, there would not be a subsequent reduction in the magnitude of depression of $\dot{V}_{max}$ because both drugs recover very slowly. In addition, antagonistic interactions require that each drug bind to a large fraction of sodium channels so that they may interfere with each other. At clinically relevant concentrations, however, the probability that two drug molecules interact simultaneously with the same receptor would be relatively small.

Finally, since disopyramide has a greater affinity for activated channels (Kodama et al., 1986 and 1987; Gruber and Carmeliet, 1989), shortening of action potential duration by MND would probably not affect depression of $\dot{V}_{max}$ produced by the parent drug.

The accumulation of significant plasma levels of MND during disopyramide therapy suggest that it may play a role in both the antiarrhythmic and toxic effects ascribed to disopyramide. Additive depression of $\dot{V}_{max}$ would occur both during normal rhythms and during a rapid tachycardia with greater depression occurring during the tachycardia. This would result in greater depression of conduction velocity both during a normal heart rate and during rapid tachycardia. Additive effects would also be observed on refractory periods. These actions should be beneficial in the abolition and prevention of reentrant ventricular arrhythmias. In fact, the mechanism of termination of reentrant excitation by disopyramide in one model was correlated with the depression of conduction (Spinelli and Hoffman, 1989). Furthermore, studies with the new antiarrhythmic agent penticainide (a disopyramide derivative) which produces electrophysiological effects similar to MND in canine ventricular tissue (Gautier et al., 1987), show that drugs with this pharmacodynamic profile are effective against chronic ventricular arrhythmias in patients (Priori et al., 1987).

However, toxicity may occur if there is excessive depression of conduction. At clinically relevant stimulation rates, recovery of $\dot{V}_{max}$ depression is minimal for both disopyramide and MND. An increase in

drug concentration can readily lead to marked slowing of conduction or conduction block. Too much depression of conduction velocity or unidirectional block could lead to or potentiate reentry and hence drug toxicity as is typically seen with Class Ic antiarrhythmic agents such as encainide or flecainide (Roden et al., 1990). Development of sustained ventricular arrhythmias have been observed previously during treatment with disopyramide (Kudenchuk et al., 1990).

As a result of reverse use-dependence, the potential Class III properties of disopyramide may be least effective during tachycardias while maximum prolongation of action potential duration would occur during bradycardia or after a long diastolic interval. Hondeghem and Snyders (1990) have suggested that reverse use-dependence may even render drugs proarrhythmic, especially after long diastolic intervals. Disopyramide has been associated with the production of prolonged QT syndrome arrhythmias such as torsade de pointes following therapeutic doses (Casedevant and Sabaut, 1975; Tzivoni et al., 1981; Schweitzer and Mark, 1982; Riccioni et al., 1983).

Although therapeutic concentrations of disopyramide shortened action potential duration at normal heart rates, we found that it produced a pronounced prolongation of repolarization following a very long diastolic interval and thus exhibits reverse use-dependent effects. Abnormal impulse generation in the form of early afterdepolarizations and triggered activity occurs following marked prolongation of action potential duration with disopyramide in the presence of slow heart rates, hypokalemia and acidosis. Pacing at normal heart rates or

addition of a therapeutic concentration of mexiletine abolishes early afterdepolarizations and triggered activity. Our study shows that triggered activity can be induced by disopyramide *in vitro* and provides a possible mechanism for the genesis of torsade de pointes arrhythmias with disopyramide *in vivo*.

The metabolite, however, might reduce this arrhythmogenic potential of the parent drug. Addition of a clincially relevant concentration of MND altered the incidence of disopyamide-induced early afterdepolarizations and triggered activity to longer cycle lengths. A similar effect *in vivo* may be beneficial in altering the conditions such that initiation of prolonged QT syndrome arrhythmias such as torsade de pointes becomes more difficult. This may explain why the incidence of torsade de pointes is less with disopyramide than it is with, for example, quinidine (Stratmann and Kennedy, 1987) In contrast, only potential arrhythmogenic effects have been observed previously with the metabolites of other antiarrhythmic drugs which produce triggered activity *in vitro* (Dangman and Hoffman, 1981; Thompson et al., 1987)

Future studies will be required to confirm our *in vitro* finding in *in vivo* models. The arrhythmogenic potential of disopyramide must be tested *in vivo* under the same conditions which precipitate long QT syndrome arrhythmias such as torsade de pointes in patients This may provide further evidence for the involvement of triggered activity in torsade de pointes and may potentially lead to development of an *in vivo* model of torsade de pointes using an antiarrhythmic drug. To date, two *in vivo* models have been available for the study of torsade de pointes

and involve the use of cesium (Brachmann et al., 1983) or anthopleurin-A (El-Sherif et al., 1988). The beneficial effects of MND against drug-induced triggered rhythms must also be confirmed using such *in vivo* models Furthermore, the discrepancy between the rate of arrhythmias *in vitro* versus events *in vivo* must be resolved before torsade de pointes arrhythmias can be attributed to triggered activity. The effects of factors such as sympathetic stimulation *in vivo* (Ben-David and Zipes, 1988) on the initiation and incidence of triggered activity or torsade de pointes must be considered. The answers to these questions will provide further insight into the mechanisms responsible for the antiarrhythmic and arrhythmogenic potential of disopyramide and the role of MND during therapy.

# Chapter 5

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