

STERESELECTIVE HPLC ANALYSIS, PHARMACOKINETICS, TISSUE DISTRIBUTION AND
PHARMACODYNAMICS OF MEXILETINE ENANTIOMERS

By

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ABSTRACT

Mexiletine [(2',6'-dimethylphenoxy)-2-amino propane] is a class I antiarrhythmic agent with a similar chemical structure and electrophysiological effects to those of lidocaine. It is a chiral drug which is used clinically in the racemic form (i.e. 50:50 ratio of two enantiomers). This thesis describes the stereoselective HPLC analysis, pharmacokinetics, tissue distribution and pharmacodynamics of mexiletine enantiomers.

The development of a highly sensitive and stereoselective HPLC assay for mexiletine enantiomers, using 2-anthroyl chloride as a derivatization reagent, was attempted. The synthesis and characterization of the acid chloride was successfully carried out. The 2-anthroyl derivatives of the enantiomers were resolved on a Pirkle^R ionic (phenyl glycine) chiral column using a mobile phase of ethyl acetate/2-propanol/Hexane (4:6:90). Detection was accomplished by fluorescence (ex = 270 nm, em = 400 nm) with a lower limit of 0.5 ng/ml. However, there was an interfering peak coeluting with S(+)-mexiletine which could not be resolved. This precluded the use of the assay for the proposed pharmacokinetic and pharmacodynamic studies. A previously developed stereoselective HPLC method, with 2-naphthoyl chloride as a derivatization reagent, was subsequently used.

The *in vitro* protein binding of mexiletine enantiomers was examined with human serum, lipoprotein deficient serum, albumin and α_1 -acid glycoprotein. The binding of the enantiomers to human serum was moderate (45 to 50%) within the therapeutic range of mexiletine. This binding was due, mainly, to albumin and α_1 -acid glycoprotein. The free fractions of the enantiomers decreased significantly ($P < 0.05$) as pH was increased from

7.0 to 8.0. Stereoselective binding was apparent at pH 8.0 such that the free fraction of S(+)-mexiletine was significantly ($p < 0.05$) greater than that of the R(-)-enantiomer. However, stereoselective binding was not observed at physiological pH (≈ 7.4). These results indicated that the serum binding of mexiletine enantiomers is pH-dependent. Binding was not concentration-dependent, nor was there any competitive binding interaction between the enantiomers, within the therapeutic range. Scatchard analysis of the binding data obtained with serum and albumin both showed the presence of 2 classes of binding sites. A high affinity, low capacity site and a low affinity, high capacity site. In contrast, α_1 -acid glycoprotein showed only 1 class of binding sites and this was a high affinity, low capacity site.

Pharmacokinetic and tissue distribution studies in rats following the administration of racemic mexiletine (10 mg/kg) indicated extensive tissue uptake and rapid elimination of the enantiomers. R(-)-Mexiletine showed a 32% greater systemic clearance (161.8 ml/min/kg vs 122.9 ml/min/kg) than the S(+)-enantiomer. The steady state volume of distribution was also greater for the R(-)-enantiomer (9.0 L/kg vs 7.4 L/kg), while the elimination half-lives of the enantiomers (1.4 and 1.3 h for R(-)- and S(+)-mexiletine, respectively) were not different. Maximum tissue concentrations were observed at 5 min in all the tissues studied (heart, brain, lung, kidney, liver and fat). These concentrations were not significantly different, except for the liver tissue where a 2.4-fold greater concentration of the S(+)-enantiomer was found. High tissue/serum ratios (>20) were observed for each enantiomer in the brain, lungs and kidneys. The brain accumulated 3-fold the heart concentrations of the

enantiomers.

Pharmacodynamic studies on the relative antiarrhythmic effects of racemic mexiletine and its enantiomers were carried out using electrical and ischaemia-induced arrhythmias in rats. Racemic mexiletine and its enantiomers significantly ($P < 0.05$) increased VFT and ERP. However, the differences between the effects of the 3 drugs on these variables were not statistically significant. R,S-, S(+)- and R(-)-mexiletine caused significant bradycardia and PR prolongation in both pentobarbitone anaesthetized and conscious rats. These effects of the drugs were also not significantly different from each other. In the ischaemic conscious rats, the 3 drugs did not significantly reduce the incidence of VT and VF, the number of PVCs nor the "arrhythmia score" when compared to saline (control). Racemic mexiletine and its enantiomers produced comparable CNS toxicity in the conscious rats.

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SYMBOLS AND ABBREVIATIONS

AAG	α_1 -Acid glycoprotein
ANOVA	Analysis of variance
AP	Action potential
ARP	Absolute refractory period
AUC	Area under the plasma concentration-time curve
AUC _T	Area under the tissue concentration-time curve
AV	Atrio-ventricular
C	Centigrade
C.V.	Coefficient of variation
CL	Systemic clearance
CL _{int}	Intrinsic clearance
CNS	Central nervous system
CSP	Chiral stationary phase
ECG	Electrocardiogram
EI	Electron impact
em	Emission
ERP	Effective refractory period
ex	Excitation
f _f	Free fraction
GLC	Gas-liquid chromatography
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
HSA	Human serum albumin
ht	Height
i.d.	Internal diameter
i.p.	Intra-peritoneal

I.S.	Internal standard
i.v.	Intravenous
IU	International unit
LAD	Left anterior descending
LP	Lipoprotein
mex	Mexiletine
MFF	Maximum following frequency
min	Minutes
ml	Millilitre
MP	Membrane potential
MS	Mass spectrometer
msec	Milliseconds
ng	Nanogram
NSVF	Non-spontaneously reverting ventricular fibrillation
OZ	Occluded zone
PE	Polyethylene
PTFE	Polytetrafluoroethylene
PVC	Premature Ventricular Contraction
R	Resolution
r^2	Coefficient of determination
RRP	Relative refractory period
R_t	Retention time
β	First order elimination rate constant
s.d.	Standard deviation
s.e.m	Standard error of the mean
sec	Seconds
SVF	Spontaneously reverting ventricular fibrillation

UV	Ultraviolet
VF	Ventricular fibrillation
VFT	Ventricular fibrillation threshold
vs	Versus
V_{ss}	Steady-state volume of distribution
VT	Ventricular tachycardia
wt	Weight
xs	Excess
α	First order distribution rate constant
μl	Microlitre
μg	Microgram

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DEDICATION

This thesis is dedicated to Dr. J.D. Kulkarni, who greatly influenced my decision to pursue graduate studies.

1. INTRODUCTION

1.1 Sudden Cardiac Death

1.1.1 Overview

Sudden cardiac death has been defined as death occurring from coronary heart disease within 1 hour of last being seen alive (Oliver, 1982). Amstrong *et al.* (1972) reported that 43% of all deaths occurring within 4 weeks of the onset of a heart attack took place within one hour and that less than 10% of these patients were seen by a medical person. Sudden cardiac death is the leading cause of death in most of the industrialized nations. According to a report by Lown and Graboys (1977), about 450,000 such deaths (nearly 25 % of all deaths) occur annually in the United States alone.

The cause of sudden cardiac death has been identified as ventricular fibrillation (VF) (Smith 1939; Miller, 1939; Bashe *et al.*, 1975; Schaffer and Cobb, 1975; Lown and Graboys, 1977; Oliver, 1982; Adgey, 1982) and sudden cardiac death and VF are viewed as being synonymous by many physicians (Oliver, 1982). VF is the most severe form of ventricular arrhythmias, characterized by a chaotic rapid contraction of the ventricular muscle fibres with no blood being pumped. It was first described by Hoffa and Ludwig (1850) who reported that electrical stimulation of the mammalian heart led to VF and death. McWilliam in 1889 suggested that VF was the likely cause of sudden cardiac death in patients with Angina Pectoris. The first electrocardiogram (ECG) of VF in the clinical setting was obtained in 1911 by Hoffman. However, it was Smith (1939) and Miller (1939) who first reported VF as the cause of sudden cardiac death in man following

myocardial infarction (MI). Acute regional ischaemia has been recognized as the basis for the development of ventricular arrhythmias leading to sudden cardiac death (Oliver, 1982). This is consistent with the finding that many patients dying suddenly had extensive arterosclerosis of one or more coronary vessel(s) upon postmortem examination (Kuller *et al.*, 1975; Perper *et al.*, 1975). However, sudden cardiac death is not always associated with identifiable MI or coronary thrombosis at autopsy (Lovegrove and Thompson, 1978). In fact, ECG evidence of MI is present in less than 20% of those patients surviving a VF attack (Cobb *et al.*, 1980). This has lead to the suggestion that vasospasm, followed by sudden reperfusion, may be a mechanism for the induction of ventricular fibrillation in man (Hellstrom, 1979; Lown, 1979). Coronary artery spasm has been clinically demonstrated in man (Oliva *et al.*, 1973; Maseri *et al.*, 1975), and the induction of VF by coronary artery occlusion, followed by sudden reperfusion, is well established in animals (Tennent and Wiggers, 1935; Penkoski *et al.*, 1978; Stockman *et al.*, 1979; Kabell *et al.*, 1980).

Epidemiological studies of survivors of MI have shown that these patients are at the greatest risk of developing VF where chronic ectopic activity is present (Coronary Drug Project Research Group, 1973; Hinkle, 1981; The Multicenter Post-Infarction Research Group, 1983). As a result of these and other studies, many drugs have been developed for the treatment and prevention of arrhythmias. However, ineffectiveness against all forms of arrhythmias, toxicity, and sometimes unfavourable pharmacokinetics have limited the therapeutic benefit of these agents. For example, quinidine in therapeutic doses may induce ventricular tachycardia and ventricular fibrillation (Selzer and Wray, 1964; Koster

and Wellens, 1976). Lidocaine, another antiarrhythmic agent, is not administered orally due to high hepatic first-pass metabolism (Singh et al., 1981). Thus, many novel drugs are being developed and tested in the continuing search for antiarrhythmic agents with better benefit-to-risk ratio, as well as convenient dosing. The subject of the present study, mexiletine, is one of the newer antiarrhythmic agents which was approved for clinical use in Canada in 1985.

1.1.2 Arrhythmias

1.1.2.1 The Cardiac Action Potential

Understanding the ionic events which take place across cardiac cell membranes during each cardiac cycle i.e action potential (AP) is necessary in rationalizing the mechanisms of formation of arrhythmias, and also the possible mechanisms of action of antiarrhythmic drugs. These ionic events have been extensively reviewed (Cranefield, 1975; Hauswirth and Singh, 1979; Perry and Illsley, 1986). The resting membrane potential (MP) of most cardiac cells is about -80 to -90 mV and is supported principally by sodium and potassium ions. By means of an active transport mechanism involving the Na^+/K^+ ATPase (sodium pump), the cell maintains a high intracellular-to-extracellular potassium concentration and a low intracellular-to-extracellular sodium concentration. At rest, the cardiac cell membrane is readily permeable to potassium ions and relatively impermeable to sodium, calcium, and chloride ions. Thus, excess intracellular K^+ can diffuse out of the cell unaccompanied by negatively charged ions, thereby, producing the above mentioned negative potential across the membrane (i.e. cells are negative on the inside with respect to the outside).

The action potential is composed of five phases that represent the changing ionic fluxes and membrane potentials of the cardiac cell. Reduction of the membrane potential to a threshold voltage, -60 to -70 mV, due to an applied stimulus or current spreading from pacemaker cells, evokes the fast inward depolarizing current carried by sodium ions (phase 0 of the action potential). The intensity of this inward current is reflected in the maximal rate of depolarization (MRD) of the transmembrane AP. Depolarization to levels positive to -55 mV inactivates the fast sodium channels. However, the membrane continues to depolarize to a level of +25 to +30 mV due to the rapid rate of depolarization from the initial portion of phase 0. A second inward current (the slow inward current) carried mostly by calcium ions and activated at a MP of about -35 mV also exists. The kinetics of this current, in terms of activation, inactivation and reactivation, are much slower than those governing the sodium current. After depolarization, repolarization takes place in three phases (phases 1, 2 and 3). Phase 1 results partly from inactivation of the fast inward current and partly from an inward movement of chloride ions. Phase 2 is characterized by inward Ca^{++} and Cl^- currents and a slow outward K^+ current. Very little net charge flows across the membrane at this point which results in the characteristic plateau of the action potential. Phase 3 is typified by inactivation of the Ca^{++} current and a rapid efflux of K^+ leading to full repolarization. Phase 4 represents the period between action potentials i.e. the resting phase of the cell. The automatic cardiac fibers (section 1.1.3.1) undergo spontaneous depolarization during phase 4.

There are two types of cardiac fibres, namely the fast and the

slow fibres. The fast fibres are the atrial and ventricular specialized conducting tissues, the distal AV node, and atrial and ventricular muscle (Wits *et al.*, 1974a; Zipes, 1984). These fibers generate fast inward current AP as described above and are characterized by rapid conduction velocity. They are sensitive (blocked) to increased extracellular potassium concentration, tetrodotoxin and local anaesthetics. The slow fibres are found in the proximal AV node, SA node, fibres of the AV ring and the mitral and tricuspid valve leaflets (Wits *et al.*, 1974a; Zipes, 1984). They generate a slow inward current AP which is mediated by calcium, has a resting membrane potential of -40 to -60 mV and are activated at a threshold of -30 to 40 mV. The AP is blocked by manganese and calcium antagonists.

The MRD during an action potential is a function of the membrane potential from which the AP takes off. The more negative the MP, the greater the MRD. This relationship is known as membrane responsiveness (Singh *et al.*, 1981). During an action potential, there is a period of time during which a second action potential cannot be produced. This period is referred to as the refractory period (Perry and Illsley, 1986). The absolute refractory period (ARP) corresponds to the period during which a stimulus cannot produce any degree of depolarization. At MP below -50 mV, transient depolarization may occur but an action potential cannot be propagated, this represents the effective refractory period (ERP). Below -60 mV a stronger than normal stimulus may generate an action potential of low amplitude. This corresponds to the relative refractory period (RRP). Action potentials generated during this period are conducted slowly, consequently, the heart is prone to arrhythmia formation (Perry and Illsley, 1986).

1.1.2.2 Electrophysiological Changes During Acute Myocardial Ischaemia

It has long been established that myocardial cells release K^+ during acute ischaemia (Harris, *et al.*, 1954). Hill and Gettes (1980) and Hirche *et al.* (1980) reported increased extracellular K^+ within 15 seconds of coronary artery occlusion in pigs (a 3-fold increase was observed by 5-8 min). The increase in extracellular K^+ was accompanied by a decrease in the resting MP due to the changed gradient of K^+ . The observed loss of K^+ from ischaemic cells has been speculated to be due to anoxia-induced increase in K^+ conductance or inhibition of the sodium pump. Simultaneous to the increase in extracellular K^+ is a decrease in the AP amplitude, MRD and action potential duration (APD). These changes would be expected to result from the decreased resting MP. However, Morena *et al.* (1980) showed that a decrease in resting MP only could not account for the marked depression of the AP upstroke characteristics observed during acute ischaemia. The results of their work indicated that the combination of lack of O_2 and substrate and lack of washout (increased extracellular K^+ and acidosis) is necessary. This combination of factors was shown to have an extra-depressant effect on the ionic mechanisms responsible for the generation of the AP upstroke. The currents generating the depressed upstroke were investigated by Cardinal *et al.* (1981) and found to be a "depressed fast response". This conclusion was deduced from results obtained in isolated hearts which showed: a) lidocaine had an extra-depressant effect on AP upstroke characteristics, and b) no response could be elicited when the resting MP was depolarized to -55 mV, at which level the fast Na^+ channel is totally inactivated. However, there is evidence that the

slow inward current is equally depressed by ischaemia (Janse, 1982). As a consequence of the reduction in APD, the ERP would be expected to be shortened. However, ERP is prolonged in acutely ischaemic cells since ERP continues beyond the point of full repolarization. This phenomenon is known as post-repolarization-refractoriness (Downar *et al.*, 1977; Lazzara *et al.*, 1978). It is important to note that these electrophysiological changes occur in different ischaemic cells to different degrees. Downar *et al.* (1977), using pig subepicardial muscle, noted that groups of cells with a difference of only a few millivolts in their resting MP varied in ERP from 180-500 msec. Thus, at a certain coupling interval, the least depolarized cell group may exhibit an AP with a low upstroke velocity which will propagate very slowly; whereas the cells with a slightly lower resting MP are at the same time inexcitable, and complete conduction block may occur. Therefore, slow conduction and conduction block, both of which are necessary conditions for re-entrant arrhythmias (section 1.1.2.3 below), are present during acute ischaemia.

1.1.2.3 Arrhythmogenesis

Arrhythmias result from disturbances in normal impulse initiation (automaticity), impulse conduction, or both (Hoffman and Rosen, 1981). Arrhythmias include ectopic beats, tachycardia, A-V block and fibrillation.

1.1.2.3.1 Impulse Initiation (Automaticity)

Automaticity is the ability of a cardiac fibre to depolarize spontaneously and generate an action potential (Wit *et al.*, 1974a; Vera

and Mason, 1981). Autonomic fibres include the SA node, distal AV node, His-Purkinje fibres, specialized atrial fibres, mitral and tricuspid valve leaflets. The SA node is normally the dominant fiber (pacemaker) and establishes the rate of impulse generation. Abnormal automaticity results when a heart tissue other than the SA node takes over the pacemaker role. The site of pacemaker activity may be shifted by 3 factors that affect the rate of depolarization: a) level of maximum diastolic potential, b) level of threshold potential and c) slope of phase 4 of the action potential (Wit *et al.*, 1974a; Vera and Mason, 1981). For example, vagal stimulation inhibits SA node automaticity (by decreasing phase 4 slope and by making the diastolic potential more negative) without affecting the Purkinje fibres. Thus pacemaker activity may be shifted to these fibres. Ischaemia increases phase 4 slope and shifts the maximum diastolic potential to a more positive value, thus enhancing automaticity.

1.1.2.3.2 Triggered Automaticity

Considerable attention has also been directed towards the phenomenon of triggered activity in the genesis of certain types of arrhythmias (Spear and Moore, 1982). Triggered automaticity has so far only been demonstrated *in vitro* in quiescent cardiac cells (Ferrier *et al.*, 1973). Certain critical characteristics of arrhythmias evoked by triggered automaticity closely resemble those induced by re-entry, notably the coupling of depolarization to a preceding beat. The after-depolarizations responsible for triggered automaticity are associated with elevated intracellular Ca^{++} concentrations. This provokes the oscillatory release of Ca^{++} from the sarcoplasmic reticulum and

activates membrane channels that permit the passage of Na^+ and K^+ . The net flux of these cations constitute a transient inward current that is responsible for the depolarization of the cell membrane. The amplitude of the after-depolarization is, thus, increased by factors that raise the intracellular Ca^{++} concentrations; such as elevated extracellular Ca^{++} or toxic levels of digitalis glycosides.

1.1.2.3.3 Impulse Conduction (Re-entry)

The concept of circus movement or re-entry as a mechanism underlying sustained arrhythmias was first introduced by Mines in 1913. Recent research (Allessie *et al.*, 1973; 1976 & 1977) has provided direct evidence for the existence of re-entrant circuits. Re-entrant arrhythmias are characterized by areas of unidirectional block such that the propagation of the normal impulses is blocked in an antegrade direction and is conducted via an alternate pathway. If conduction along this pathway is slow enough to allow the zone of block to recover excitability, then retrograde conduction through the block zone will occur and the wavefront will re-excite fibers at the site of origin of the impulse (Wit *et al.*, 1974b; Vera and Mason, 1981). This type of re-entry is referred to as macro re-entry. The conduction pathway will be functionally long if conduction is depressed. On the other hand, if the refractory period in the antegrade pathway is shortened, then the re-entrant circuit need not be long. The later type of re-entry is called micro re-entry (Sasyniuk and Mendez, 1971; Kramer *et al.*, 1985). The re-entry phenomenon can result in either single premature beats or repetitive ectopic activity (Perry and Illsley, 1986). Conduction is sufficiently slow in cardiac fibers with slow-current action potential

(SA and AV nodes) to allow re-entry to occur, or in fast fibers where normal fast current action potential has been slowed by disease or drugs (Witt *et al.*, 1974b).

1.1.2.4 Mode of Action of Antiarrhythmic agents.

Most antiarrhythmic agents have major electrophysiological effects on myocardial cells. Based on these effects, they have been classified into 4 main groups (Vaughan Williams, 1974 & 1975).

Class I antiarrhythmic drugs have a local anaesthetic action on the nerve and myocardial cell membranes. However, their effects on cardiac cells are often observed at lower concentrations than those on nerves. Drugs in this group block the fast inward current carried by Na^+ , thus depressing the MRD in cardiac tissues. The reduction in MRD is associated with an increase in the threshold of excitability, a depression in conduction velocity, a prolongation in the effective refractory period and an inhibition of spontaneous diastolic depolarization in automatic fibres (Singh, 1978). These actions lead to the suppression of automaticity or re-entrant arrhythmias. Class I agents include mexiletine, tocainide, quinidine, lidocaine, disopyramide, encainide, flecainide and propafenone. A further classification of these agents has been proposed based on their effects on the cardiac action potential (Vaughan Williams, 1974 & 1975). Quinidine, procainamide and disopyramide which prolong the action potential duration form class IA. The class IB drugs shorten the action potential duration and include mexiletine, tocainide and lidocaine. Flecainide and encainide which have little effect on the action potential duration make up class IC. It is interesting to note

that a more recent classification based on the kinetics of the interaction of these drugs with the fast sodium channels (Vaughan Williams, 1984) produced a similar grouping to that above.

The class II antiarrhythmic agents consist of drugs that antagonize cardiac sympathetic drive, either by pre-synaptic receptor blockade or by a central action (Jewitt and Singh, 1974). Clinically, life threatening arrhythmias often occur associated with over-activity of sympathetic out-flow (Winslow, 1984). Catecholamines can exacerbate after-potentials in damaged myocardium and increase the slope of diastolic depolarization, thus, provoking tachyarrhythmias (Jewitt and Singh, 1974). The major clinical electrophysiologic effect of β -blockers is the depression of phase 4 depolarization (Singh *et al.*, 1981). However, long term treatment with β -blockers has been reported to produce a large prolongation of atrial and ventricular APD (Vaughan Williams, 1978), which may in itself be antiarrhythmic. Examples of drugs in this class are propranolol, practolol and sotalol.

Class III drugs delay repolarization, thereby, causing a prolongation of the APD and ARP (Singh and Vaughan Williams, 1970; Vaughan Williams, 1977). Drugs in this class include amiodarone and bretylium.

Class IV antiarrhythmic agents restrict the slow inward current carried by calcium (Singh and Vaughan Williams, 1972a; Singh, 1978). They are referred to as calcium antagonists and include verapamil, nifedipine and diltiazam. The action potentials in all cardiac tissues, apart from the SA and AV nodes, are normally mediated by fast inward sodium current. However, in ischaemia or other situations where partial diastolic depolarization and inactivation of fast current occur, the

slow inward current may take over, permitting the activation of slowly conducting AP which can initiate re-entrant arrhythmias. The antiarrhythmic action of calcium antagonists is attributed to abolishing these abnormal slow depolarizations.

1.1.2.5 Experimental Arrhythmias

Arrhythmogenic stimuli in animals fall into 3 main categories: electrical, chemical and mechanical (Winslow, 1984).

1.1.2.5.1 Electrically-induced arrhythmias.

The induction of arrhythmias by the application of electric current is based on the premise that following normal excitation, there is a period of inhomogeneity of recovery of excitability in the cardiac fibers, i.e. the vulnerable period (Mines, 1913; Moe *et al*, 1964). This corresponds to the downward slope of the T-wave in the ECG. An extra-stimulus of sufficient intensity applied during this period will precipitate fibrillation. Antiarrhythmic drugs are expected to increase the intensity of the current necessary to evoke fibrillation. Fibrillation has been produced by single or serial shocks with progressively increasing intensity. The use of electrical threshold for the measurement of antiarrhythmic effects has many advantages. Its effects are fully reversible (except for extremely intense stimuli), the analogy with the natural impulse is closer than all the other methods, each animal serves as its own control, and the parameters characterizing the stimulus (strength and duration) can be accurately determined and controlled (Szekeres and Papp, 1971). Details of this arrhythmia model has been described by Wiggers and Wegria (1940) and Han (1969).

1.1.2.5.2 Chemically-Induced Arrhythmias

Many chemical agents alone or in combination are used to induce arrhythmias in animals. Lawson (1968) reported that chloroform alone produced VF in mice. The animals were placed in a closed container with cotton wool soaked in chloroform. Recently, chloroform-induced arrhythmias in rats sensitized with theophylline has been reported (Baker and Erker, 1980). Intravenous aconitine has also been shown to induce arrhythmias in anaesthetized rats (Szekeres and Papps, 1971). Many publications have reported arrhythmias induced by cardiac glycosides (usually ouabain) in animals including guinea pigs (Sekiya and Vaughan Williams, 1963), cats (Raper and Wall, 1968), and dogs (Luchessi and Hardman, 1961). Other chemical agents that are known to be arrhythmogenic include barium chloride, calcium chloride, adrenaline, adrenaline and insulin alone or in combination with glucose and acetylcholine (Winslow, 1984)

1.1.2.5.3 Mechanically-Induced Arrhythmias

Occlusion of a major coronary artery (ischaemia) is known to lead to arrhythmias, including VF, in various animals. The most popular ischaemia model is the Harris two-stage coronary artery occlusion technique in the dog (Harris, 1950). In this model, an initial partial occlusion of the left anterior descending (LAD) coronary artery was performed, followed 30 min later by complete occlusion. Arrhythmias develop within 4-7 h and reach a peak 24-48 h after occlusion. The two-step occlusion avoids the development of acute VF (Szekeres and Papp, 1971) and is mainly used for the study of late arrhythmias. Ischaemia-induced arrhythmias have also been reported in the cat (Ritchie *et al.*,

1977 & 1979). Meesman and co-workers developed a model of early arrhythmias in the dog in which mortality from VF was 100% (Meesman *et al.*, 1970; Abendroth *et al.*, 1977; Menken *et al.*, 1979). This involved occlusion of the left circumflex coronary artery under morphine-urethane-chlorolose anaesthesia. In the pig, whose coronary tree resembles that of man, occlusion of the LAD coronary artery results in death within 2 hours from VF (Verdouw *et al.*, 1978). However, by lowering blood flow to 25% of normal, a less severe but consistent model was developed. Early studies on ischaemic arrhythmias in anaesthetized rats were carried out by Heimbürger (1946) and Selye *et al.* (1960). In this model, a silk suture was placed around the LAD coronary artery following left thoracotomy (under anaesthesia) and the artery occluded. Consistent arrhythmias which included premature ventricular contractions, ventricular tachycardia and fibrillation were produced. This model has been utilized to assess the antiarrhythmic actions of several agents (Au *et al.*, 1979; Clark *et al.*, 1980; Kane *et al.*, 1980). Recently, ischaemic arrhythmias have been demonstrated in conscious chronically prepared rats (Johnston *et al.*, 1983; Curtis *et al.*, 1984 & 1986). This model has the advantage of circumventing the effects of anaesthesia and recent surgery on the outcome of coronary occlusion.

1.1.2.5.4 Reperfusion-Induced Arrhythmias

Arrhythmias have been induced in dogs by sudden reperfusion after complete coronary artery occlusion for 30-45 min (Fiedler *et al.*, 1979; Stockman *et al.*, 1979; Martorana *et al.*, 1980; Kabell *et al.*, 1980). The reported incidence of arrhythmias in this model is 63 to 87%. The arrhythmias are almost immediate in onset, and in the absence of VF

terminate within 10 min. Reperfusion arrhythmias have also been demonstrated in the cat (Penkoske *et al.*, 1978) and in Langendoff perfused rat heart (Lubbe *et al.*, 1978). The electrophysiological basis for these arrhythmias seem to differ from those due to MI (they are associated with a rapid idio-ventricular rate which is usually normal in MI). It has been suggested that the differences in the rates at which cells in the ischaemic zone regain or improve their electrical activity after reperfusion may be the important factor that triggers the arrhythmias (Downar *et al.*, 1977).

1.2 Mexiletine

1.2.1 Chemistry

Mexiletine, 1-(2',6'-dimethylphenoxy)-2-aminopropane, was synthetically derived from the phenethanolamine compound, Phenmetrazine, in an attempt to produce an anorexic agent with reduced CNS side effects (Koppe, 1977). The resultant compound was an agent that exhibited anticonvulsant activity. Subsequent studies in animals showed that mexiletine also had antiarrhythmic properties (Allen *et al.*, 1972). Mexiletine is a basic drug with a pKa of 8.8 (Merck Index, 1983). It is a chiral compound, composed of equal proportions of two enantiomers. Mexiletine is used clinically as the hydrochloride salt of the racemate (Mexitil^R) which is an almost white crystalline substance, soluble in water, methanol, ethanol and chloroform and practically insoluble in ether (Merck Index, 1983).

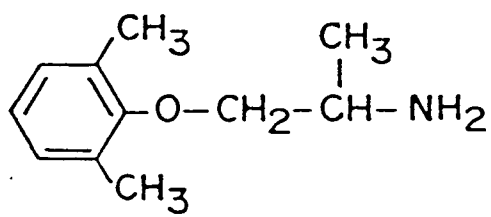


Figure 1. The structure of mexiletine [(2',6'-dimethylphenoxy)-2-amino propane]

1.2.2 Pharmacology

1.2.2.1 Animal Studies

Early studies on the antiarrhythmic effects of mexiletine in animals were carried by Allen *et al.*, (1972) and Singh and Vaughan Williams (1972b). Mexiletine was found to be effective against ventricular arrhythmias induced by digitalis in dogs. The effective plasma concentration was 0.6 $\mu\text{g/ml}$. In the canine 24 h two-stage coronary occlusion arrhythmia, mexiletine administered intravenously restored sinus rhythm at a plasma concentration of 5.3 $\mu\text{g/ml}$. Mexiletine was also shown to be effective against arrhythmias induced by halothane and epinephrine in dogs. Further studies in canine arrhythmia models (Hashimoto *et al.*, 1984) have confirmed the antiarrhythmic efficacy of mexiletine. Minimum plasma concentrations of 1.8, 1.9 and 3.7 $\mu\text{g/ml}$ were associated with suppression of arrhythmias induced by digitalis, coronary occlusion and epinephrine, respectively. In a recent study (Uprichard *et al.*, 1988), mexiletine was not effective against arrhythmias evoked by programmed electrical stimulation in dogs. CNS toxicity limited the dose of mexiletine given to the dogs in this study.

The other pharmacological properties of mexiletine include strong local anaesthetic (Singh and Vaughan Williams, 1972b; Danneberg and Shelley, 1977) and anticonvulsant (Danneberg and Shelley, 1977) actions.

1.2.2.2 Clinical Effectiveness

Numerous human studies have reported the effectiveness of mexiletine against arrhythmias originating from digitalis intoxication,

myocardial infarction and cardiac surgery (Campbell *et al.*, 1973 & 1978a; Talbot *et al.*, 1973 & 1976; Zipes and Troup, 1978; Singh *et al.*, 1980; Bury *et al.*, 1982 and IMPACT, 1984).

1.2.2.3 Plasma Concentration-Clinical Effect Relationships

Talbot *et al.* (1973) reported that mexiletine concentrations of 0.5 to 2.0 $\mu\text{g/ml}$ were associated with greater than 95% reduction of ventricular ectopic beats in 37 patients. However, clear separation between therapeutic and toxic ranges was not observed in this study since adverse effects sometimes occurred at concentrations as low as 0.3 $\mu\text{g/ml}$. The relationship between plasma concentrations of mexiletine and clinical effects was investigated in 149 patients by Campbell *et al.*, (1978a). Suppression of premature ventricular contractions was observed in 77% of the patients when plasma concentration was maintained between 0.74 and 1.0 $\mu\text{g/ml}$. An 80% response was observed when plasma concentrations were 2 $\mu\text{g/ml}$ or above. However, adverse effects developed in 30% of recipients at these levels with 19% developing severe adverse reactions such as hypotension, vomiting, tremor, toxic confusional states and atrioventricular dissociation. Studies on the efficacy of long term oral mexiletine treatment showed adequate suppression of ventricular arrhythmias at serum concentrations of 0.9 to 2.6 $\mu\text{g/ml}$ (Talbot *et al.*, 1976). Further studies during short term oral mexiletine testing in cases with persisting ventricular ectopic beats reported mexiletine levels between 0.38 to 2.76 $\mu\text{g/ml}$ in 30 responders whose premature ventricular contractions were reduced by more than 50%. Effective plasma concentrations during maintenance treatment were in the same range, 0.44 to 2.0 $\mu\text{g/ml}$ (Podrid and Lown, 1981). However, serum

mexiletine concentrations in non-responding patients were not different from those of the responders.

1.2.3 Mechanism of Action

The major electrophysiologic action of mexiletine is blockade of the fast sodium channels. This action results in a decrease in the phase 0 maximal upstroke velocity (V_{\max}) or membrane responsiveness in atrial, ventricular and Purkinje AP (Vaughan Williams, 1977 and Yamaguchi *et al.*, 1979). The decrease in V_{\max} is accompanied by electrophysiologic effects which vary in different tissues. In isolated atrial and ventricular myocardial tissue preparations, mexiletine depressed conduction velocity, increased the threshold of excitability and produced a marked prolongation of the ERP (Allen *et al.*, 1972; Singh and Vaughan Williams, 1972b; Vaughan Williams, 1977; Arita *et al.*, 1979). These alterations, occurring without a significant change in either the resting MP or APD are associated with the depression of spontaneous diastolic depolarization in automatic fibres. In isolated Purkinje fibres of the dog, a concentration dependent decrease in action potential duration was observed (Arita *et al.*, 1979; Weld *et al.*, 1979 and Yamaguchi *et al.*, 1979). This was accompanied by a shortening of the effective refractory period (Yamaguchi *et al.*, 1979) but the ratio of the ERP to that of the APD was consistently increased. Thus, there is less time during an action potential for an ectopic impulse to initiate another action potential. It has been suggested that mexiletine may have an exaggerated effect on partially depolarized tissue since it blocks activated and inactivated sodium channels more than the resting channels (Arita *et al.*, 1979; Hering *et al.*, 1983;

Hohnloser *et al.*, 1982 and Sada *et al.*, 1980). For example, Sada *et al.* (1980) showed that 5 $\mu\text{g/ml}$ of mexiletine decreased V_{max} of guinea pig ventricular muscle only 4% at a resting MP of -96 mV. However, the same concentration of mexiletine was shown by Hohnloser *et al.* (1982) to decrease V_{max} 24% and 33% at resting MP of -76 and -69 mV, respectively. It has also been demonstrated that the sodium channel blocking effect of mexiletine is potentiated in *in vitro* ischaemic models and in hypoxic conditions (Frame *et al.*, 1982).

The threshold effective concentrations of mexiletine used in the *in vitro* animal studies above ranged from 1 to 10 $\mu\text{g/ml}$ (Iwamura *et al.*, 1976; Singh and Vaughan Williams, 1972b; Weld *et al.*, 1979; Arita *et al.*, 1979; Sada *et al.*, 1980; Campbell *et al.*, 1983a & 1983b; Hering *et al.*, 1983). Mexiletine has no effect on the calcium channel or the adrenergic β -receptor (Singh *et al.*, 1980).

In humans, the electrophysiologic effects of mexiletine have been shown to be somewhat variable. It was found to have no consistent effect on sinus rate, atrial refractoriness, and AV or His-Purkinje conduction times (Ross *et al.*, 1977; McCormish *et al.*, 1977). However, the functional refractory period of the AV node was increased by mexiletine, with a variable effect on the effective refractory period of the His-Purkinje system; lengthening it in some patients (Ross *et al.*, 1977), while shortening it in others (McCormish *et al.*, 1977). However, if the His-Purkinje conduction is impaired by disease, the drug always lengthens the AV interval, suggesting that mexiletine may produce AV block in patients with underlying conduction disturbances (Ross *et al.*, 1977; Singh *et al.*, 1981).

1.2.4 Comparison with Other Antiarrhythmic Agents.

Horowitz *et al.* (1981), in a controlled study, compared mexiletine and lidocaine over a period of 48 h in patients who developed ventricular arrhythmias within 2 days of the onset of acute MI. The two drugs comparably reduced ectopic beats over the first 24 h. However, a significantly lower frequency of arrhythmias was observed in patients on mexiletine, thereafter. In another comparative study, (Arakawa *et al.*, 1984), mexiletine (3 mg/kg i.v.) was equi-effective with procainamide (10 mg/kg i.v.) in suppressing ventricular premature contractions in cardiac patients. Similar results have previously been reported by Jewitt *et al.* (1977) and Campbell *et al.* (1981). In the study by Arakawa *et al.*, mexiletine and procainamide were more potent than β -adrenoceptor antagonists in suppressing ventricular arrhythmias. Fenster *et al.* (1981) compared the efficacy of mexiletine and quinidine in a double-blind dose-ranging study using 26 ambulatory patients with chronic ventricular arrhythmias. Mexiletine was found to be as effective as quinidine in the suppression of ventricular ectopic beats. Recently, Singh *et al.* (1984) reported comparable antiarrhythmic efficacy of oral mexiletine and quinidine in a single-blind randomized trial using 26 cardiac patients. Other studies have reported comparable antiarrhythmic efficacy for mexiletine and disopyramide (Breithardt *et al.*, 1982; Trimarco *et al.*, 1983). However, a more recent study in 160 patients with chronic ventricular arrhythmias of various etiologies found mexiletine to be slightly more active than disopyramide (Kato *et al.*, 1984).

1.2.5 Prevention of Sudden cardiac death

The benefits of antiarrhythmic drug therapy with respect to the prevention of sudden cardiac death remains to be clearly established. A review of the results of 20 randomized, controlled, clinical trials showed that beneficial effects on patients' survival could not be demonstrated either during the early hospital phase following acute myocardial infarction or after discharge (Furberg *et al.*, 1983). In a study on the effect of mexiletine on mortality in 344 patients, Chamberlain *et al.*, (1980) were not able to show any significant reduction in death rate. Recently, in another study in cardiac patients, the IMPACT research group reported that there were more deaths in the mexiletine group (7.6%) when compared to a placebo group (4.8%) (IMPACT, 1984). However, the difference between the two groups was not statistically significant. In another study in 240 patients with myocardial infarction (Smyllie *et al.*, 1984), mexiletine did not prevent primary fibrillation. The mortality at 6 weeks in this study was not significantly different from that of the placebo group. However, one study (Stein *et al.*, 1984) reported a significant reduction in the death rate following mexiletine therapy in patients who had ventricular tachycardia and fibrillation with syncope.

1.2.6 Clinical Indications

The clinical indications for mexiletine are ventricular arrhythmias such as ventricular tachycardia and premature ventricular ectopic beats (unifocal premature ventricular beats, salvos, and R-on-T phenomenon) (Bradley, 1983; Kreeger and Hammill, 1987). Mexiletine is

also useful in the prophylactic treatment of ventricular arrhythmias after myocardial infarction (Bradley, 1983).

1.2.7 Toxicology

Between 47 and 60% of patients on mexiletine therapy experience adverse effects (DiMarco *et al.*, 1981; Podrid and Lown 1981; Waspe *et al.*, 1983; Cetnarowski and Rihn, 1985). Discontinuation of therapy has occurred in up to 30% of patients due to adverse effects (Kupersmith *et al.*, 1985; Shrader and Bauman, 1986). The neurologic side effects are dizziness, lightheadedness, tremor, ataxia and convulsions. The gastrointestinal side effects include nausea, vomiting and dyspepsia. The cardiovascular side effects are transient hypotension and bradycardia.

1.2.8 Pharmacokinetic Parameters

1.2.8.1 Pharmacokinetics of racemic mexiletine

Mexiletine is rapidly absorbed after oral administration with peak plasma levels normally achieved within 1 to 4 h (Singh *et al.* 1981). Unlike lidocaine, mexiletine undergoes minimal (< 10%) first-pass elimination (Woosley *et al.*, 1984). Bioavailability ranges from 80 to 88% (Prescott *et al.*, 1977; Haselbarth *et al.*, 1981 and Ohashi *et al.*, 1984). The total volume of distribution is large and highly variable (5.5 to 9.5 l/kg) (Campbell *et al.*, 1978b; Prescott *et al.*, 1977; Haselbarth *et al.*, 1981), reflecting extensive tissue uptake of the drug. The elimination half-life ranges from 6.3 to 11.8 h (Campbell *et al.*, 1978b; Danillo, 1979; Prescott *et al.*, 1977 and Haselbarth *et al.*, 1981). The total body clearance is large, 6.1 to 7.1 ml/min/kg (Paalman

et al., 1977; Campbell *et al.*, 1978b). The mean renal clearance in normal subjects is 0.58 ± 0.30 ml/min/kg (Grech-Belanger *et al.*, 1985), and is increased by urinary acidosis. At a urinary pH of 5, renal clearance is reported to increase by a factor of 3 to 4 (Kiddie *et al.*, 1974; Prescott *et al.*, 1977). However, the effect of normal physiologic fluctuation in urinary pH is not conclusive. While Prescott *et al.* (1977) reported that normal urinary pH fluctuations are clinically unimportant, Johnston *et al.* (1979) found a significant change in urinary excretion and an increase of up to 50% in plasma concentrations of mexiletine due to spontaneous fluctuation in urinary pH. Approximately 9% of the administered dose of mexiletine is excreted unchanged in urine of healthy subjects (Prescott *et al.*, 1977 and Campbell *et al.*, 1978b).

1.2.8.2 Effects of Disease on Pharmacokinetics

The pharmacokinetic parameters of mexiletine are affected by many disease conditions. Patients with hepatic impairment have been reported to have decreased plasma clearance of mexiletine (Nitsch *et al.*, 1981). This is not surprising since the liver is the major site of metabolism of the drug. Renal disease has no significant effect on the plasma clearance, except at creatinine clearance values below 10 ml/min (El Allaf *et al.*, 1982). Delayed and incomplete absorption, as well as increased elimination half-life, were associated with acute myocardial infarction (Prescott *et al.*, 1977; Pottage *et al.*, 1978 and Pentikainen *et al.*, 1984). Prolongation of half-life has also been reported in patients with severe left ventricular failure (Leahey *et al.*, 1980a).

1.8.2.3 Pharmacokinetics of Mexiletine Enantiomers

The pharmacokinetics of mexiletine enantiomers has been reported from our laboratory (Igwemezie *et al.*, 1989) and by one other group (Grech-Belanger *et al.*, 1986). Both investigations involved single, oral dose studies in healthy human subjects. In our study, a statistically significant difference in the elimination half-lives of the two enantiomers (9.1 ± 2.9 h for R(-)-mexiletine and 11.0 ± 3.8 h for S(+)-mexiletine) was observed. The apparent volume of distribution of S(+)-mexiletine (7.3 ± 2.4 l/kg) was significantly greater than that of R(-)-mexiletine (6.6 ± 2.6 l/kg), while apparent oral clearance and plasma area under the concentration-time curve (AUC) of the two enantiomers were not significantly different. These results differ from those of Grech-Belanger *et al.* (1986) who reported a significant difference in serum AUC and no difference in the elimination half-lives of the enantiomers. However, both studies found a stereoselective renal excretion which favoured the S(+)-enantiomer. The salivary excretion of S(+)-mexiletine was significantly greater than that of the R(-)-enantiomer (Igwemezie, 1986).

1.2.9 Dosage

The reported therapeutic plasma concentration range of racemic mexiletine is 0.5 to 2.0 $\mu\text{g/ml}$ (Campbell *et al.*, 1975; Talbot *et al.*, 1976). This concentration range is usually achieved with oral doses of 200 to 400 mg of mexiletine hydrochloride given every six to eight hours. For intravenous dosing, Prescott *et al.*, (1977) recommended the following dosing regimen: 150 to 200 mg infused over 2 to 5 minutes, followed by a steadily decreasing loading infusion over 11 hours (250 mg

in 30 min, 260 mg in 2.5 h, 500 mg in 8 h) and a maintenance dose of 500 to 1000 mg over 24 h.

1.2.10 Metabolism

Mexiletine is eliminated from the body by extensive hepatic metabolism and renal excretion of the metabolites and unchanged drug (Beckett and Chidomere, 1977a & 1977b). The major metabolites are para-hydroxy mexiletine, hydroxymethyl mexiletine and their corresponding alcohols, and mexiletine glucuronide (Prescott *et al.*, 1977; Beckett and Chidomere, 1977b; Grech-Belanger *et al.*, 1985). Minor metabolites include the hydroxylamine and ketone, and the N-methylated metabolites (Beckett and Chidomere 1977a & 1977b) and 2,6-dimethyl phenol (Grech-Belanger *et al.*, 1987). None of these metabolites has been shown to possess any antiarrhythmic activity (Brown and Shand, 1982). The glucuronidation of mexiletine enantiomers in humans has been shown to occur stereoselectively in favour of the R(-)-enantiomer (Grech-Belanger *et al.*, 1986). The stereoselectivity of the other metabolic pathways has not been documented.

1.2.11 Interactions of Mexiletine with Other Drugs

The potential for interaction between mexiletine and other drugs is great because patients who have significant ventricular arrhythmias often have heart disease and other cardiovascular problems. Thus, these patients concomitantly receive other cardiovascular, as well as non-cardiovascular drugs. The absorption of mexiletine after oral dosing has been shown to be delayed by drugs which slow gastric emptying such as narcotic analgesics (Prescott *et al.*, 1977), antacids (Herzog *et al.*,

1982), and atropine-like agents (Wing *et al.*, 1980). Metoclopramide, on the other hand, increases the absorption rate (Wing *et al.*, 1980).

Bioavailability, however, is not altered by any of these agents.

Mexiletine has no effect on digoxin serum levels (Leahey *et al.*, 1980b).

This is in contrast to many antiarrhythmic drugs including amiodarone, propafenone, quinidine, and verapamil, which are known to increase the plasma concentrations of digoxin (Hager *et al.*, 1979; Leahey *et al.*, 1981; Moysey *et al.*, 1981., Klein *et al.*, 1982; Pedersen *et al.*, 1981 and Pederson *et al.*, 1982). Since mexiletine undergoes extensive hepatic metabolism in man, drugs which induce the hepatic enzyme systems are expected to enhance its non-renal clearance. This has been reported for rifampicin (Pentikainen *et al.*, 1982) and phenytoin (Begg *et al.*, 1982). Cimetidine, contrary to the expected inhibition of mexiletine metabolism, was reported to have no effect on the disposition of oral mexiletine in normal subjects (Klein *et al.*, 1985). Leahey *et al.*

(1980c) observed a beneficial drug interaction between mexiletine and the β -adrenergic receptor blocking drug, propranolol. They showed that mexiletine and propranolol are effective in suppressing ventricular premature depolarization and ventricular tachycardia when mexiletine alone had failed to do so. Propranolol did not significantly increase the plasma concentration of mexiletine in this study, hence the authors concluded that mexiletine and propranolol had "cooperative"

antiarrhythmic effects involving a pharmacodynamic interaction. A similar beneficial interaction between mexiletine and quinidine has been reported (Duff *et al.*, 1983).

1.2.12 Serum Protein Binding

The serum protein binding of racemic mexiletine has been reported to be 70% in healthy subjects (Talbot *et al.*, 1973). In acute myocardial infarction patients, binding was reported to be 64% (Pentikainen *et al.*, 1984). No significant difference was noted in the serum binding between the acute and recovery stages in these patients. Binding studies on the individual enantiomers carried out in our laboratory showed stereoselective binding to serum proteins with free fractions of $28.3 \pm 1.4\%$ for S(+)-mexiletine and $19.8 \pm 1.5\%$ for R(-)-mexiletine (McErlane *et al.*, 1987).

1.2.13 Tissue Distribution of Mexiletine

Thus far, only one study on the tissue distribution of racemic mexiletine in rats has been published (Barrigon *et al.*, 1983). These authors showed that following i.v. administration, the tissue concentrations of mexiletine reached peak values that were 12 to 24-fold higher than plasma concentrations in various parts of the brain, 6-fold higher in the heart, 33-fold higher in the lungs and a comparably high ratio in the liver and kidneys. These high tissue/serum ratios reflect the large volume of distribution of mexiletine in both animals (Barrigon *et al.*, 1983) and man (Prescott *et al.*, 1977; Haselbarth *et al.*, 1981). The high distribution of mexiletine into the brain could explain the CNS side effects often observed during mexiletine therapy.

1.2.14 Analytical Methods for Mexiletine

The assay of racemic mexiletine in human biological fluids has been carried out by several techniques. These include a number of gas-

liquid chromatographic (GLC) and high-performance liquid chromatographic (HPLC) procedures. The GLC methods measured mexiletine with a nitrogen selective detector without derivatization (Smith and Meffin, 1980; Vasiliades *et al.*, 1984); after derivatization with a nitrogen-selective detection (Kelly *et al.*, 1973; Bradbrook *et al.*, 1977; and Elfing *et al.*, 1981), flame ionization detection (Perchalski *et al.*, 1974; Kelly, 1977; Holt *et al.*, 1979; Kacprowicz, 1982 and Grech-Belanger, 1984), and electron-capture detection (Frydman *et al.*, 1978). The lowest amount detected was reported to be 5 ng/ml with the nitrogen-selective detector (Smith and Meffin, 1980). These GLC methods differ in their mode of detection, volume of sample required for analysis, extraction procedures, derivatization reagent and the nature of the stationary phase. The method described by Holt *et al.*, 1979, has been adapted for the routine serum monitoring of mexiletine. The HPLC methods measured mexiletine either directly using UV detection (Kelly *et al.*, 1981); Mastropolo *et al.*, 1984) or after derivatization followed by UV (Breithaupt and Wilfling, 1982) or fluorescence detection (White and Farid, 1983; Grech-Belanger *et al.*, 1984). Most of the UV techniques do not have the required sensitivity for studying single dose kinetics of mexiletine in humans.

1.2.15 Stereoselective Analysis of Mexiletine

Grech-Belanger *et al.* (1985) first reported a procedure for the HPLC measurement of mexiletine enantiomers using a method of derivatization with 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl-isothiocyanate (GITC), followed by resolution of the enantiomers on a reverse-phase (octadecylsilane) column and detection by UV absorbance.

The lowest amount of each enantiomer detected was 50 ng/ml. We have developed a stereoselective HPLC assay procedure (McErlane *et al.*, 1987) in which mexiletine enantiomers were resolved on a chiral stationary phase (Pirkle^R ionic phenyl glycine column) with subsequent detection of the 2-naphthoyl derivatives by fluorescence. The lower limit of detection in plasma was 5 ng/ml, which is a considerable improvement from that reported above.

1.3 Chirality

1.3.1 Introduction

Chirality is the property displayed by any molecule which is not superimposable on its mirror image. The two non-superimposable forms are referred to as enantiomers, enantiomorphs or optical isomers. A vast number of drugs of synthetic as well as natural origin exhibit the property of chirality. While the natural synthesis of chiral drugs produces only one of two possible enantiomers, their laboratory synthesis usually produces a racemate (50:50 ratio of the two enantiomers). Enantiomers are characterized by the same physical and chemical properties except for the direction in which they rotate the plane of polarized light, and their interaction with other chiral compounds. During evolution, living systems progressively became chirally enriched such that they contain only D-sugars and L-amino acids in their chemical make-up (Mason, 1984 & 1988). Thus, it is not surprising that living organisms are able to discriminate between enantiomers at the molecular level. This often leads to differences (stereoselectivity) in the disposition of the two enantiomers of a chiral drug.

1.3.2 Stereoselective drug Disposition

1.3.2.1 Pharmacodynamics

The R(-)-enantiomer of the antiarrhythmic agent, tocainide, has been shown to be three times more potent than the S(+)-enantiomer against chloroform-induced arrhythmias in the mouse (Bynes *et al.*, 1979; Block *et al.*, 1988) and arrhythmias evoked by programmed electric stimulation (Uprichard *et al.*, 1988). (-)Verapamil is four times more potent than its opposite enantiomer in suppressing ischaemia-induced arrhythmias in conscious rats (Curtis and Walker, 1986). The antiarrhythmic activity of disopyramide has been reported to reside mainly with the S(+)-enantiomer in both animal (Mirro *et al.*, 1981) and human (Pollick *et al.*, 1982) studies. A recent study (Lima and Boudoulas, 1987) noted that S(+)-disopyramide has less negative inotropic effect than the R(-)-enantiomer in humans. The authors concluded that it may be possible to remove up to 72% of the negative inotropic effect associated with racemic disopyramide by the administration of the pure S(+)-enantiomer. Most of the therapeutic actions of propranolol, including the antihypertensive effects, are mediated by the S(-)-enantiomer (Nies and Gerber, 1980; Rahn *et al.*, 1974). The anticoagulant potency of S-warfarin is approximately 6 times that of the R-enantiomer (Eble *et al.*, 1966). The action of the non-steroidal antiinflammatory drug, ibuprofen, shows stereoselectivity in that only the S(+)- enantiomer inhibits prostaglandin synthesis (Kazuna *et al.*, 1974).

1.3.2.2 Absorption

The absorption of the enantiomers of a chiral drug may be stereoselective if the drug undergoes active transport across the intestinal mucosa. Examples are dopa (Wade *et al.*, 1973) and methotrexate (Hendel and Brothagen, 1984) whose L-isomers have been shown to be absorbed more rapidly than the D-isomers.

1.3.2.3 Distribution

The distribution of enantiomers may differ as a result of differences in either tissue and/or plasma protein binding. It has been shown that S(-)-propranolol is more rapidly taken up by the heart tissue than its antipode in rats (Kawashima *et al.* 1976). Similarly, the extravascular binding of S(-)-propranolol is greater than that of the R(+)-enantiomer in dogs (Bai *et al.*, 1983). It has also been demonstrated that (-)timolol is more rapidly taken up by lung, heart, and brain tissue fractions than the (+)enantiomer. The latter was found to bind only to non-specific sites (Tocco *et al.*, 1976).

The serum proteins consist of chiral amino acids and possesses chiral secondary structures. Thus, they provide chiral environments for small bioactive molecules. For example, the essential amino acid, L-tryptophan, binds to human serum albumin (HSA) with an affinity about 100 times that of D-tryptophan (McMenamy and Oncley, 1958). The first drug that was shown to exhibit stereoselective binding to HSA was oxazepam succinate. Muller and Wollert (1975) found that (+)oxazepam bound to HSA with an affinity 40 times that of its enantiomer. S-Warfarin has also been shown to bind to HSA with a greater affinity than the R-enantiomer (Brown *et al.*, 1977). α_1 -acid glycoprotein (AAG)

is the major serum binding protein for many basic drugs and its binding has been shown to be stereoselective for such agents as propranolol (Bai *et al.*, 1983; Walle *et al.*, 1983; Albani *et al.*, 1984) and disopyramide (Valdivieso *et al.*, 1983; Lima *et al.*, 1984; Lima and Boudoulas, 1986).

1.3.2.4 Metabolism

Vogelgesang *et al.* (1984) reported an interesting stereoselective first-pass metabolism for verapamil. They showed that the systemic availability of the more active (-)-enantiomer was 2 to 3 times smaller than that of the (+)-enantiomer. Thus, their data explained the earlier discrepancy observed by Eichelbaum *et al.*, (1980) that (\pm)-verapamil's effect on atrio-ventricular conduction in man is 2 to 3 fold less after oral administration than after i.v. administration when doses which produced equal plasma concentrations of racemic drug were used. Enantiomeric differences in pre-systemic metabolism have also been reported for propranolol enantiomers in man (Silber and Reigelman, 1980; Von Bahr *et al.*, 1982). The 2-arylpropionic acids provide a good example of stereoselective metabolic activation. The inactive R(-)-enantiomers undergo metabolic inversion in humans, *in vivo*, to the active S(+)-enantiomer (Adams *et al.*, 1976; Kripalani *et al.*, 1976; Bopp *et al.*, 1979; Lee *et al.*, 1985). The progressive enrichment of the active S(+)-enantiomer means that its enantiomeric ratio must be determined if meaningful concentration-effect relationships are to be determined. Enantiomers may also interact with each other at the metabolic level. For example, the analgesic activity of levomethorphan is significantly enhanced and prolonged by the co-administration of its antipode, the inactive dextromethorphan, due to metabolic inhibition (Cooper and Anders, 1974). The review papers published by Jenner and

Testa (1980), Testa (1986) and Caldwell *et al.* (1988) contain many other examples of stereoselective metabolism.

1.3.3 Stereoselective Drug Analysis

1.3.3.1 Introduction

Until recently, there has been a paucity of information in the literature on the importance of stereochemistry in drug disposition. This can be attributed to the lack of analytical methodology appropriate for the determination of the enantiomeric composition of chiral drugs at therapeutic concentrations. The traditional and classical method of resolving enantiomers involves their conversion to diastereoisomeric salts by reaction with an optically active compound, and subsequent resolution by fractional recrystallization. Resolution is possible because diastereoisomers have different chemical and physical properties, including solubility in a given solvent. This traditional method, however, is often laborious, inefficient, does not yield optically pure samples and is essentially restricted to carboxylic acids and amines (Blaschke, 1980). More importantly, it is not amenable to pharmacokinetic studies. Thus, most of the recent advances in enantiomer separation technology have been made in the area of chromatography. Chromatographic methods have the advantage of small sample size, speed, efficiency, independence from the magnitude of specific rotation and the presence of other optically active species in the sample (Lochmuller and Souter, 1975). Enantiomers are resolved chromatographically as the diastereoisomers on an achiral stationary phase or by direct resolution on a chiral stationary phase (CSP).

1.3.3.2 Resolution of Enantiomers as Diastereoisomers

The enantiomers of the antiarrhythmic agent, tocainide, have been resolved by the formation of diastereoisomers using S(-)-2-methoxy-2-trifluoromethylphenylacetyl chloride by GLC (Gal *et al.*, 1982). S(-)-1-(4-Nitrophenylsulfonyl)prolyl chloride was successfully applied as a chiral derivatization reagent for the separation of amphetamines and ephedrine by HPLC (Clark and Blacksdale, 1984). Similarly, the 2-arylpropionic acids were resolved with S(+)-2-octanol by HPLC (Johnson *et al.*, 1979; Lee *et al.*, 1984), and with optically active amphetamine by GLC (Singh *et al.*, 1986). Isocyanates such as phenylethylisocyanate, have been utilized to resolve many amino compounds by HPLC. These include amphetamines (Miller *et al.*, 1984), propranolol (Wilson and Walle, 1984) and ephedrine (Gal and Sedman, 1984). Extensive review papers on the resolution of enantiomers using chiral derivatization reagents have been published by several authors (Lochmuler and Souter, 1975; Tamegai *et al.*, 1979; Lindna, 1982 & 1988).

Considerable advances toward understanding the separation mechanism for diastereoisomers have been made by Rose *et al.* (1966). In their study with diastereoisomeric esters of 2-acetoxypropionic acid, they observed that the size of the substituents at the asymmetric carbon, the distance between the optical centres in the ester, as well as the polarity of the stationary phase were critical for resolution. There are, however, drawbacks associated with the resolution of enantiomers by formation of their diastereoisomers. These include the requirement of an active functional group on the enantiomer, differences in the reaction kinetics of enantiomers with the chiral reagent which may result in quantitation error, and the requirement of an optically

pure reagent (Konig *et al.*, 1977; Frank *et al.*, 1978; Liu and Ku, 1983). In addition, the diastereoisomers must be chemically and stereochemically stable under the chromatographic conditions.

1.3.3.3. Resolution of Enantiomers on Chiral Stationary Phases

The direct approach to the resolution of enantiomers using a chiral stationary phase does not have any of the disadvantages of the above methods and in some instances, the enantiomers can be resolved without prior derivatization. Resolution depends on the formation of transient diastereoisomeric complexes (via π -bond, hydrogen bonds, electrostatic bonds and steric interactions) between the enantiomers and the CSP (Feibush and Grinberg, 1988). The relative stability of these complexes leads to the resolution of the enantiomers. Chirasil-Val^R (L-valine-tert-butylamide-carboxyalkyl-methyl-siloxane) was the first stable GLC CSP to become available commercially (Frank *et al.*, 1977). Resolution of enantiomers on the phase was based mainly on H-bonding and steric interactions. This chiral GLC phase was successfully used to resolve the enantiomers of amino acids, sugars, aromatic and aliphatic hydroxy-acids and amines (Frank *et al.*, 1977; Frank *et al.*, 1979; Frank *et al.*, 1980). This phase has also been used in the resolution of the enantiomers of tocainide in reports from our laboratory (McErlane and Pillai, 1983). The high thermal stability of Chirasil-Val^R made it possible, for the first time, to employ a mass-spectrometer, coupled to a gas chromatograph, for the analysis of enantiomeric drugs and metabolites (Frank *et al.*, 1978). XE-60 valine-S-phenylethylamide is another commercially available GLC chiral phase which has been used to resolve chiral alcohols, hydroxyacids and

carbohydrates by GLC (Konig and Sievers, 1980; Konig *et al.*, 1981; Konig *et al.*, 1982).

The development of chiral HPLC phases has seen a much faster growth than GLC phases and more than 22 are now commercially available. The resolution of enantiomers on the α - and β -cyclodextrins (cycloheptaamylase and cyclooctaamylase) involves inclusion complexes in which the enantiomers enter exclusively into chiral cavities within the CSPs. Stereoselectivity results from a difference in the fit of the enantiomers (Armstrong and Damond, 1984; Armstrong *et al.*, 1986). A similar mechanism has been proposed for the chiral phases, cellulose triacetate (Hesse and Hegel, 1976) and poly(triphenylmethylmethacrylate) (Okamoto *et al.*, 1981; Okamoto and Hatada, 1986). The protein-bonded CSPs contain mainly bovine serum albumin (Allenmark *et al.*, 1983) or α_1 -acid glycoprotein (Hermansson, 1983) attached to silica. Both CSPs have been successfully used for the resolution of many enantiomeric drugs (Wainer *et al.*, 1986). The retention mechanism of these phases involve a combination of electrostatic, H-bonding and hydrophobic interactions (Schill *et al.*, 1986; Allenmark, 1986). By far, the most versatile of all the chiral HPLC phases are the Pirkle^R chiral phases. These columns contain amino acid derivatives such as 3,5-dinitrobenzoyl derivatives of (R)- and (S)-phenylglycine, (S)-leucine and (R)- and (S)-naphthylalanine. The proposed mechanism of resolution by these phases are π -acid π -base, dipole-dipole and steric interactions (Pirkle and Welch, 1984). The Pirkle^R chiral phases have proven to be particularly valuable for the resolution of a variety of compounds such as amines (Pirkle and Welch, 1984; Pirkle *et al.*, 1984), amino acids and alcohols (Pirkle and Welch, 1984), benzodiazepines (Pirkle and Psipouras, 1984)

and acyclic alkyl carbinols (Weems and Yang., 1982). In our own work, the Pirkle^R ionic (phenyl glycine) CSP has been used to resolve the enantiomers of mexiletine (McErlane *et al.*, 1987). The applications of chiral stationary phases have been reviewed (Louchmuller and Souter, 1975; Blaschke, 1980; Liu and Ku, 1980; Armstrong, 1984; Wainer and Alembik, 1988).

1.3.3.4 Resolution of Enantiomers Using Chiral Eluents

With a suitable chiral component in the mobile phase, enantiomers can be resolved on an achiral HPLC support. Two possible mechanisms may result in enantiomer resolution. The chiral component present in the mobile phase can interact with enantiomers to form diastereoisomeric complexes or with the stationary phase to produce essentially a chiral stationary phase. Examples in the literature include the resolution of D and L amino acids using an optically active Cu^{++} -proline complex (Poole and Schuette, 1984) and enantiomeric amines with a chiral counter ion of (+)-10-camphor sulfonic acid (Poole and Schuette, 1984).

1.4 Rationale

- a) Rationale for development of assay of mexiletine enantiomers with 2-anthroyl chloride as a derivatization reagent.

Two studies (Grech-Belanger *et al.*, 1986; McErlane *et al.*, 1987) have reported the resolution and quantitation of mexiletine enantiomers by HPLC. The sensitivity limit (lower) of the assay by Grech-Belanger *et al.* (1986) was 50 ng/ml of each enantiomer. The method developed by McErlane *et al.* (1987) using 2-naphthoyl chloride as a derivatization reagent improved sensitivity of detection by 10-fold (lower limit of detection was 5 ng/ml of each enantiomer). However, adequate delineation of the pharmacokinetics, including serum protein binding, of mexiletine enantiomers in rats where relatively small biological samples and faster drug elimination were anticipated indicated the need for a more sensitive assay. Evaluation of the stereochemical interactions between the 2-naphthoyl derivatives of the enantiomers and the Pirkle^R chiral stationary phase (Igwezie, 1986; McErlane *et al.* 1987) lead to the proposal that sensitivity could be improved while preserving selectivity by using 2-anthroyl chloride, instead of 2-naphthoyl chloride, as a derivatization reagent (the fluorescence quantum yield of the anthracene ring is 1.5-fold that of the naphthalene ring).

- b) Rationale for the study of the binding of mexiletine enantiomers to serum and isolated serum proteins.

The free form of a protein bound drug is believed to be in equilibrium with the target tissue sites since it is the form that can diffuse out of the vascular system. Intuitively, the serum free drug

concentration of drugs should correlate better with pharmacological effects than the total drug concentration. This has been reported for propranolol (McDevitt *et al.*, 1976) and disopyramide (Lima *et al.*, 1981). The serum binding of racemic mexiletine is reported to be 70% in healthy human subjects (Talbot *et al.*, 1973). This study was carried out by ultrafiltration technique with only one drug concentration. Preliminary studies on the binding of mexiletine enantiomers in our laboratory showed stereoselective binding with bound fractions of 81 and 72% for R(-)- and S(+)-mexiletine, respectively. However, the binding parameters were not determined since the range of concentrations studied was small (0.2-2 $\mu\text{g/ml}$). Both studies indicated relatively high binding for mexiletine. The present study was proposed to fully characterize the binding kinetics of mexiletine enantiomers as well as evaluate those factors known to affect serum protein binding. Furthermore, most basic drugs are known to bind to α_1 -acid glycoprotein (Piafsky and Knoppert, 1978). The concentration of this protein is reported to be increased in many disease conditions including acute MI. Since mexiletine may be used to control arrhythmias in these patients, it is pertinent to identify the protein(s) responsible for the binding of the enantiomers in serum. This will make it possible to predict the effect of disease on the pharmacokinetics of mexiletine enantiomers.

c) Rationale for studying tissue distribution kinetics of mexiletine enantiomers.

Barrigon *et al.* (1983) studied the tissue distribution kinetics of racemic mexiletine in the rat and reported extensive uptake into various tissues. Tissue/serum ratios of 12-24 was found in different parts of the brain, 6 in the heart, and up to 33 in the lungs. These high tissue

uptakes suggest the possible involvement of a facilitated uptake mechanism. Stereoselective distribution into tissues including the heart has been reported for propranolol (Bai et al. 1983) and timolol (Tocco et al., 1976). However, the relative tissue uptake of mexiletine enantiomers has not been reported. The brain uptake of the enantiomers is of particular interest since it has been reported that a significant number of patients on mexiletine therapy experience CNS side effects, necessitating withdrawal of the drug in some of the patients (Kreeger and Hammill, 1987). If the brain uptake of mexiletine enantiomers is stereoselective, then, removing the enantiomer that accumulates more, may reduce the severity of the CNS side effects associated with mexiletine therapy.

d) Rationale for the pharmacodynamic studies on the relative antiarrhythmic activity of racemic mexiletine and its enantiomers.

It is well established that drug enantiomers often differ in their pharmacological properties (Simonyi, 1984). With respect to mexiletine, the relative antiarrhythmic activity of the enantiomers has never been reported. However, the antiarrhythmic effects of other class I agents which share the same mode of action with mexiletine have been shown to be stereoselective. For example, R(-)-tocainide is significantly more potent than the S(+)-enantiomer in protecting against chloroform induced arrhythmias in mice (Bynes et al., 1979; Block et al., 1988) and electrically-induced arrhythmias in dogs (Uprichard et al., 1988). The (+)-enantiomer of disopyramide has been shown to prolong action-potential duration in canine cardiac purkinje fibres, while the (-)-enantiomer shortens it (Mirro et al., 1981). These results suggest

that the antiarrhythmic actions of mexiletine enantiomers may also be different.

1.5 Specific Objectives

- a) To develop a sensitive and stereoselective HPLC assay for the measurement of mexiletine enantiomers in biological fluids and tissue homogenates.
- b) To study the binding characteristics of mexiletine enantiomers to human serum, isolated human serum albumin and α_1 -acid glycoprotein.
- c) To determine the tissue distribution kinetics of the enantiomers of mexiletine in the heart, brain, liver, lung, kidney and fat tissues in rats.
- d) To determine the relative efficacy of the enantiomers of mexiletine against electrical and ischaemia-induced arrhythmias in rats, and to establish plasma concentration-effect relationships.
- e) To evaluate the relative effects of mexiletine enantiomers on the haemodynamic and ECG parameters in rats.

2. EXPERIMENTAL

2.1 Supplies

2.1.1 Drugs

The following drugs were used: R,S-mexiletine hydrochloride¹, R(-)², and S(+)-mexiletine Hydrochloride³, KOE 2963 (internal standard)⁴, pentobarbitone sodium⁵, halothane⁶, Cicatrin⁷, Marcaine⁸ and heparin⁹.

2.1.2 Chemicals and Reagents

The following chemicals and reagents were used: 2-naphthoyl chloride¹⁰, anthraquinone-2-carboxylic acid¹¹, oxalyl chloride¹², calcium chloride¹³, potassium permanganate¹⁴, trichloroacetic acid¹⁵, aluminium hydroxide (alumina)¹⁶, zinc dust¹⁷, sodium hydroxide¹⁸, barium hydroxide octahydrate¹⁹, zinc sulphate heptahydrate²⁰, hydrochloric acid²¹, sodium chloride²², monosodium phosphate monohydrate²³, disodium phosphate heptahydrate²⁴, concentrated ammonia solution²⁵ and sulphuric acid²⁶.

2.1.3 Solvents

The following solvents were used: dichloromethane²⁷, n-hexane²⁸, 2-propanol²⁹, diethyl ether³⁰, chloroform³¹,

1-4 Boehringer Ingelheim Ltd., Burlington, Ontario,
Canada.

ethanol³², acetic acid³³, methanol³⁴, acetonitrile³⁵, ethyl acetate³⁶.

2.1.4 Human Serum Proteins

These were albumin³⁷, α_1 -acid glycoprotein³⁸ and lipoprotein-deficient serum³⁹.

2.2 Chromatographic Stationary Phases and Columns

2.2.1 HPLC Columns

a) A Pirkle^R ionic chiral column⁴⁰ (25 x 0.46 cm i.d.) with a stationary phase consisting of (R)-N-3,5-dinitrobenzoylphenyl glycine ionically bonded to Γ -aminopropyl silica was used.

b) A Pirkle^R ionic chiral column⁴¹ (25 x 0.46 cm i.d.) with a stationary phase consisting of (S)-N-3,5-dinitrobenzoyl leucine ionically bonded to Γ -aminopropyl silica was used.

c) A HPLC silica guard column⁴² (1.5 x 0.32 cm i.d.) was used in front of the Pirkle^R column.

d) Whatman Preparative C18 column⁴³ (25 x 0.94 cm i.d.).

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- | | |
|-------|---|
| 5 | M.T.C. Pharmaceuticals, Mississauga, Ontario, Canada. |
| 6 | Ayerst Laboratories, Montreal, Quebec, Canada. |
| 7 | Wellcome Inc., Kirkland, Quebec, Canada. |
| 8 | Winthrop Laboratories, Aurora, Ontario, Canada. |
| 9 | Glaxo Ltd., Toronto, Ontario, Canada |
| 10-14 | Aldrich Chemical Co., Milwaukee, Wisconsin, USA. |
| 15-16 | Fischer Scientific Co. Fair Lawn, New Jersey, USA. |

2.3 Equipment

2.3.1 High-Performance Liquid Chromatograph

A Gilson model 302 high-performance liquid chromatograph⁴⁴ equipped with a Gilson model 602 data master, a NEC model PC-8023A-C data terminal⁴⁵, a 20 μ l loop injector⁴⁶ and a Schoeffel model 970 fluorometer⁴⁷ was used.

2.3.2 Gas-Chromatograph/Mass Spectrometer

A Hewlett Packard model 5987A GC/MS⁴⁸ equipped with a model 5880 gas chromatograph was used.

2.3.3 Polygraph

A model 79D Grass polygraph⁴⁹ was used.

2.3.4 Electric Stimulator

A model SD9 Grass electric stimulator⁵⁰ was used.

17-18 BDH Chemicals Ltd., Poole, England, UK.

19-36 BDH Chemicals Ltd., Vancouver, British Columbia, Canada.

37-38 Terochem Laboratories Ltd., Edmonton, Alberta, Canada.

39 Sigma Chemical Co., St. Louis, Mo, USA

40-41 Regis Chemical Co., Morton Grove, Illinois, USA.

42 Rainin Instruments Inc., Woburn, Massachussetts, USA.

2.4 Assay of Mexiletine Enantiomers Using 2-Anthroyl Chloride as a Derivatization Reagent

2.4.1 Synthesis of 2-Anthroyl Chloride from Anthraquinone-2-Carboxylic Acid

The synthesis of 2-anthroyl chloride was accomplished by the reduction of anthraquinone-2-carboxylic acid with dilute ammonia and zinc dust to anthracene-2-carboxylic acid, followed by conversion of the acid to the acid chloride with oxalyl chloride.

2.4.1.1 Synthesis of Anthracene-2-Carboxylic Acid

Anthraquinone-2-carboxylic acid (1.0203 g) was suspended, with stirring, in 80 ml of dilute ammonia (9.5% ammonia solution) in a 250 ml round bottom glass flask. To this suspension was added 2 g of zinc dust and the mixture was heated in a water bath at 100°C. The reaction mixture which was initially blood-red in colour turned to pale yellow at the end of the reaction (approximately 45 minutes). The mixture was allowed to cool and was filtered through a Whatman (No 1) filter paper.

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- 43 Whatman Inc., Clifton, New Jersey, USA.
44-45 Mandel Scientific Co. Ltd., Edmonton, Alberta, Canada.
46 Rheodyne Inc., Berkeley, California, USA.
47 Kratos (Schoeffel Instruments), Westwood,
 New Jersey, USA.
48 Hewlett Packard, Avondale, Pennsylvania, USA.
49-50 Grass Instruments Co., Quincy, Massachusetts, USA.

The filtrate was acidified to pH ≈ 7 with 80 ml of 2 M HCl, filtered and the residue was discarded. The filtrate was further acidified to pH ≈ 1 with 20 ml of 2 M HCl and a yellow precipitate of anthracene-2-carboxylic acid appeared. The aqueous portion was filtered out by suction. The resulting anthracene-2-carboxylic acid was purified by HPLC (section 2.4.1.2 below).

2.4.1.2 Purification of Anthracene-2-Carboxylic Acid

The synthetic acid was purified by reverse phase HPLC using a Whatman Preparative C18 column. The mobile phase was methanol/acetic acid/chloroform (2:1:97) pumped isocratically at a flow of 0.5 ml/min. Detection was accomplished by UV at 262 nm. The anthracene-2-carboxylic acid thus isolated ($R_t = 8$ min) was subsequently recrystallized from ethanol (95%).

2.4.1.3 Characterization of Anthracene-2-Carboxylic Acid

a) **Melting point:** The melting point of the recrystallized anthracene-2-carboxylic acid was 278-279°C.

b) **Structure of Anthracene-2-carboxylic Acid:** The structure of anthracene-2-carboxylic acid was confirmed by direct probe electron-impact mass-spectrometry (EI-MS). The following EI-MS conditions were employed: source temperature, 240°C; probe temperature programme, 50°C for 1 min to 300°C for 10 min at a rate of 30°C per min; electron beam voltage, 70 eV; emission current, 300 uA; and multiplier voltage, 2500 V.

2.4.1.4 Synthesis of 2-Anthroyl Chloride

2-Anthroyl chloride was prepared by refluxing 25 mg of anthracene-2-carboxylic acid in 10 ml of purified anhydrous dichloromethane with 200 μ l of oxalyl chloride (freshly distilled) for 5 hours in a 250 ml round bottom flask fitted with a reflux condenser and a drying tube. At the end of the reaction, the mixture was allowed to cool and was filtered. Excess reagent and the reaction solvent were removed by rotary evaporation under reduced pressure. The residue, which was greenish-yellow, was dried overnight under vacuum (1 mm Hg). The synthetic acid chloride was purified by HPLC (section 2.4.1.5 below).

2.4.1.5 Purification of 2-Anthroyl Chloride

The synthetic 2-anthroyl chloride was purified by preparative HPLC. The stationary phase was a Whatman C18 reverse phase column and the mobile phase was 100% acetonitrile pumped isocratically at a flow rate of 2 ml/min. Detection of the acid chloride was accomplished with a UV detector set at 230 nm. The R_t of the acid chloride was 12 min.

2.4.1.6 Characterization of 2-Anthroyl Chloride

The structure of the synthetic 2-anthroyl chloride was confirmed by direct probe EI-MS. The MS conditions employed were the same as those used for the anthracene-2-carboxylic acid.

2.4.2 Development of Assay of Mexiletine Enantiomers

2.4.2.1 Derivatization of Mexiletine Enantiomers with 2-Anthroyl Chloride

The derivatization of mexiletine enantiomers with 2-anthroyl chloride was carried out according to the Schotten-Baumann reaction (Vogel, 1964). An aqueous solution of R,S-mexiletine hydrochloride (200 μ l), containing 50 ng equivalent of the base, was transferred into a 10 ml glass tube. The solution was basified with 100 μ l of 2 M NaOH and 15 μ l of 2-anthroyl chloride solution (1 mg/ml in dichloromethane) was added and mixed by vortex for 5 min at room temperature. The derivatives formed were extracted into 0.5 ml of mobile phase (section 2.4.2.2 below) and analyzed by HPLC.

The optimum reaction time was evaluated by derivatization of a given amount of R(-)-mexiletine and the internal standard over various time intervals (2, 5, 7.5, 10, 15 and 30 min) and comparing the absolute peak heights.

2.4.2.2 Chromatographic Resolution of Mexiletine Enantiomers

The enantiomer derivatives were resolved by HPLC using the Pirkle^R ionic (phenyl glycine) chiral column. The optimum mobile phase was 2-propanol/ethyl acetate/hexane (6:4:90) pumped isocratically at a flow rate of 1.7 ml/min. This mobile phase was arrived at after an extensive assessment of many other solvent combinations. These included 2-propanol/hexane, 2-propanol/chloroform/hexane, ethanol/hexane, ethanol/acetonitrile/hexane, ethanol/chloroform/hexane, ethanol/acetonitrile/ethylacetate/hexane, etc. Each of these mobile

phases was assessed at different solvent ratios to produce different polarities, as well as at different flow rates.

2.4.2.3 Sensitivity

The detection of the enantiomer derivatives was accomplished with a fluorescence detector using optimum excitation and emission wavelengths of 270 and 400 nm, respectively. These wavelengths were determined with a fluorescence spectrophotometer⁵¹.

2.4.2.4 Structure of 2-Anthroyl Derivative of Mexiletine

The structure of the 2-anthroyl derivative of mexiletine (mexiletine-2-anthramide) was confirmed by direct probe EI-MS using the same conditions as those employed for the anthracene-2-carboxylic acid.

2.4.2.5 Extraction Solvent

The solvent used for the extraction of mexiletine enantiomers and the internal standard from aqueous solutions and plasma was diethyl ether. The suitability of this solvent had been established in a prior study (Igwezie, 1986).

2.4.2.6 Selection of Internal Standard

The internal standard used was KOE 2963 [(2',6'-dimethylphenoxy)-2-aminoethane], which is an analogue of mexiletine. The choice of this internal standard was based on its structural similarity to mexiletine.

2.4.2.7 HPLC Analysis of Mexiletine Enantiomers

An aqueous solution of R,S-mexiletine (1 ml) containing 250 ng equivalent of the base was transferred into a 10 ml PTFE-lined screw-capped culture tube. To this tube was added 50 μ l of an aqueous solution of the internal standard (containing 62 ng equivalent of the base). The pH was adjusted above 12 by the addition of 0.4 ml of 2 M NaOH and extracted twice with 5 ml portions of diethyl ether. The organic extracts were combined and evaporated to approximately 1 ml under a gentle stream of nitrogen at 37°C. The resulting solution was shaken with 200 μ l of 0.1 M HCl and the ether layer removed and discarded. The aqueous layer was again basified with 100 μ l of 2 M NaOH and 15 μ l of 2-anthroyl chloride (1 mg/ml) was added and mixed vigorously for 5 minutes at room temperature. The 2-anthroyl derivatives of the enantiomers were extracted into 0.5 ml of the mobile phase and a 20 μ l aliquot injected onto the HPLC column.

The HPLC analysis showed the presence of an extraneous peak co-eluting with the S(+)-enantiomer.

2.4.2.8 Attempted Resolution/Removal of the Interfering Peak

a) **Variation of Mobile Phase Polarity:** The first approach to solving the interfering peak problem was to try and resolve it from the enantiomer peaks. Thus, extensive variation of the mobile phase

polarity was undertaken using different binary, ternary and quaternary combinations of hexane and 2-propanol, ethyl acetate, chloroform, acetonitrile and ethanol. The flow rate was also varied with each mobile phase.

b) Extraction Solvent: The extraction solvent was changed to hexane and dichloromethane to determine if the interfering substance could be removed by selective solubility.

c) Purification of solvents and Reagents: To rule out the possibility of the source of the interfering peak being an impurity present in the solvents or reagents, an extensive purification of each solvent and reagent was carried out according to protocols described by Perrin *et al.* (1966).

i) Oxalyl chloride: by distillation

ii) Diethyl ether: by sequential washing with strongly alkaline potassium permanganate and concentrated sulphuric acid, followed by drying over sodium wire and distillation.

iii) Hexane: by elution through activated alumina, followed by distillation.

iv) Dichloromethane: by treatment with concentrated sulphuric acid and sodium hydroxide, sequentially, followed by drying with calcium chloride and distillation.

v) Anthracene-2-carboxylic acid: by HPLC (section 2.4.2.2.).

vi) 2-Anthroyl chloride: by HPLC (section 2.4.2.5.).

d) Pirkle^R (S)-Leucine Chiral Column: The stationary phase used for the HPLC analysis was changed to the Pirkle^R (S)-leucine chiral column.

2.5 Assay of Mexiletine Enantiomers using 2-Naphthoyl Chloride as a Derivatization Reagent

The assay of mexiletine enantiomers using 2-naphthoyl chloride as a derivatization reagent has been previously described (Igwemezie, 1986; McErlane *et al.*, 1987).

2.5.1 Extraction, Derivatization and HPLC Analysis

An aliquot of biological fluid or tissue homogenate was transferred into a 10 ml PTFE-lined screw-capped culture tube. To this tube was added mexiletine and the internal standard. The plasma proteins were precipitated with 1 ml of barium hydroxide (0.15 M) and 1 ml of zinc sulfate (0.15 M) solutions. The pH of all biological samples was adjusted above 12 by the addition of 0.4 ml of 2 M NaOH and extracted twice with 5 ml portions of diethyl ether. The organic extracts were combined and evaporated in a water bath at 37°C under a gentle stream of nitrogen to a volume of approximately 1 ml. The resulting solution was acidified with 0.2 ml of 0.1 M HCl, shaken, and the ether layer was removed and discarded. The aqueous layer was again basified with 0.2 ml of 2 M NaOH and 15 µl of the 2-naphthoyl chloride solution was added and mixed vigorously on a vortex mixer for 2 min. The derivatives formed were extracted into 0.5 ml of the mobile phase used for the HPLC analysis and a 20 µL aliquot was injected onto the HPLC column.

The HPLC conditions were : stationary phase, Pirkle^R ionic (phenyl glycine) chiral phase; mobile phase, chloroform/2-propanol/Hexane (7:7:86); flow rate, 1.2 ml/min; and detection, fluorescence at 230 nm (ex) and 370 nm (em).

2.5.2 Calibration Curves and Assay Precision in Human Plasma

To five 1 ml aliquots of blank plasma were added 20, 40, 100, 400 and 1000 ng (equivalent of the base) of racemic mexiletine along with 50 ng of KOE 2963 (internal standard). The samples were subsequently treated as described under "Extraction, Derivatization and HPLC Analysis" (section 2.5.1). The calibration curves were constructed by plotting the peak height ratios of each enantiomer to that of the internal standard against the known concentration of the enantiomer. Inter-assay variability was determined by triplicate preparation and analysis of each of the samples used for the standard curve.

The standard curves for mexiletine enantiomers in rat plasma, serum and tissue homogenates were prepared in a similar manner.

2.6 *In Vitro* Serum Protein Binding of Mexiletine Enantiomers in Humans

2.6.1 Serum Collection

Six healthy subjects were used for this study. The blood chemistry of the subjects were assessed prior to sample collection. Approximately 100 ml of venous blood was collected from each subject using 10 ml glass syringes. Blood was immediately transferred into 10 ml glass tubes with PTFE lined caps, allowed to clot at room temperature for 2 h, centrifuged at 2500 x g for 15 min and the serum separated.

2.6.2 Serum pH Adjustment

Adjustment of serum pH to ≈ 7.4 was necessary to ensure that the

binding experiments were done under physiological conditions. The following approaches were used:

- a) bubbling 5% CO₂/O₂ into serum
- b) bubbling 100% CO₂ into serum
- c) dissolving phosphate buffer salts in serum. The concentration of the salts used were 5.02 mg monobasic sodium phosphate monohydrate and 32.55 mg dibasic sodium phosphate heptahydrate per 1 ml of serum (0.12 M).

The last approach was found to be the most effective in maintaining the serum pH at physiological values and was subsequently used for the binding experiments.

2.6.3 Purified Human Serum Protein Solutions

Solutions of purified human serum proteins were prepared by dissolving the proteins in phosphate buffer (pH = 7.4) to yield concentrations of 4% and 0.1% w/v for human serum albumin (HSA) and α_1 -acid glycoprotein (AAG), respectively. The contribution of serum lipoproteins to the binding of mexiletine enantiomers was assessed using lipoprotein deficient human serum.

2.6.4 Sample Preparation

To 4 ml aliquots of serum was added racemic mexiletine in phosphate buffer (50 μ l) to yield a series of concentrations; 0.2, 0.5, 1.0, 2.0, 10, 40, 100, 200, 1000, 2000, 3000, and 4000 μ g/ml. The serum was mixed on a rotator for 10 minutes. Duplicate 1 ml samples were used to determine the total drug concentrations while a second set of duplicate 1 ml samples were subjected to ultrafiltration to determine

free drug concentrations. The binding studies were replicated with phosphate buffer solutions (pH = 7.4) of isolated HSA and AAG.

2.6.5 Ultrafiltration

Ultrafiltration was carried out with the MPS-1 micropartition system⁵² and a 30,000 Dalton molecular weight cut-off YMT ultrafiltration membrane⁵³. The centrifuge (Beckman Model J2-21)⁵⁴ had a 35° angle head rotor (Beckman model JA-17)⁵⁵ which was equilibrated to 37°C before introduction of the samples. Centrifugation time was 15 min at a relative centrifugal force of 1650 x g. The centrifugation parameters were those recommended by the manufacturer of the ultrafiltration system.

2.6.6 Analysis of Free and Total Mexiletine Enantiomers in Serum and Protein Solutions.

The concentrations of mexiletine enantiomers in serum and protein solutions, and their respective ultrafiltrates (free enantiomer concentrations) were determined using the HPLC method described in section 2.5.

2.6.7 Analysis of Binding Data

The free fraction, expressed as a percentage, was the ratio of the free to the total enantiomer concentration. Initial estimates of the equilibrium association (affinity) constant (K) and the capacity constant (N) were determined graphically for the enantiomers by fitting

52-53 Amicon Canada Ltd., Oakville, Ontario, Canada.

54-55 Beckman Instr. Inc., Fullerton, California, USA

the experimental data to the "Rosenthal" equation (Rosenthal, 1967):

$$B/F = \frac{N_1 K_1}{1 + K_1 F} + \frac{N_2 K_2}{1 + K_2 F}$$

(where B and F are the molar concentrations of bound and free drug, respectively. N is also the product of the number of binding sites per mole of protein (n) and the molar concentration of the protein(s)). Thus, from the binding data obtained from the isolated proteins, n was determined. The initial estimates of the capacity and affinity constants were used by the non-linear least squares program, ENZFITTER (Leatherbarrow, 1987), to yield the final values of these parameters.

2.6.8 Statistical Data Analysis

The Student's t-test (paired and independent groups) and one way ANOVA ($\alpha = 0.05$) were used.

2.7 Tissue Distribution Kinetics of Mexiletine Enantiomers in Rats

2.7.1 Drug Administration and Sample Collection

Male Sprague-Dawley rats (weight = 150-200 g) were administered 10 mg/kg of racemic mexiletine through a tail vein using a butterfly cannula (30 gauge) while each rat was temporarily restrained in a Perspex restrainer. Drugs were injected over a 1 min period. Groups of animals (4-5 rats per group) were sacrificed by decapitation at 0.08,

0.25, 0.5, 1, 2, 4 and 6 hours following drug administration and the brain, heart, lung, liver, kidney and epididymal fat tissues were rapidly excised. The organs were cut into small pieces, blotted with filter paper, weighed and homogenized in ice cold 0.1 M HCl. Blood was also collected at the same time as the tissues and serum was obtained for free and total concentration measurements. The serum and tissue homogenates were stored at -20°C until required for analysis.

2.7.2 Analysis of Serum and Tissue Samples

The concentrations of the enantiomers of mexiletine in both serum and the tissue homogenates were determined using the HPLC assay described in section 2.5.

2.7.3 Efficiency of Recovery of Mexiletine Enantiomers from Tissue Homogenates

The efficiency of recovery of the enantiomers from tissue homogenates was determined by the addition of 500 and 2000 ng of R,S-mexiletine to two 1 ml aliquots of the homogenates (containing 100 mg of tissue). The samples were extracted, derivatized and assayed as earlier described except that the internal standard was added just prior to the derivatization step. The resulting peak height ratios were expressed as a percentage of those obtained with identical amounts of R,S-mexiletine and the internal standard which were derivatized directly without prior extraction.

2.7.4 Serum Protein Binding

The *in vivo* free fractions of the enantiomers were determined by ultrafiltration technique as described in the serum protein binding

section (2.6).

2.7.5 Pharmacokinetic Data Analysis

The serum and tissue concentration time data were best described by a bi-exponential function of the form $C = Ae^{-\alpha t} + Be^{-\beta t}$ ($\alpha > \beta$) where C is the serum or tissue concentration at time, t , A and B are constants. α and β are the first order distribution and elimination rate constants, respectively. The initial estimates of the rate constants were obtained using the computer program, AUTOAN (Sedman and Wagner, 1976). These initial estimates were used by the iterative non-linear regression program, NONLIN (Metzler, 1974) to obtain the final values. The remaining pharmacokinetic parameters were obtained using formulae reported in Gibaldi and Perrier (1982). The distribution half-life [$t_{1/2}(\alpha)$] was determined from the formula: $t_{1/2}(\alpha) = 0.693/\alpha$, while the terminal elimination half-life [$t_{1/2}(\beta)$] was calculated from the formula: $t_{1/2}(\beta) = 0.693/\beta$. The area under the serum concentration-time curve (AUC) was determined from the trapezoidal rule with extrapolation to infinity from the last data point. The systemic clearance (CL) was calculated from the formula: $CL = \text{dose}/AUC$. The steady-state volume of distribution (V_{ss}) was determined from the formula: $V_{ss} = \text{dose} \cdot AUMC / AUC^2$ where AUMC is the total area under the first moment curve (Benet and Galeazzi, 1979).

2.7.6 Statistical Data Analysis

The Student's paired t-test ($\alpha=0.05$) was used to assess the statistical significance of differences in the serum and tissue concentrations obtained for the enantiomers.

2.8 Antiarrhythmic Activity of Racemic Mexiletine and its Enantiomers

The antiarrhythmic effects of racemic mexiletine and its enantiomers were assessed using 2 models. These were electrical-induced arrhythmias in pentobarbitone anaesthetized rats and ischaemia-induced arrhythmias in both conscious and pentobarbitone anaesthetized rats. All the experiments were carried out blind and randomized.

2.8.1 Electrically-Induced Arrhythmia

2.8.1.1 Preparation of Rats

Male Sprague-Dawley rats (wt = 250-350 g) were anaesthetized with pentobarbitone sodium (45 mg/kg i.p.). The left carotid artery was cannulated for blood pressure recording and sample acquisition, while a second cannulae was placed in the right jugular vein for drug administration. ECG leads (lead II) were implanted subcutaneously. A left thoracotomy was performed on the rats between the 5th and 6th intercostal ribs and the heart exposed. A Pair of teflon coated silver wire electrodes were placed 0.3 cm apart in the left ventricular wall. Stimulation of the left ventricle with square wave pulses was accomplished using a Grass Electric Stimulator.

2.8.1.2 Experimental End-points

Permanent records of the ECG and blood pressure were obtained using a Grass Polygraph. The ECG was also continuously monitored throughout the experiment with a delayed loop oscilloscope (Honeywell Type E for M). Discrimination of the end-points was carried out using the oscilloscope.

2.8.1.3 Variables Related to Antiarrhythmic Effects

The variables were: Current Threshold for Capture, Current Threshold Pulse Width, Maximum Following Frequency, Effective Refractory Period and Ventricular Fibrillation Threshold. Each variable was measured 3 times and a mean value obtained. The procedure for the measurements has been described (Curtis et al., 1984 & 1986; Euler and Scanlon, 1988).

a) **Current Threshold for Capture:** Current threshold for capture was determined at 1 ms and 7.5 Hz. It is the minimum current required to capture the heart.

b) **Threshold Pulse width:** The threshold pulse width for capture was determined at 7.5 Hz and twice the current threshold.

c) **Effective Refractory Period (ERP):** ERP was determined by the extra-stimulus method. The heart was paced at 7.5 Hz and a single extra stimulus was applied at varying intervals behind the pacing stimuli. The shortest interval between the pacing stimuli and the extra-stimulus in which an extra-systole was obtained was taken as the ERP.

d) **Maximum Following Frequency (MFF):** MFF was determined at twice the current and pulse width thresholds. MFF was taken as that point when the heart failed to follow, on a 1:1 basis, a steadily increasing frequency of stimulation from 7 to 20 Hz. This was readily seen as a sudden increase in blood pressure after an initial sustained drop.

e) **Ventricular Fibrillation Threshold (VFT):** VFT was determined at 60 Hz and twice pulse width threshold. The high frequency was used to ensure that a pulse was delivered during the vulnerable period, i.e.

the terminal portion of the QT interval in the ECG. The maximum current which elicited sustained fibrillation with a precipitous fall in blood pressure was taken as the fibrillation threshold.

2.8.1.4 Other Variables

The other variables measured included heart rate, blood pressure and the ECG parameters (PR, QRS and QT_c intervals)

2.8.1.5 Drug Administration

The rats were divided into 4 treatment groups with 8 rats per group. Each group received only one of the three forms of mexiletine (R,S-, S(+)- and R(-)-mexiletine) dissolved in saline, or saline as a control. After establishing a stable base line value for the measured variables, a 4 mg/kg dose was given intravenously over 1 min. Blood (0.5 ml) was withdrawn into plastic vials containing a drop of heparin (50 IU/ml) 10 min post-dose for determination of drug concentrations. ERP, MFF and VFT were determined between 10 and 15 min and a fast ECG trace (150 mm/min) was obtained for the determination of heart rate and the ECG parameters. The subsequent dose was then administered at 20 min. Dosages were assigned to produce a cumulative doubling of the previous dose i.e. 4, 8, 16 and 32 mg/kg. After the final measurements were taken, blood (3 ml) was collected from the rats for determination of the serum protein binding of racemic mexiletine and the individual enantiomers.

2.8.1.6 Analysis of Plasma Samples

The blood collected from the rats was centrifuged for 5 min and

the plasma separated and stored at -20°C until required for analysis. The concentrations of the enantiomers of mexiletine were determined using the stereoselective HPLC assay described in section 2.5. For the rats given racemic mexiletine, the individual enantiomers were measured and added to obtained the concentration of the racemate. The serum binding of the enantiomers was determined by ultrafiltration as described in section 2.6.

2.8.1.7 Statistical Analysis

Differences in the measured variables between the groups were assessed using "Repeated Measures" ANOVA ($\alpha=0.05$). The changes from pre-drug values were analyzed as absolute values (rather than as percent change). The *in vivo* free fractions of racemic mexiletine and the enantiomers were compared by one-way ANOVA.

2.8.2 Coronary Artery Occlusion in Conscious Rats

Coronary artery occlusion has been used by many investigators for the assessment of the antiarrhythmic efficacy of several drugs (Selye *et al.*, 1960; Clark *et al.*, 1980; Kane *et al.*, 1980; Johnston *et al.*, 1983; Curtis *et al.*, 1984). The present study was carried out in conscious rats according to the method described by Johnston *et al.* (1983) and Curtis (1986).

2.8.2.1 Preparation of Rats

The experiments were carried out using male Sprague-Dawley rats (wt = 350-450 g). All the lines, leads and occluder were sterilized in 70% ethanol in distilled water. Anaesthesia was induced in the rats in

a glass jar with 5% halothane/oxygen delivered via a vapourizer. The rats were subsequently intubated (for artificial respiration) with a 14 gauge human intravenous catheter with the aid of a paediatric laryngoscope and maintained on 1% halothane throughout the rest of the surgery.

2.8.2.2 Preparation of Occluder

The occluder was made up of an 11 cm length of PE-10 (polyethylene) tubing. One end of the tubing was flared by brief exposure to heat. About 1 cm from the other end, a flange was made by briefly melting the tube (with a wire inserted to prevent blockage of the lumen) by rotating it in front of a jet of hot air and then pressing it together (the jet of hot air was created by passing pressurized air through a thin copper tube held over a Bunsen burner). The tubing was used as a guide for a 5.0 gauge prolene suture threaded through with the needle end of the suture at the flared end of the guide.

2.8.2.3 Preparation of Lines

A 14 cm length of PE-50 tubing was welded onto a 10 cm length of PE-10 tubing by melting their tips using the jet of hot air described above and then pressing them together. A length of thin wire was passed through the tubings prior to the welding process to prevent the lumen of the tubes from getting blocked. The PE-10 end of the line was shaped into a coil by looping it around a glass rod and submerging it in boiling water for 3 seconds. The coil was fixed by submerging it in ice-cold water.

2.8.2.4 Implantation of Lines

Following midline laparotomy, the inferior vena cava and the abdominal aorta were cannulated for drug administration and blood pressure recording, respectively. The PE-10 ends of the lines were placed proximal to the blood vessels and the small diameter ensured that the aorta and vena cava were not blocked. These vessels were used because they are large, easily accessible vessels suitable for cannulation with chronic indwelling non-occluding lines. The lines were routed subcutaneously and exteriorized between the shoulder blades of the animal with the aid of a trocar. The abdomen was dusted with Cicatrin^R antibiotic powder and the body wall closed with running stitches. The skin was subsequently closed with interrupted stitches and the wound was infiltrated with Cicatrin^R and Marcaine^R. Approximately 0.3 ml of saline was injected into the vena cava through the exteriorized end of the cannula and the line abruptly clamped with a pair of atraumatic forceps. The open end of the cannula was then sealed with the aid of a lighter flame and the forceps were released. This treatment kept the line patent. The aortic line was treated in a similar fashion.

2.8.2.5 Implantation of the Occluder

A left thoracotomy was performed by blunt dissection through the 4th intercostal space while rats were respired artificially (stroke volume = 10 ml/kg at a stroke rate of 60/min). Retractors were used to widen the incision and the heart exposed. The prolene suture of the occluder was placed around the left anterior descending coronary artery such that the suture emerging from the guide tubing passed under the

artery and back through the tip of the guide tubing making a loose snare. The needle end of the suture was cut off and the remaining length carefully melted down to form a ball, thus, preventing the suture from being pulled back through the flared tip of the guide tubing. The prolene suture extended the full length of the guide tubing. The size of the snare was adjusted such that the flared end of the guide tubing was positioned adjacent to the atrial appendage. The distal end of the suture was then melted down to a small ball. The *pectoralis* muscle was sutured lightly to the *rectus abdominus* muscle with silk forming a purse string suture around the occluder. At the time the chest was closed, the pneumothorax was evacuated by applying negative pressure through a length of PE-90 tubing. The occluder was routed subcutaneously to the subscapular region using a trocar and exteriorized between the shoulder blades. The chest wound was infiltrated with Cicatrin^R and Marcaine^R and the skin closed with interrupted suture.

2.8.2.6 Implantation of the ECG Leads

The ECG leads were prepared from teflon coated stainless steel wire. The insulation was removed from approximately 1 cm of one end of each lead with the aid of a lighter flame. A tight coil was made in one end of one lead by wrapping it around a 21 gauge hypodermic needle. This end was then tied through the pectoralis muscle (chest lead) and exteriorized with the occluder. Limb leads were placed subcutaneously in the 2 fore limbs and the left hind limb. The ECG measured was approximately V3.

After implanting all the leads, lines and occluder, the subscapular wound was infiltrated with Cicatrin^R and Marcaine^R and the

respiratory pump disconnected. The leads, lines and occluder were exteriorized no more than 1 cm behind the shoulder of the animal. The animals were allowed to recover in individual cages. The incidence of infection or mortality following surgery was less than 1%.

2.8.2.7 Coronary Artery Occlusion

Rats were allowed to recover for one week. On the day of the occlusion experiment, the rats were weighed and their weights recorded. The lines and the ECG leads were connected and stable blood pressure recording and ECG were obtained for at least 15 min. Drug was then infused over a 10 min period and the coronary artery occluded 5 min after the end of drug administration. To occlude the coronary artery, the occluder guide was held by the Spenser Wells forceps with the atraumatic tips just above the flange located approximately 1 cm from the end distal to the heart. A second pair of forceps was used to firmly grip the inner snare of the occluder and traction was applied smoothly between the inner snare and the outer guide tubing to produce occlusion. When the sudden ECG and blood pressure changes characteristic of occlusion were observed, the atraumatic forceps were clamped down firmly on the occluder. The exposed length of the inner snare of the occluder was then melted down with a soldering iron to form a bulb adjacent to the distal end of the outer guide tubing, fixing it in place.

2.8.2.8 Drug Administration and Plasma Concentration Measurement

A 20 mg/kg dose of drug (R,S- or R(-)- or S(+)-mexiletine) in saline, or saline as control, was administered to each rat by constant

rate intravenous infusion over 10 min. A second dose was started at 1.5 h after occlusion and infused over 30 min. Blood (≈ 0.3 ml) was collected at -5 and 20 min and at 1, 1.5, 2, 3 and 4 h (with respect to initiation of coronary occlusion) into plastic vials containing a drop of heparin (50 IU/ml). The plasma was separated and analyzed for mexiletine enantiomer concentrations as described earlier (section 2.5).

2.8.2.9 Response to Drug Treatment and Occlusion

The ECG and blood pressure were recorded for 30 min before and 4 hr after coronary artery occlusion. Fast ECG traces (150 mm/min) were obtained just before the beginning of drug infusion and at 1 min before occlusion for the determination of the effect of treatment on heart rate and ECG parameters. Fast traces were also obtained at 1, 2, 5, 10, 15 and 30 min and at 1, 1.5, 2, 3 and 4 h after occlusion

2.8.2.10 Occluded Zone

The animals were sacrificed 4 h after coronary artery occlusion by stunning and decapitation. The heart was excised and perfused through the aorta with saline. When blood was no longer present in the perfusate, perfusion was continued with saline containing indocyanine green (0.5 g/l) via a 2-way stop-cock for 2-3 min. After perfusion, the atria, aorta and pulmonary vessels were excised and discarded. The remaining ventricular myocardium was readily differentiated into normal (green) and occluded (pink) tissue by visual inspection. The two zones were separated with a pair of scissors, blotted with filter paper and weighed.

2.8.2.11 Occlusion-Induced Arrhythmia

The following definitions were used in the diagnosis of arrhythmias following coronary occlusion in rats.

a) **Premature Ventricular Contraction (PVC):** PVC was defined as a premature QRS complex occurring independent of the P wave. A PVC was generally accompanied by a transient drop in aortic blood pressure.

b) **Ventricular Tachycardia (VT):** VT was defined as a run of 4 or more consecutive premature ventricular contractions. No restriction was made on the associated heart rate. VT's were associated with a decrease in mean arterial blood pressure which was sustained through the duration of the VT.

c) **Ventricular Fibrillation (VF):** VF was defined as a chaotic ECG tracing without recognizable QRS complexes and accompanied by a precipitous fall in blood pressure. VF was further subdivided into spontaneously reverting (SVF) i.e. fibrillation lasting less than 10 sec and non-spontaneously reverting (NSVF) i.e. those lasting more than 10 sec. In the later case, the arrhythmia was terminated by precordial taps.

2.8.2.12 Evaluation of Antiarrhythmic Efficacy

Antiarrhythmic efficacy was evaluated by determining the incidence of VT and VF, and the number of PVCs following coronary occlusion.

"Arrhythmias Score" (Johnston *et al*, 1983) was used to summarize and grade the arrhythmias.

This score is as follows:

0 = No more than 49 PVCs,

1 = 50 - 499 PVCs

- 2 = No more than 1 episode of SVT or SVF and/or greater than 499 PVCs.
- 3 = More than 1 episode of VT and/or VF of less than 60 sec duration,
- 4 = VT and/or VF of 60 - 119 sec total duration
- 5 = VT and/or VF of greater than 119 sec total duration
- 6 = Fatal VF occurring 15 min - 4 h after occlusion
- 7 = Fatal VF occurring 4 min - 14 min 59 sec after occlusion
- 8 = Fatal VF occurring 1 min - 3 min 59 sec after occlusion
- 9 = Fatal VF occurring before 1 min after occlusion

All the results were subsequently divided into early (0-0.5 h) and overall (0-4 h) arrhythmias.

2.8.2.13 Statistical Data Analysis

Differences in the binomially distributed variables (incidence of arrhythmias) were assessed by Chi-square test with the aid of Mainland's contingency table (Mainland *et al.*, 1956). One-way ANOVA was used to compare the normally distributed variables (arrhythmia score, blood pressure, heart rate and the ECG parameters). The number of PVCs is not a Gaussian distributed variable (Johnston *et al.*, 1983). Therefore, data obtained for this variable was \log_{10} transformed before being used for statistical tests. When a significant F ratio was obtained from ANOVA, subsequent analysis using Duncan's and Newman Keul's multiple comparison tests were carried out to determine which treatment groups were significantly different. The value of α was 0.05 for all the statistical tests.

2.8.2.14 Exclusion Criteria

To avoid sources of variance that may influence the precision and accuracy of the experimental results, a set of exclusion criteria reported by Curtis (1986) was used to exclude animals from the study. The set of criteria were applied blindly and rats were excluded before, during or after occlusion based on the following abnormalities.

I) Pre-occlusion Abnormalities

These were designed to exclude rats with previous occlusion.

- a) The presence of a Q-wave. This is because a Q-wave is only seen in chest leads in rats following coronary occlusion.
- b) The presence of more than 5 PVCs during the 15 min period prior to drug administration. This may indicate the presence of a lesion in the myocardium which may influence the outcome of occlusion.
- c) More than 25% weight loss between surgery and coronary occlusion. Rats were excluded if weight loss was associated with other signs of illness such as diarrhea and/or pre-drug mean arterial blood pressure of 85 mmHg or less.
- d) Signs of lung infection such as exudate around snout and/or noisy respiration.
- e) Hypertension (mean blood pressure greater than 125 mmHg for longer than 10 min before drug administration). Rats were excluded only if postmortem examination revealed renal necrosis.

II) Post-occlusion Abnormalities

These evaluations were designed to exclude rats without full occlusion, rats which experienced reperfusion, and rats with misplaced occluders.

a) No increase in R wave, and/or ST elevation. Rats were excluded if they had unacceptably low OZ, if death occurred within 4 hours and pus or scarring was found in the heart, if autopsy showed occluder was loose, if sudden ECG changes typical of occlusion occurred later than 3 min after occlusion (posturally induced full occlusion).

b) Loss of R wave increase and/or ST elevation leading to ECG resembling pre-occlusion ECG with accompanying recovery of blood pressure. Rats were excluded if OZ was unacceptably low, if the occluder was loose even if OZ was normal.

c) Death associated with immediate calamitous hypotension. Rats were excluded if thorax was filled with blood and the rat had not received chest taps.

III) Postmortem Abnormalities

Rats were excluded if they had:

- a) Small OZ defined as less than 25% of ventricular weight.
- b) Scar tissue greater than 5% of the ventricle.
- c) Pus of 10% or more ventricular weight at the occlusion site.

2.8.3 Coronary Artery Occlusion in Pentobarbitone Anaesthetized Rats

The study of the antiarrhythmic effects of racemic mexiletine in pentobarbitone anaesthetized rats was carried out as described for the conscious rats with the following modifications:

- a) Rats were acutely prepared under pentobarbitone anaesthesia (45 mg/kg i.p.) and artificially respired throughout the duration of the

experiment.

b) The carotid artery and jugular vein were cannulated for blood pressure monitoring and drug administration, respectively.

c) Lead II ECG was recorded.

d) Response to coronary artery occlusion was monitored for only 30 min which covered the early phase of arrhythmias.

e) Three groups of rats ($n = 9$ per group) were given either saline or R,S-mexiletine (20 or 40 mg/kg) in a blind and random manner.

f) Blood was collected at -5, 15 and 30 min post-occlusion for the determination of drug levels.

3. RESULTS and DISCUSSION

3.1 Assay of Mexiletine Enantiomers by HPLC using 2-Anthroyl Chloride as a Derivatization Reagent

3.1.1 Synthesis of 2-Anthroyl Chloride

The synthesis of 2-anthroyl chloride undertaken in this study was necessary since neither the acid chloride nor the precursor acid could be obtained commercially. 2-Anthroyl chloride was synthesised by a 2-step reaction according to the scheme shown in figure 2. The first step involved the selective reduction of anthraquinone-2-carboxylic acid to anthracene-2-carboxylic acid using dilute ammonia and zinc dust. This reaction proceeded smoothly, going to completion within 45 min. The product isolated by preparative HPLC was recrystallized from ethanol (95%) and was found to have a melting point of 278-279°C. This agrees with the reported literature value which is 280°C (Weast, 1976). The structure of the acid was confirmed by direct probe EI-MS. The total ion current chromatogram and the mass spectrum are shown in figure 3. The major fragment ions were diagnostic of the acid with M^+ ($m/z=222$), M^+-OH ($m/z=205$), and M^+-COOH ($m/z=177$) mass ions present.

The second step in the reaction scheme (fig. 2) involved the conversion of anthracene-2-carboxylic acid to 2-anthroyl chloride by treatment with oxalyl chloride. The synthetic acid chloride was isolated by preparative HPLC and the structure confirmed by direct probe EI-MS. Figure 4 shows the total ion current chromatogram and the mass spectrum of 2-anthroyl chloride. The molecular ion peaks were present in the mass spectrum at $m/z=240$ and 242 from the natural isotope ratio

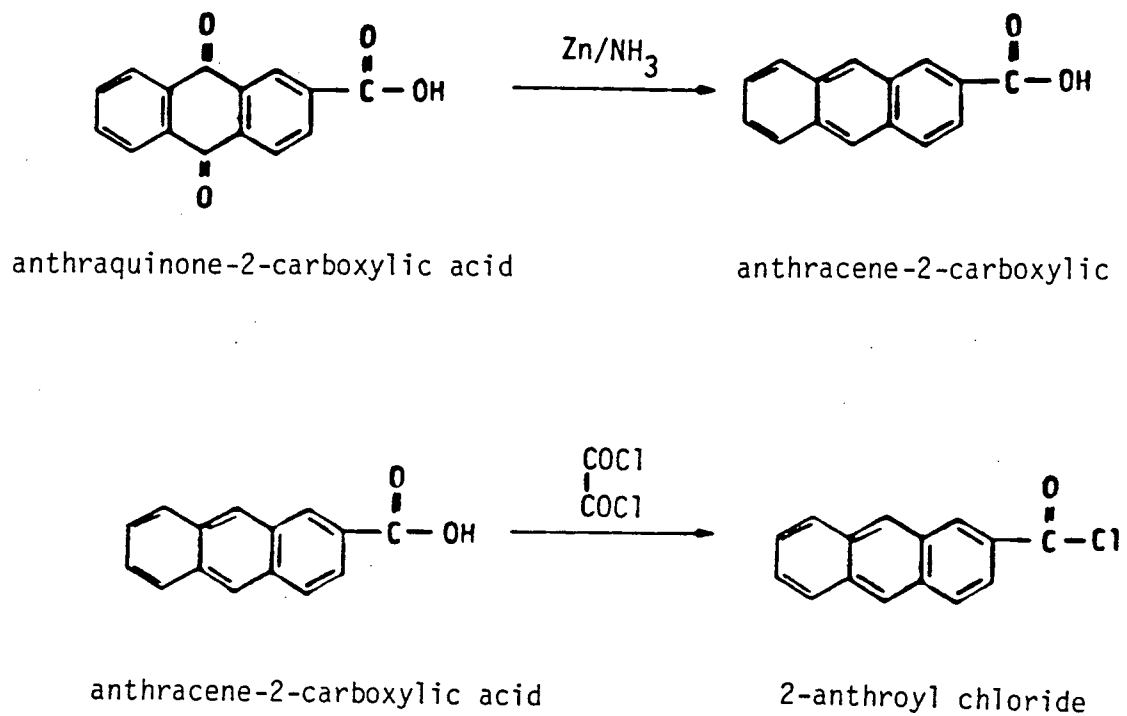


Figure 2. Scheme for the synthesis of 2-anthroyl chloride from anthraquinone-2-carboxylic acid.

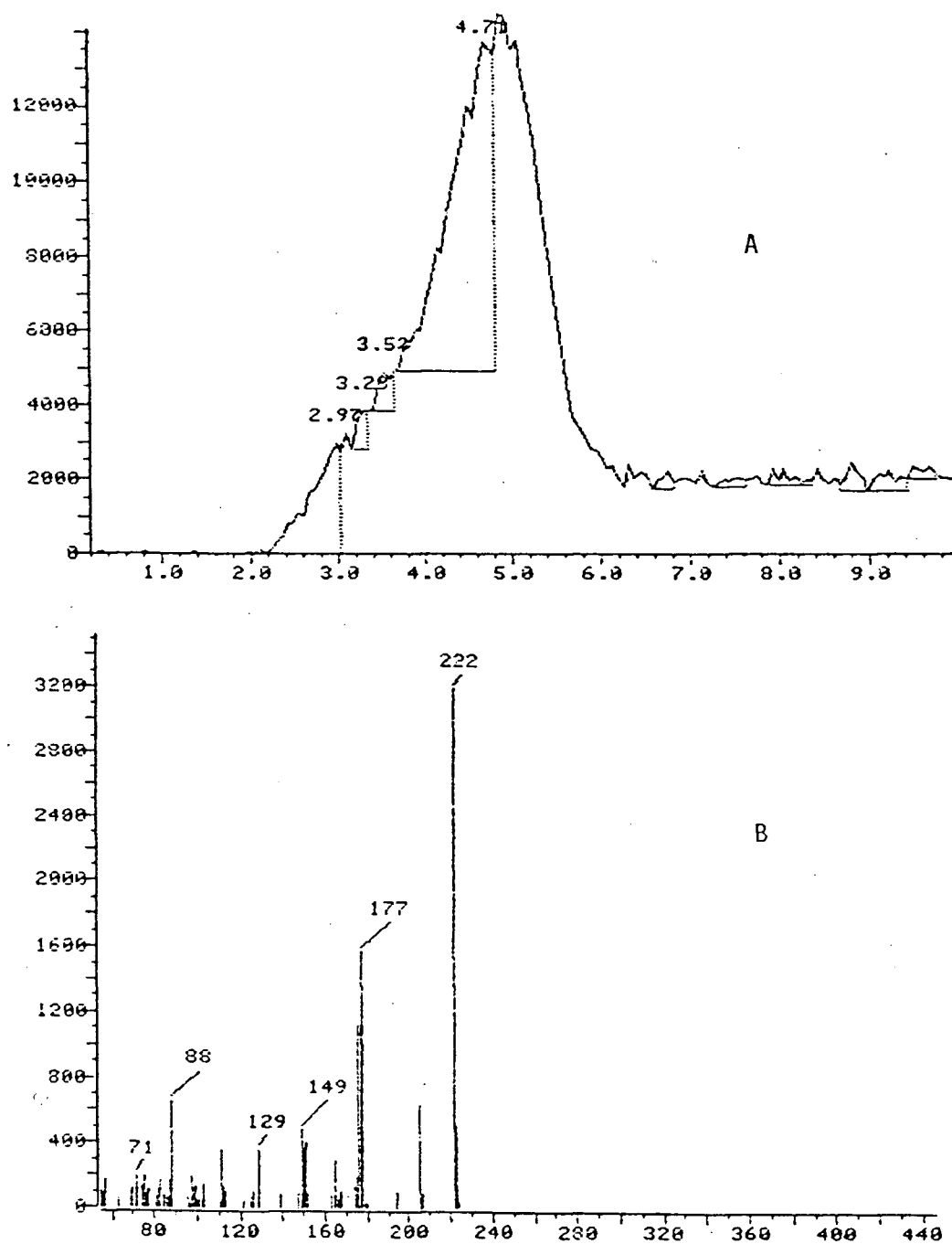


Figure 3. The total ion current chromatogram (A) and the mass spectrum (B) of anthracene-2-carboxylic acid. The following EI-MS conditions were employed: source temperature, 240°C ; probe temperature programme, 50°C for 1 min to 300°C for 10 min at the rate of 30°C per min; electron beam voltage, 70 eV; emission current, 300 μA and multiplier voltage, 2500 V.

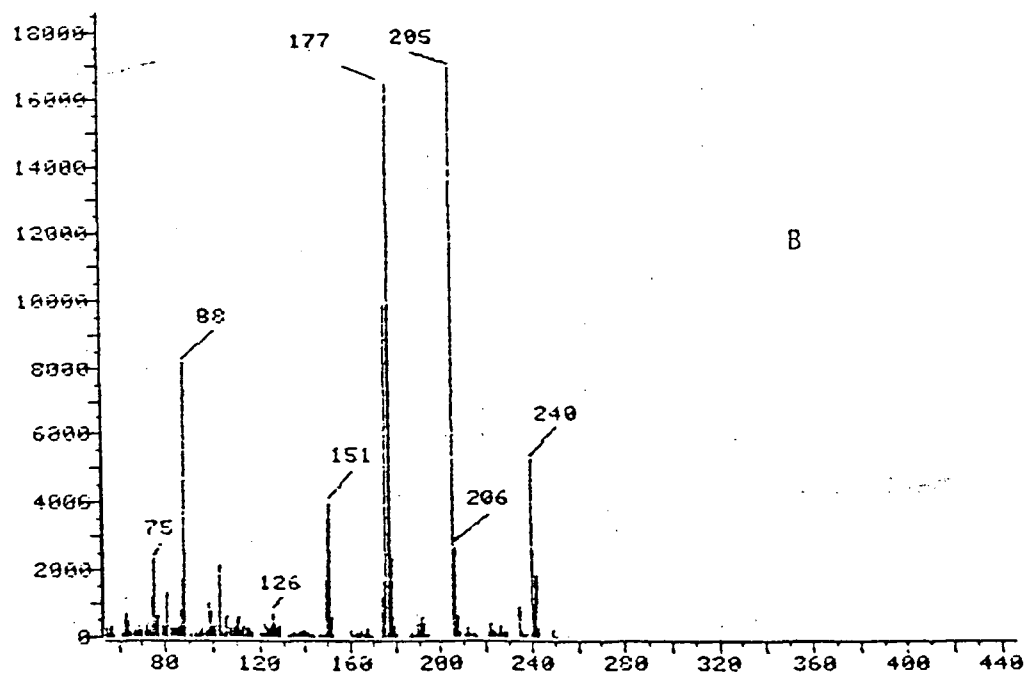
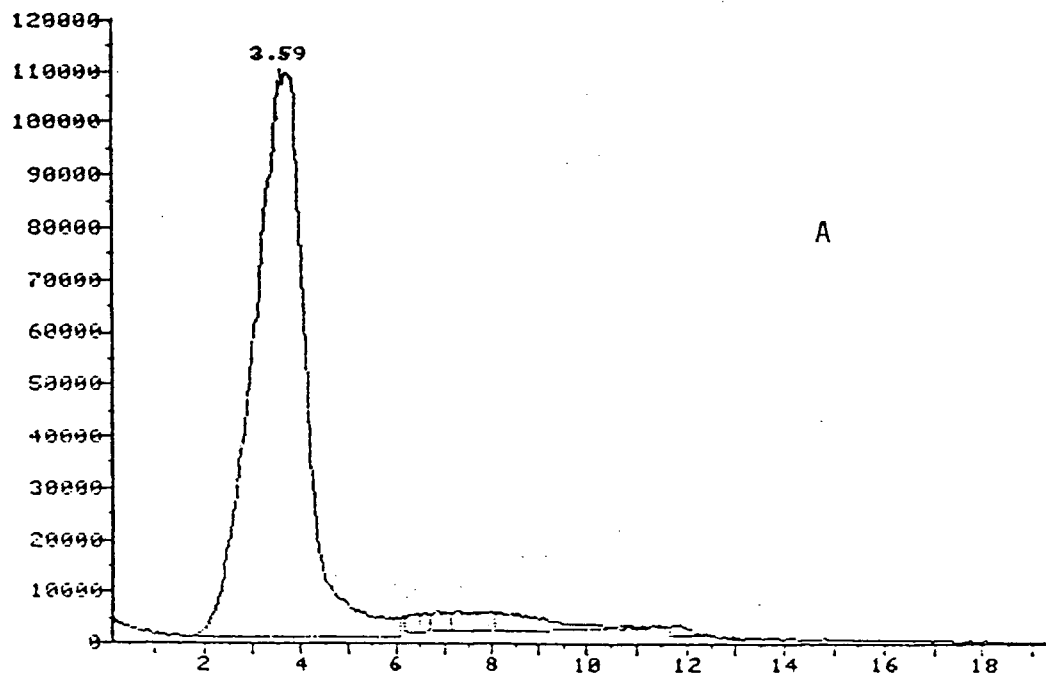


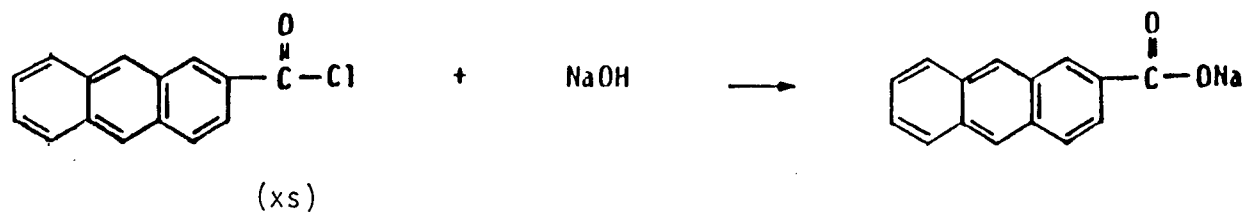
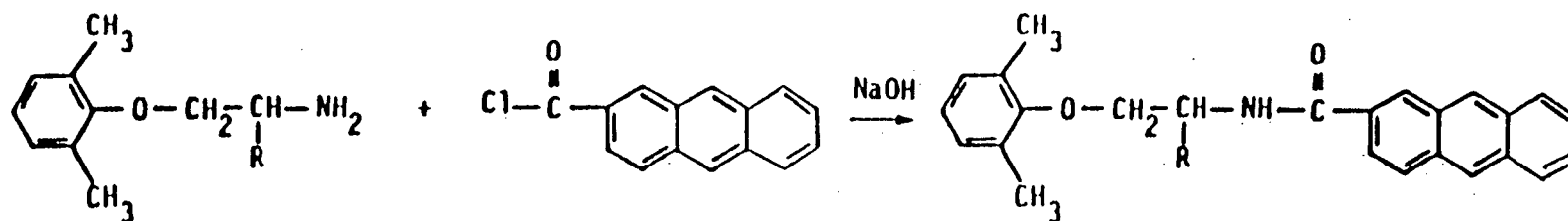
Figure 4. The total ion current chromatogram (A) and the mass spectrum (B) of 2-anthroyl chloride. The following EI-MS conditions were employed: source temperature, 240°C; probe temperature programme, 50°C for 1 min to 300°C for 10 min at the rate of 30°C per min; electron beam voltage, 70 eV; emission current, 300 μ A and multiplier voltage, 2500 V.

of chlorine (3:1). Other important fragment ions observed were M^+-Cl ($m/z=205$) and M^+-COCl ($m/z=177$).

3.1.2 Development of Assay of Mexiletine Enantiomers

3.1.2.1 Derivatization of Mexiletine Enantiomers with 2-Anthroyl Chloride

A number of properties are required of an ideal derivatization reagent for the HPLC resolution and quantitation of mexiletine enantiomers using the Pirkle^R ionic (phenyl glycine) chiral phase. These properties include a functional group that will react with the primary amino group of mexiletine, a strong fluorophore for sensitive detection of the enantiomer derivatives and functional groups that aid in the chiral recognition process on the Pirkle^R chiral phase. In a previous study, 2-naphthoyl chloride was found to have these necessary characteristics and was successfully used to resolve and quantitate mexiletine enantiomers using the Pirkle^R ionic (phenyl glycine) chiral column (Igwemezie, 1986; McErlane *et al.*, 1987). Examination of the chiral recognition process required for the resolution of the 2-naphthoyl derivatives of the enantiomers lead to the proposal that selectivity could be preserved, while improving sensitivity by the use of 2-anthroyl chloride as the derivatization reagent (the fluorescence quantum yield of naphthalene is 0.19 while that of anthracene is 0.30 in cyclohexane at 25°C) (Froehlich, 1985). The derivatization of mexiletine enantiomers with 2-anthroyl chloride was accomplished using the Schotten-Baumann reaction (Vogel, 1964) as shown in figure 5. The amide derivatives of the enantiomers were insoluble in the aqueous



$\text{R} = \text{CH}_3$ (Mexiletine)
 $\text{R} = \text{H}$ (I.S.)

Figure 5. Reaction scheme for the derivatization of mexiletine enantiomers and the internal standard with 2-anthroyl chloride

reaction medium and therefore readily isolated by extraction with the mobile phase.

3.1.2.2 Structure of the 2-Anthroyl Derivative of Mexiletine

The structure of the 2-anthroyl derivative of mexiletine (mexiletine-2-anthramide) was confirmed by direct probe EI-MS. The total ion current chromatogram (fig. 6A) showed the presence of two peaks. The mexiletine-2-anthramide peak had a retention time of 5.21 min and the mass spectrum (fig. 6C) exhibited major fragment ions which were diagnostic as shown in figure 7. The identity of the peak at 3.28 min (fig. 6A) could not be determined from its mass spectrum (fig. 6B). However, the presence of mass ions at 149 and 279 suggested that it may be a phthalate contaminant (Watson, 1985).

3.1.2.3 Resolution of Mexiletine Enantiomers

Following derivatization with 2-anthroyl chloride, the enantiomers of mexiletine were resolved on a Pirkle^R ionic (phenyl glycine) chiral stationary phase (see fig. 9). The optimum mobile phase was ethyl acetate/2-propanol/hexane (4:6:90) delivered isocratically at a flow rate of 1.7 ml/min. The retention times of the enantiomers were 16.68 and 17.69 min for R(-)- and S(+)-mexiletine, respectively (fig. 8). The elution order of the peaks was determined by comparing the retention time of the individual enantiomer derivatives with those of the racemate peaks and also from the anticipated stereochemical interactions between the enantiomers and the stationary phase (section 3.1.2.5). The

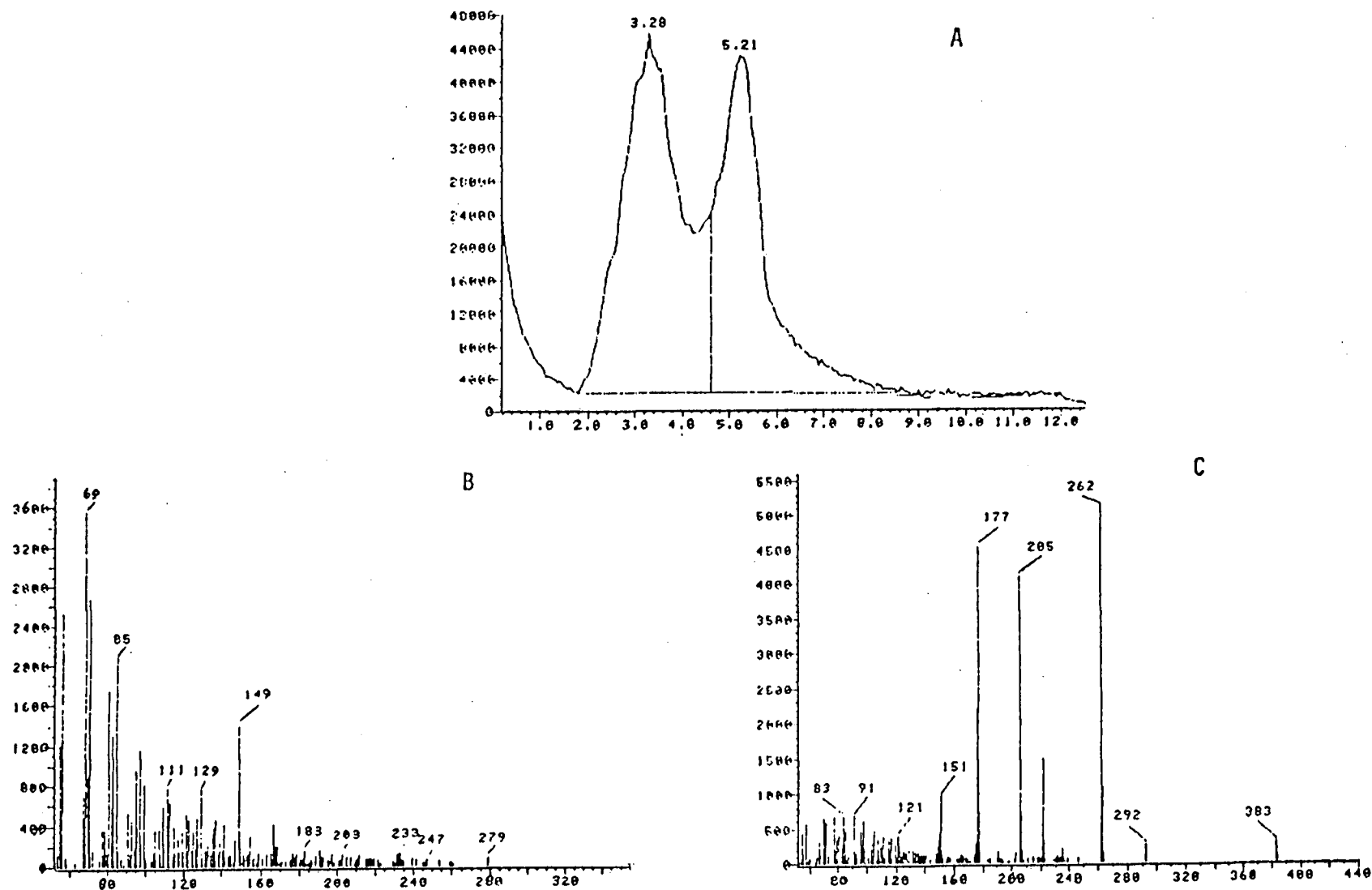
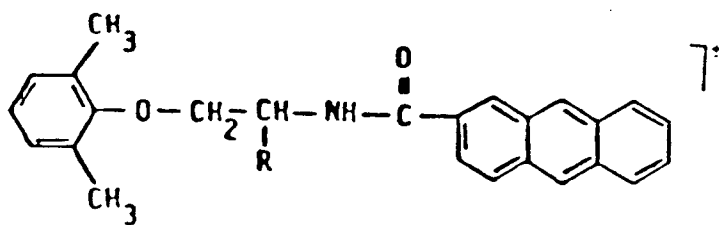
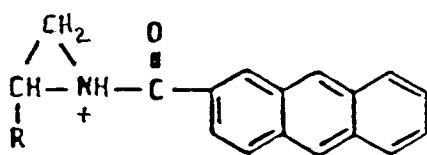


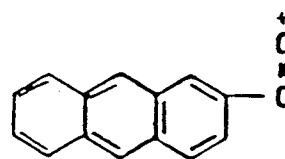
Figure 6. The total ion current chromatogram (A) and the mass spectra (B and C) obtained from the 2-anthroyl derivative of mexiletine. The following EI-MS conditions were employed: source temperature, 240°C; probe temperature programme, 50°C for 1 min to 300°C for 10 min at 30°C per min; electron beam voltage, 70 eV; emission current, 300 uA and multiplier voltage, 2500 V



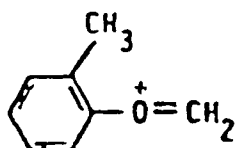
m/z 383 (M^+)



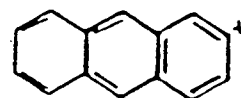
m/z 262



m/z 205



m/z 121



m/z 177

Figure 7. The major fragment ions of the 2-anthroyl derivative of mexiletine.

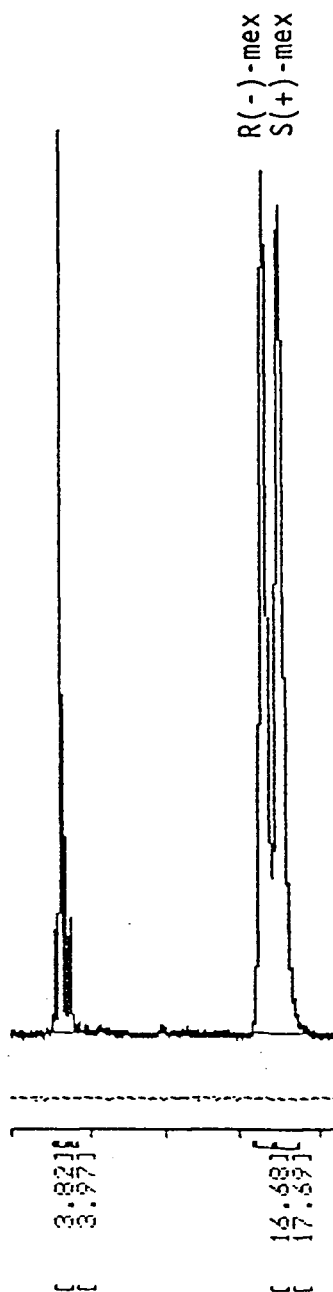


Figure 8. Chromatogram of the 2-anthroyl derivative of mexiletine enantiomers. Chromatographic conditions were: stationary phase, Pirkle[®] ionic (phenyl glycine) chiral phase; mobile phase, ethyl acetate/2-propanol/hexane (4:6:90); flow rate, 1.7 ml/min; detection, fluorescence at 270 nm (ex) and 400 nm (em).

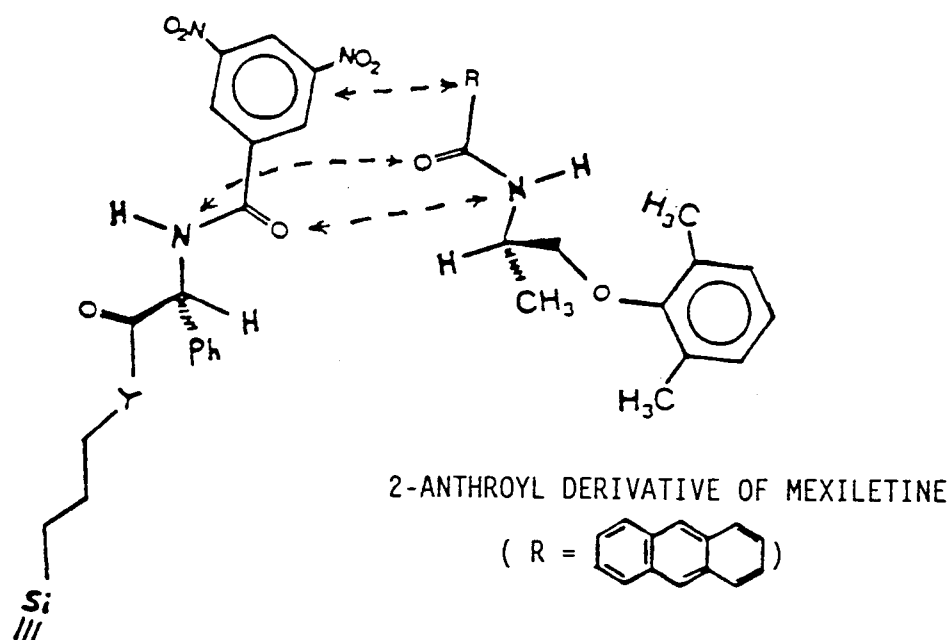
resolution of the enantiomers achieved was considered satisfactory ($R = 1.4$) for peak height measurement.

3.1.2.4 Sensitivity of the 2-Anthroyl Derivative of Mexiletine

The detection of the enantiomer derivatives was accomplished by fluorescence ($\text{ex}=270 \text{ nm}$ and $\text{em}=400 \text{ nm}$). The minimum detectable quantity of each enantiomer was 0.5 ng/ml , representing 50 pg at the detector (signal-to-noise ratio of 5:1). Thus, 2-anthroyl chloride provided a 10-fold increase in sensitivity over a previously reported assay (Igwezie, 1986; McErlane *et al.*, 1987) in which mexiletine enantiomers were derivatized with 2-naphthoyl chloride.

3.1.2.5 Mechanism of Resolution of Mexiletine Enantiomers

To resolve enantiomers on a chiral stationary phase (CSP), a minimum of 3 simultaneous interactions between the enantiomers and the CSP is necessary (Feibush and Grinberg, 1988). One of these interactions also has to be stereochemically dependent and could be an attraction or a repulsion. These interactions result in the formation of transient diastereoisomeric complexes whose stabilities are different, resulting in a difference in the migration rate of the enantiomers through the CSP. Figure 9 shows the structure of the Pirkle^R ionic (phenyl glycine) chiral phase and the 2-anthroyl derivative of mexiletine. The enantiomer derivatives are envisaged to interact with the CSP by means of π - π bonding between the π -basic anthroyl group and the π -acidic 3,5-dinitrobenzoyl group of the CSP, electrostatic bonding of the amide dipoles (broken arrows in figure 9),



PIRKLE^R IONIC CHIRAL STATIONARY
PHASE (Y= O^-NH_3^+)

Figure 9. The structure of the Pirkle^R ionic chiral stationary phase [(R)-3,5-dinitrobenzoylphenyl glycine ionically bonded to Γ -amino propyl silica] and the 2-anthroyl derivative of mexiletine. The broken arrows show the envisaged interactions.

and a steric interaction of the methyl and xylyloxymethyl groups at the chiral centre of mexiletine with the proximate portion of the CSP. Due to the presence of the bulky phenyl group below the plane of the CSP, the analyte molecule can only approach the CSP from the top. The analyte-CSP interactions will produce a more stable diastereoisomeric complex with S(+)-mexiletine-2-anthramide where the methyl group lie below the plane of the analyte molecule. The R(-)-mexiletine-2-anthramide, with the bulkier xylyloxymethyl group below the plane of the analyte molecule, will be more sterically hindered. The result is a faster elution of this enantiomer through the CSP. This stereochemical model of interaction of mexiletine enantiomer derivatives with the Pirkle^R CSP was derived from the "dipole stacking" chiral recognition model of Pirkle and Welch (1984). The model has been used to determine the elution order of enantiomeric amines (Pirkle and Welch, 1984) and mexiletine enantiomers as the 2-naphthoyl derivatives (McErlane *et al.*, 1987) on the Pirkle^R CSP.

3.1.2.6 HPLC Assay of Mexiletine Enantiomers

Mexiletine enantiomers and the internal standard were extracted from an aqueous solution with diethyl ether prior to derivatization. Figure 10 shows representative chromatograms of extracts of distilled water containing racemic mexiletine and the internal standard, and blank distilled water. The latter chromatogram (fig. 10B) indicated the presence of an extraneous peak which co-eluted with the S(+)-enantiomer.

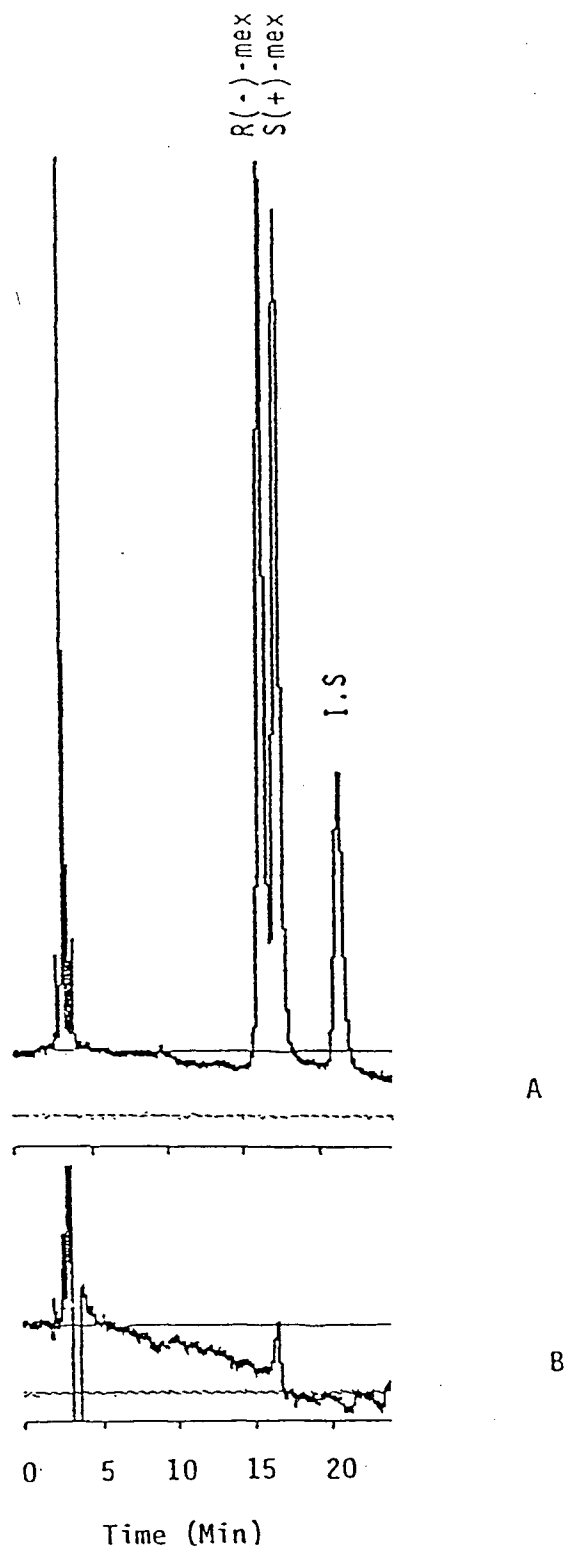


Figure 10. Chromatograms of extracts of distilled water containing mexiletine enantiomers (125 ng/ml each) and the internal standard (60 ng/ml) (A), and distilled water (B). Chromatographic conditions were: stationary phase, Pirkle[®] ionic (phenyl glycine) chiral phase; mobile phase, ethyl acetate/2-propanol/hexane (4:6:90); flow rate, 1.7 ml/min; detection, fluorescence at 270 nm (ex) and 400 nm (em).

3.1.2.7 Attempted Resolution/Removal of the Interfering Peak

a) **Variation of Mobile Phase Polarity:** Extensive variations of the mobile phases polarity as described in section 2.4.2.8 did not significantly change the retention time of the interfering compound relative to that of the enantiomers. The extent to which polarity could be increased was limited by the fact that the Pirkle^R ionic chiral column can only tolerate the equivalent of 20% 2-propanol/hexane with respect to polarity.

b) **Extraction Solvent:** A comparison of the chromatograms obtained from the extracted and unextracted R(-)-enantiomer (100 ng/ml) and the internal standard (100 ng/ml) demonstrated that the size of the interfering compound was significantly greater when an extraction step was involved (fig. 11). Thus, the possibility of a reaction between the 2-anthroyl chloride and an impurity in the extraction solvent was suspected. Two other solvents, hexane and dichloromethane were investigated. As shown in figure 12, the interfering peak was present with each solvent.

c) **Purification of Solvents and Reagents:** To determine if the interfering peak was due to an impurity present in the solvents or reagents, extensive purification of each solvent and reagent was carried out as described in section 2.4.2.8. These treatments did not eliminate the interfering compound.

d) **Pirkle^R (S)-Leucine Chiral Column:** In order to resolve the interfering peak from those of mexiletine enantiomers, the stationary phase was changed to the Pirkle^R (S)-leucine phase. However, the

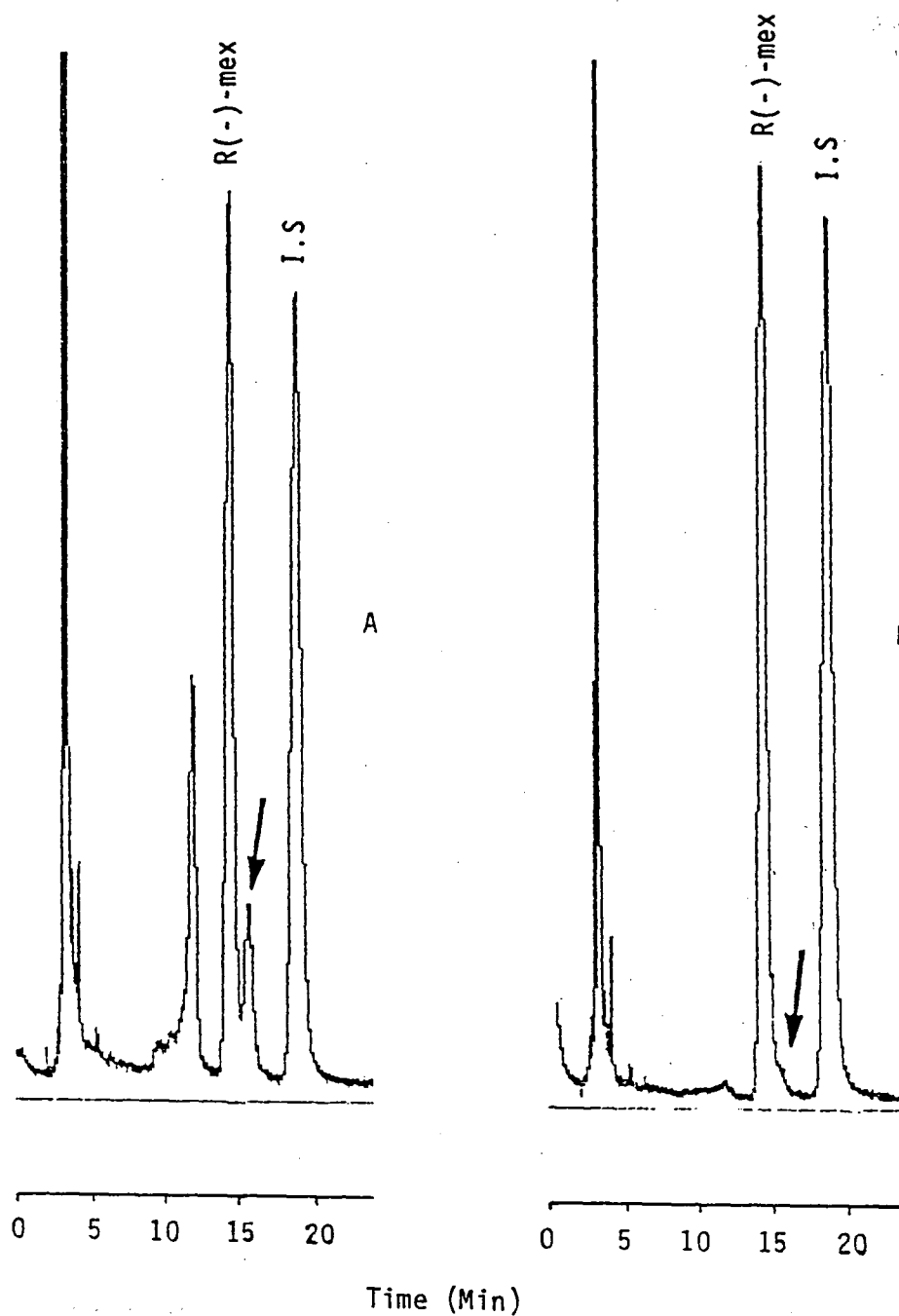


Figure 11. Chromatograms of R(-)-mexiletine (100 ng/ml) and the internal standard (100 ng/ml) derivatized with 2-anthroyl chloride after extraction from an aqueous solution with diethyl ether (A), and the same concentrations of R(-)-mexiletine and the internal standard derivatized without prior extraction (B).

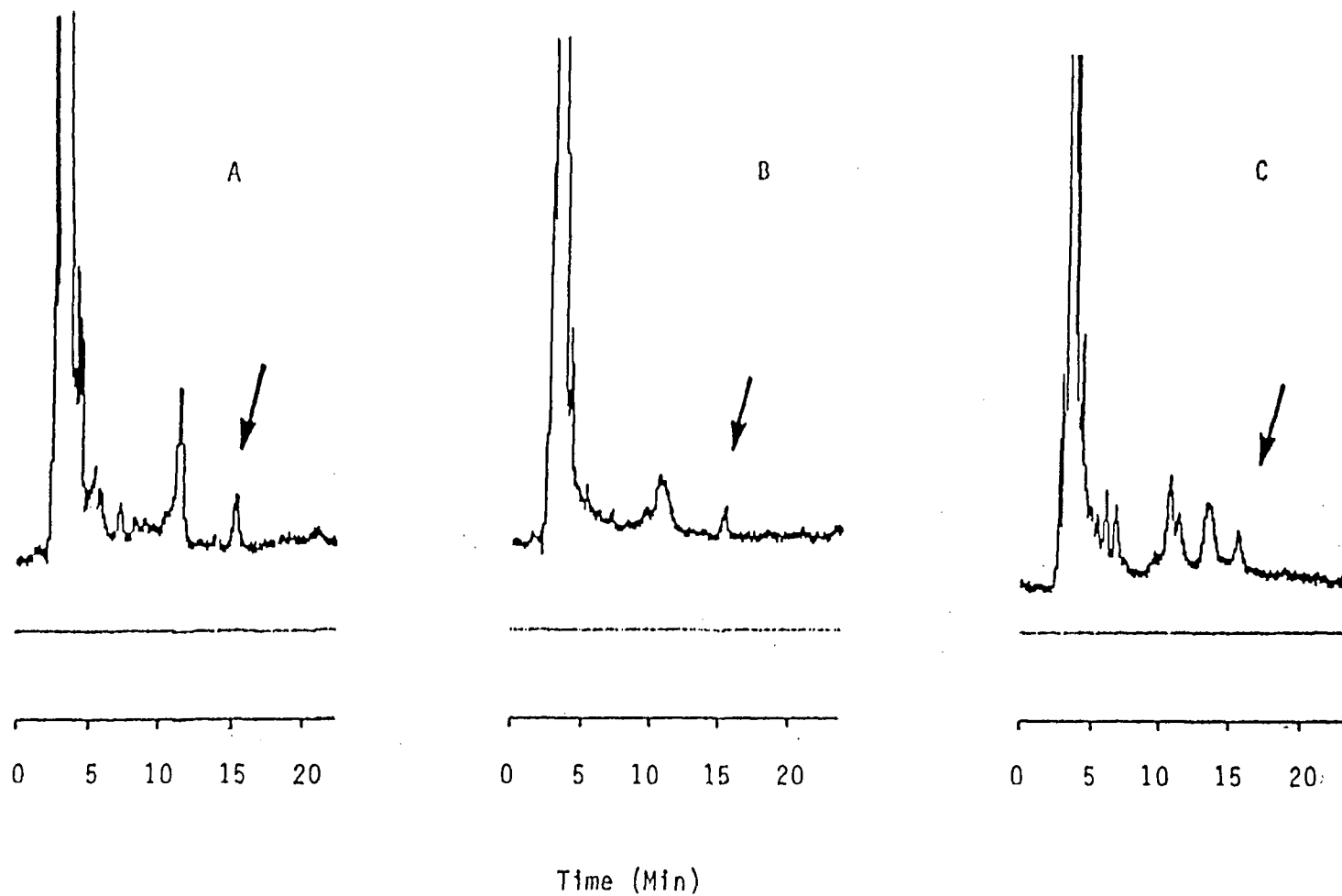


Figure 12. Chromatograms obtained after extraction of 1 ml aliquots of distilled water with different extraction solvents: diethyl ether (A), hexane (B) and dichloromethane (C). The arrows indicate the retention time of the interfering substance.

anthroyl derivatives of mexiletine enantiomers were unresolved on this stationary phase.

Since all attempts to solve the interfering peak problem were successful, a previously developed stereoselective assay for mexiletine enantiomers using 2-naphthoyl chloride as derivatization reagent (see below) was used for the proposed pharmacokinetic and pharmacodynamic studies.

3.2 Assay of Mexiletine Enantiomers by HPLC with 2-Naphthoyl Chloride as a Derivatization reagent

Figure 13 shows the resulting chromatograms of mexiletine enantiomers (50 ng/ml each) and the internal standard (100 ng/ml) isolated from human plasma and blank plasma and assayed as their 2-naphthamide derivatives. There was no evidence of any interfering peak with those due to the enantiomers or the internal standard. The minimum detection limit of the assay was 5 ng/ml of each enantiomer in plasma, at a signal-to-noise ratio of 5:1. Table 1 shows the calibration curve data for the enantiomers in plasma. A coefficient of determination (r^2) greater than 0.99 was obtained for both enantiomers and the inter-assay variability (of 3 determinations) was less than 10%.

Representative calibration curves of the enantiomers in rat plasma (concentration range = 10 to 250 ng/ml of each enantiomer) gave the following linear least squares regression equations: $Y = 0.024X + 0.027$ ($r^2=0.999$) for R(-)-mexiletine and $Y = 0.022X + 0.022$ ($r^2=0.999$) for S(+)-mexiletine. Similar calibration curves were also obtained for the enantiomers in the various rat tissue homogenates.

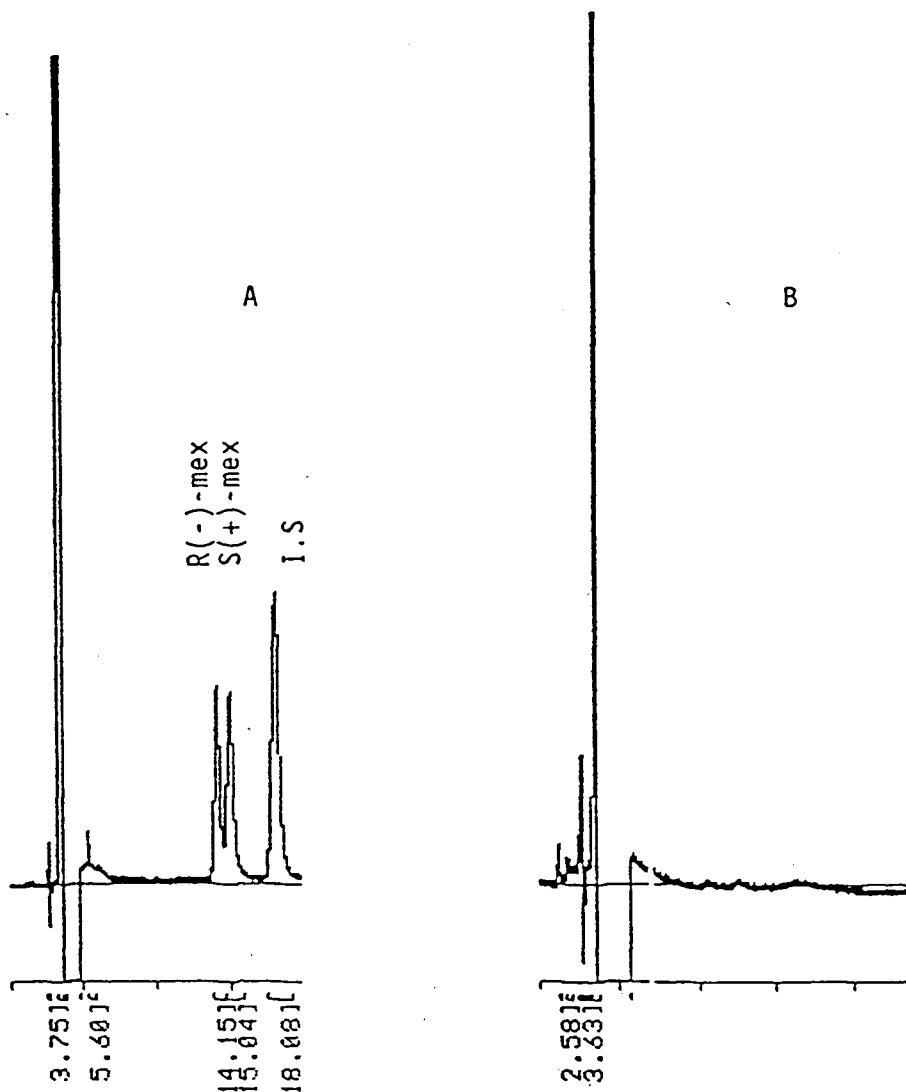


Figure 13. Chromatograms of 2-naphthoyl derivatives of mexiletine enantiomers (50 ng/ml each) and the internal standard (100 ng/ml) isolated from human plasma (A), and blank plasma (B). The chromatographic conditions were: stationary phase, Pirkle[®] ionic (phenyl glycine) chiral phase; mobile phase, chloroform/2-propanol/hexane (7:7:86); flow rate, 1.2 ml/min; detection, fluorescence at 230 nm (ex) and 370 nm (em).

Table 1. Calibration curve data for mexiletine enantiomers in human plasma

Wt of each enantiomer (ng)	Peak-ht ratio R(-)-mex/I.S.*	C.V. (%)**	Peak-ht ratio S(+)-mex/I.S.*	C.V. (%)**
500	12.50 \pm 0.04	1	12.10 \pm 0.06	1
200	4.98 \pm 0.14	3	4.82 \pm 0.18	4
50	1.24 \pm 0.02	2	1.18 \pm 0.02	2
20	0.50 \pm 0.01	1	0.48 \pm 0.01	2
10	0.26 \pm 0.02	8	0.24 \pm 0.02	9
slope	0.025		0.024	
intercept	-0.003		-0.014	
r ²	0.999		0.999	

* Mean \pm s.d., n = 3 determinations.

** Percent coefficient of variability. The amount of internal standard (I.S.) used was 50 ng.

3.3 *In Vitro* Serum Protein Binding of Mexiletine Enantiomers

3.3.1 Factors Affecting Serum Protein Binding

The *in vitro* serum protein binding of mexiletine enantiomers was carried out using serum from healthy human subjects as well as commercially obtained α_1 -acid glycoprotein (AAG), human serum albumin (HSA) and lipoprotein deficient serum. To ensure that the binding results were meaningful, methodological factors which may influence serum protein binding were evaluated. These factors included non-specific adsorption to the ultrafiltration system, pH-dependent binding and competitive binding. The influence of other factors such as binding displacement by tris(butoxyethyl) phosphate from Vacutainer^R caps and the age of serum were avoided by collecting blood in glass syringes and using the serum shortly after collection.

3.3.1.1 Non-specific Binding

Table 2 shows the percent recovery of mexiletine enantiomers during ultrafiltration. The recovery was in excess of 90% for both enantiomers indicating that non-specific binding to the ultrafiltration system was minimal. However, the free fractions obtained were corrected for the small adsorptive loss.

3.3.1.2 Effect of Serum pH

The effect of pH on the binding of mexiletine enantiomers to human serum was evaluated at 3 different pH values (7.0, 7.4 and 8.0). As shown in Figure 14, the free fraction of each enantiomer decreased

Table 2. Percent recovery of mexiletine enantiomers during ultrafiltration

Concentration of each enantiomer ($\mu\text{g/ml}$)	R(-)-mex	S(+)-mex
1.00	96 \pm 2	94 \pm 1
0.25	91 \pm 2	92 \pm 3
0.10	92 \pm 2	91 \pm 2

Values are means \pm s.e.m of triplicate determinations.
Percent recovery was determined from the ratio of the
concentration of the enantiomers (in phosphate buffer, pH = 7.4)
before and after ultrafiltration.

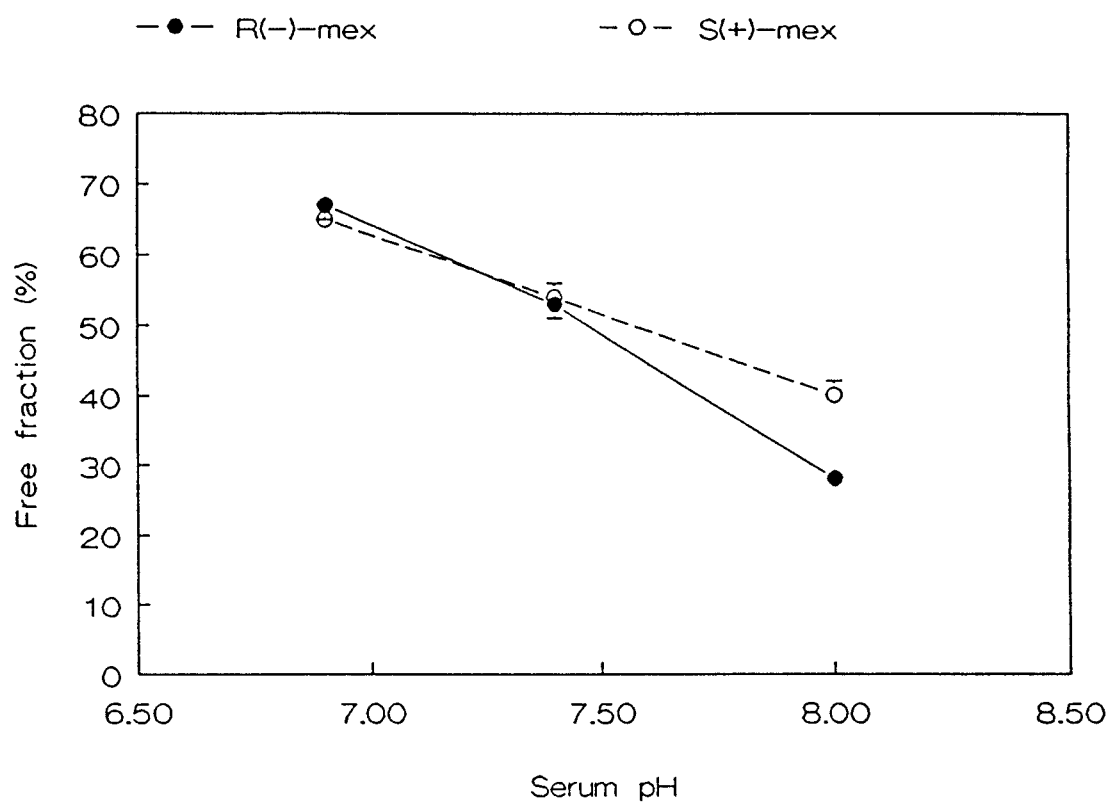


Figure 14. The relationship between percent free fraction (mean \pm s.e.m., $n=6$ subjects) and serum pH.

significantly ($p < 0.05$) with increasing pH. Binding was found to be stereoselective as the pH was raised above 7.4 and was greater for the R(-)-enantiomer. There were no significant differences between the free fraction of the enantiomers at pH 7.4 and 7.0. A change in the free fraction of a drug occurring with a change in pH can be explained on the basis of changes in the ionization of the binding protein and/or the ionization of the drug (McNamara *et al.*, 1981). A similar decrease in free fraction with increase in pH was reported for lidocaine, a structural analogue of mexiletine, by Burney *et al.* (1978) and McNamara *et al.* (1981). Both investigators explained the changes in binding to be due to a change in the ionization of lidocaine as pH was increased. With respect to mexiletine, a change in pH from 8.0 to 7.0 should not markedly change the ratio of the ionized to the unionized forms since the pK_a of mexiletine is 8.8 (Merck Index, 1983) (at a pH of 8.0, mexiletine is approximately 84% ionized). Furthermore, since enantiomers have the same chemical properties, their ionization constant should be the same and the ratio of their free fractions should remain constant as pH is decreased from 8.0 to 7.0. The present results, therefore, suggest that the pH-dependent changes in free fraction and stereoselectivity of the enantiomers is most likely a reflection of pH-dependent changes in the binding protein(s). The results also indicate that to obtain meaningful binding data for either racemic mexiletine or its enantiomers, serum pH must be adjusted and maintained at ≈ 7.4 during the binding experiment. Serum pH as high as 8.5 was observed in the present studies before pH adjustment.

3.3.1.3 Competitive Binding (Enantiomer-Enantiomer Interaction)

Lima (1987) reported competitive interaction between the enantiomers of disopyramide resulting in a greater than 2-fold increase in the free fraction of each enantiomer. The possibility of such an interaction was investigated for mexiletine enantiomers. Table 3 shows the free fractions of the enantiomers obtained when binding studies were carried out with the individual enantiomers or the racemate. There were no significant differences in the free fractions obtained for each enantiomer (free fractions were compared by independent groups t-test). This suggests the absence of enantiomer-enantiomer interaction, which could affect protein binding, within the therapeutic range of mexiletine.

3.3.2 Methods of Serum pH Adjustment

The pH-dependent binding described above (section 3.3.1.2) necessitated the adjustment of serum pH before carrying out binding experiments. Table 4 shows the methods investigated for the adjustment and maintenance of serum pH at approximately 7.4 during ultrafiltration. Both 100% CO₂ and buffer salts adequately maintained serum pH at ≈ 7.4 . The results obtained by these two methods indicated that buffer salts did not affect the free fraction values obtained. Buffer salts were subsequently used in this study because this approach was more adaptable to the large number of samples involved in the binding experiments. Serum pH was not satisfactorily controlled by using 5% CO₂/O₂ as shown in table 4.

Table 3. The percent free fractions of mexiletine enantiomers from serum containing racemic mexiletine or the individual enantiomer

Concentration of each enantiomer ($\mu\text{g/ml}$)	R(-)-mex [*]	S(+)-mex [*]	R(-)-mex	S(+)-mex
1.00	53 \pm 1	54 \pm 2	59 \pm 4	48 \pm 3
0.25	49 \pm 2	50 \pm 2	57 \pm 4	52 \pm 3

* Free fractions from racemic mexiletine. Values are means \pm s.e.m, n = 5-6 subjects.

Table 4. Methods used to adjust serum pH to physiological value (≈ 7.4) and the corresponding percent free fractions obtained.

Method	pH before adjustment	pH after adjustment	pH after filtration	Free fraction	
				R(-)-mex	S(+)-mex
5% CO ₂ /O ₂	8.05	7.47	7.65 \pm 0.01	44 \pm 1	52 \pm 2
100% CO ₂	8.05	7.35	7.41 \pm 0.02	52 \pm 1	58 \pm 1
phosphate* buffer salts	8.05	7.35	7.35 \pm 0	48 \pm 1	51 \pm 1

Drug concentration = 2 μ g/ml R,S-mexiletine. Values are means \pm s.e.m., n = 5-6 determinations.

* Concentration of the buffer salts were 5.02 mg monobasic sodium phosphate and 23.55 mg dibasic sodium phosphate per 1 ml of serum (0.12 M).

3.3.3 Binding of Mexiletine Enantiomers to Serum and to Various Serum Proteins

Table 5 summarizes the data obtained for the free fractions of mexiletine enantiomers when protein binding was determined using serum, lipoprotein deficient serum, AAG and HSA. The 2 concentrations of the enantiomers evaluated were within the therapeutic range of mexiletine. The results indicated that the binding of mexiletine enantiomers to serum was only moderate, with free fractions ranging from 50 to 55%. These results are in disagreement with those in the literature that reported lower free fractions (24 to 30%) (Talbot *et al.*, 1973; McErlane *et al.*, 1987). The disagreement is thought to be due to the fact that serum pH was not controlled in these studies. The results of the present study also indicated that the *in vitro* binding of mexiletine enantiomers to human serum is not stereoselective. As shown in table 5, the binding of the enantiomers to HSA was approximately 30% and was not stereoselective. The binding of the enantiomers to AAG showed significant ($p < 0.05$) stereoselectivity, with binding being greater for R(-)-mexiletine. This is probably due to the significantly ($p < 0.05$) greater binding affinity constant of R(-)-mexiletine relative to its enantiomer (3.3.4.2). With lipoprotein deficient serum, binding was slightly lower in comparison to that obtained with serum, suggesting a possible small contribution of the lipoproteins to the binding of mexiletine enantiomers. This binding was found to be significantly ($p < 0.05$) greater for S(+)-mexiletine. The data in table 5 show that the binding of the enantiomers within the therapeutic range can be accounted for mainly by the binding to HSA and AAG.

Table 5. The contributions of the major drug binding proteins to the serum binding of mexiletine enantiomers

Concentration of each enantiomer ($\mu\text{g/ml}$)		Serum	HSA	AAG	LP def. serum
1.0	R(-)-mex	52 \pm 1	73 \pm 2	77 \pm 1	63 \pm 0
	S(+)-mex	53 \pm 2	72 \pm 2	84 \pm 0*	56 \pm 1*
0.5	R(-)-mex	52 \pm 1	70 \pm 1	74 \pm 1	58 \pm 1
	S(+)-mex	51 \pm 1	68 \pm 1	84 \pm 2*	50 \pm 0*

Values are means \pm s.e.m. of percent free fractions ($n = 6$ experiments). HSA, AAG and LP def. serum refer to human serum albumin, α_1 -acid glycoprotein and lipoprotein deficient serum, respectively. Concentrations of the enantiomers used were those within the therapeutic range.

* Significant ($p < 0.05$).

Since many disease conditions are known to influence the binding of drugs to AAG and HSA (Routledge, 1985; Kremer *et al.*, 1988), the present results suggest that the binding of the enantiomers may change in disease states. However, Pentikainen *et al.* (1984) found no significant differences in the free fractions of racemic mexiletine in myocardial infarction patients, during the acute and recovery phases. The moderate binding of the enantiomers observed in this study, and their large volume of distribution in humans (6.6 ± 2.6 and 7.3 ± 2.4 L/kg for R(-)- and S(+)-mexiletine, respectively) (Igwemezie *et al.*, 1989), suggest that serum protein binding may not be an important factor in the pharmacodynamics of racemic mexiletine and its enantiomers.

3.3.4 Determination of Binding Constants

3.3.4.1 Serum Binding Data

To determine the constants characterizing the binding of the enantiomers to serum proteins, the binding of a wide range of total drug concentrations (0.1-2000 $\mu\text{g/ml}$ of each enantiomer) was investigated. The binding data obtained are shown in table 6. The results indicate that the binding of mexiletine enantiomers was not concentration-dependent within the therapeutic range of 0.5-2.0 $\mu\text{g/ml}$ of racemic mexiletine in man (the free fractions at total enantiomer concentrations of 0.25, 0.5 and 1.0 $\mu\text{g/ml}$ were compared by one-way ANOVA). However, significant increases in the free fractions of the enantiomers was evident at 50 $\mu\text{g/ml}$ and above, indicating a possible saturation of the binding sites on the protein(s). Figure 15 is a representative "Rosenthal" plot of the binding data obtained for each enantiomer in a

Table 6. The percent free fractions of mexiletine enantiomers in serum from healthy human subjects

Concentration of enantiomer in serum ($\mu\text{g/ml}$)	Free Fraction*	
	R(-)-mex	S(+)-mex
0.10	0.42 ± 0.02	0.44 ± 0.04
0.25	0.49 ± 0.01	0.49 ± 0.02
0.50	0.52 ± 0.01	0.52 ± 0.01
1.0	0.52 ± 0.01	0.51 ± 0.02
5.0	0.55 ± 0.01	0.58 ± 0.01
20.0	0.59 ± 0.02	0.60 ± 0.01
50.0	0.62 ± 0.02	0.62 ± 0.02
100.0	0.62 ± 0.02	0.61 ± 0.02
500.0	0.68 ± 0.02	0.65 ± 0.02
1000.0	0.70 ± 0.02	0.69 ± 0.02
1500.0	0.77 ± 0.02	0.75 ± 0.02
2000.0	0.77 ± 0.01	0.75 ± 0.01

* Free fraction values are means \pm s.e.m., n = 6 subjects.

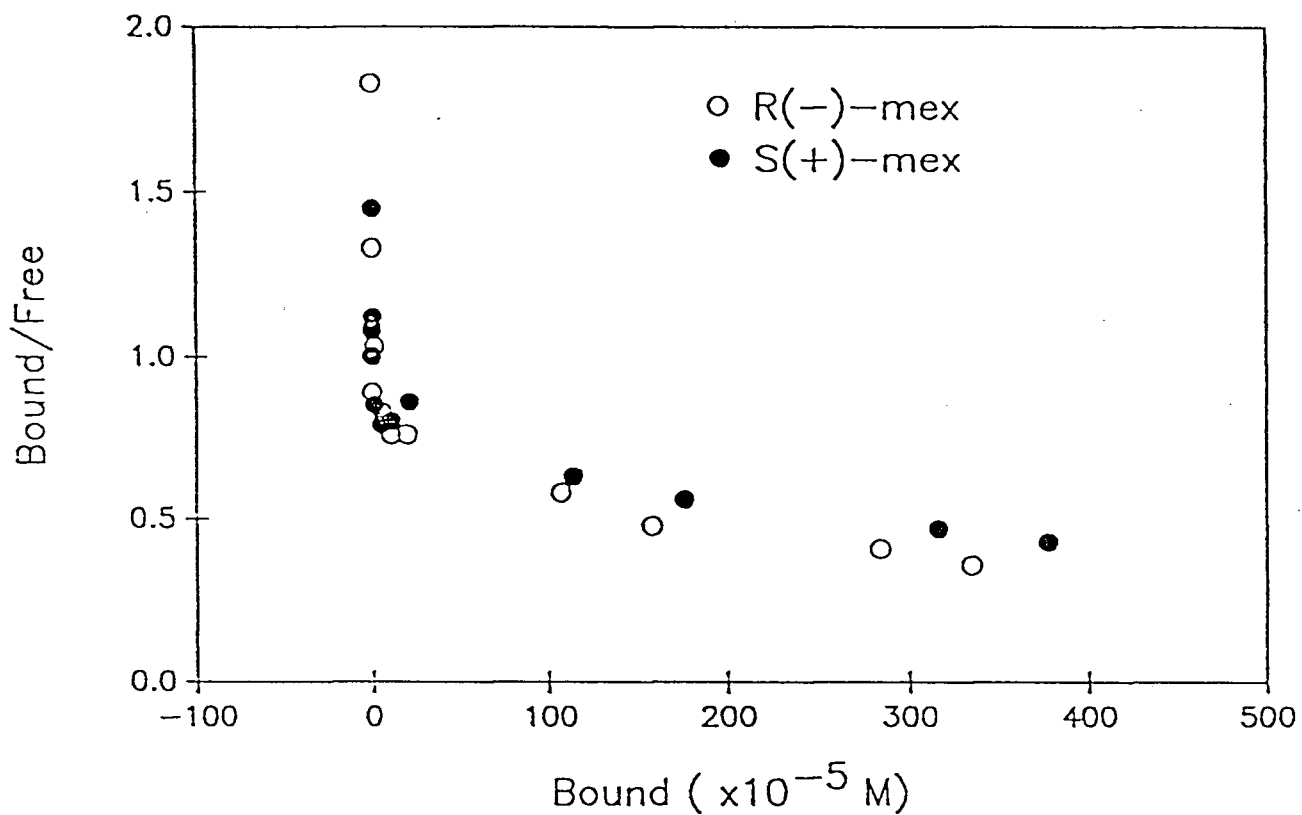


Figure 15. A representative "Rosenthal" plot of the binding of mexiletine enantiomers to serum from a healthy subject.

healthy subject. Analysis of the data using the non-linear least squares regression program "ENZFITTER" (Leatherbarrow, 1987) indicated the presence of two classes of binding sites. A high affinity, low capacity class of binding sites and a low affinity, high capacity class of binding sites. The affinity and capacity constants determined for these classes of binding sites are shown in table 7. There were no significant differences in the binding constants of the enantiomers.

3.3.4.2 α_1 -Acid Glycoprotein Binding Data

A similar analysis was carried out on the binding data obtained with AAG. The binding constants derived are shown in table 8. AAG showed only one class of binding sites. This was a high affinity, low capacity binding site and probably accounts for the high affinity binding of serum (table 8). The binding capacity of the enantiomers were not significantly different (mean values were $1.9 \times 10^{-5}M$ and $1.8 \times 10^{-5}M$ for R(-)- and S(+)-mexiletine, respectively). However, R(-)-mexiletine had a significantly ($p < 0.05$) greater affinity than the S(+)-enantiomer (mean values were $2.0 \times 10^4 M^{-1}$ vs $1.2 \times 10^4 M^{-1}$). The number of binding sites per mole of protein (n) was approximately 1 for both enantiomers. This is consistent with the reports of other studies on the binding of basic drugs to AAG (Muller et al., 1985; Kremer et al., 1988). It is generally accepted in the literature that only one high affinity class of binding sites exist on AAG (Muller et al., 1985).

Table 7. Binding constants of mexiletine enantiomers in serum from healthy subjects

Subject	R(-)-mex				S(+)-mex			
	n_1P_1 ($\times 10^{-6}M$)	K_1 ($\times 10^5M^{-1}$)	n_2P_2 ($\times 10^{-3}M$)	K_2 ($\times 10^2M^{-1}$)	n_1P_1 ($\times 10^{-6}M$)	K_1 ($\times 10^5M^{-1}$)	n_2P_2 ($\times 10^{-3}M$)	K_2 ($\times 10^2M^{-1}$)
1	1.5	9.9	6.2	1.2	1.7	4.2	7.4	1.1
2	1.3	7.0	4.4	1.9	1.2	12.2	6.2	1.2
3	2.1	3.1	5.0	1.3	2.1	3.1	4.7	1.4
4	3.9	2.3	5.6	0.7	7.8	1.1	5.7	0.8
5	3.0	2.9	5.9	0.9	3.2	2.9	9.7	0.5
6	2.7	2.1	4.7	1.3	3.0	1.2	5.4	1.2
mean	2.4	4.5	5.3	1.2	3.2	4.1	6.5	1.0
±s.e.m	0.4	1.3	0.3	0.2	1.0	1.7	0.7	0.1

K and nP refer to the affinity and capacity constants, respectively. Binding constants were generated using the non-linear least squares regression program "ENZFITTER".

Table 8. Binding constants of mexiletine enantiomers in isolated human α_1 -acid glycoprotein (AAG)

	nP ($\times 10^{-5}M$)	n	K ($\times 10^4 M^{-1}$)
R(-)-mex	1.9 \pm 0.1	0.8	2.0 \pm 0.1
S(+)-mex	1.8 \pm 0.1	0.8	1.2 \pm 0.1*

Values are means \pm s.e.m, n = 4 experiments. K and nP refer to the binding affinity and capacity constants, respectively.

AAG concentration used was 0.1% w/v. The molecular weight of AAG used for the calculation of n (the number of binding sites per mole of protein) was 44,000 Daltons.

* Significant ($p < 0.05$).

3.3.4.3 Albumin Binding Data

In contrast to α_1 -acid glycoprotein, the binding data from albumin showed the presence of two classes of binding sites (table 9). The low affinity sites had comparable binding constants to those of the corresponding sites in serum for each of the enantiomers.

R(-)-Mexiletine had a slightly greater capacity constant than S(+)-mexiletine (mean values were 4.0 and 3.2×10^{-3} M). However, the affinity of R(-)-mexiletine was significantly ($p < 0.05$) less than that of its antipode (mean values were 0.5 and $1.2 \times 10^2 \text{M}^{-1}$). The mean number of binding sites per mole of protein were 6.6 and 5.4 for R(-)- and S(+)-mexiletine, respectively. The high affinity class of binding sites had binding constants that were comparable to the same class of binding sites in serum. There were no significant differences in the binding constants of the enantiomers. The mean number of binding sites obtained for the high affinity sites were 0.006 and 0.001 for R(-)- and S(+)-mexiletine, respectively. The magnitude of these n values suggest that the high affinity site may not represent a true binding site on albumin. It may be speculated that the high affinity binding could have resulted from the presence of AAG contaminant in the HSA.

3.4 Tissue Distribution Kinetics of Mexiletine Enantiomers in Rats

The tissue distribution kinetics of mexiletine enantiomers was studied in male Sprague-Dawley rats after administration of a single i.v. dose (10 mg/kg) of racemic mexiletine.

Table 9. Binding constants of mexiletine enantiomers in isolated human serum albumin

	$n_1 P_1$ ($\times 10^{-6} M$)	n_1	K_1 ($\times 10^5 M^{-1}$)	$n_2 P_2$ ($\times 10^{-3} M$)	n_2	K_2 ($\times 10^2 M^{-1}$)
R(-)-mex	3.4 ± 0.7	0.006	0.9 ± 0.3	4.0 ± 0.4	6.6	0.5 ± 0.1
S(+)-mex	2.0 ± 0.3	0.001	1.2 ± 0.2	3.2 ± 0.4	5.4	$1.2 \pm 0.1^*$

Values are means \pm s.e.m, $n = 4$ experiments. Albumin concentration was 4% w/v. The molecular weight of albumin used for the calculation of n (the number of binding sites per mole of protein) was 66,000 Daltons.

* Significant ($p < 0.05$).

3.4.1 Serum Levels

The mean serum concentrations of mexiletine enantiomers plotted as a function of time are shown in figure 16. The concentration-time decay curve was biexponential. S(+)-Mexiletine had a greater serum concentration at each sampling time than the R(-)-enantiomer but this did not achieve statistical significance until 1 h after drug administration. However, the ratio of the enantiomers remained constant between 1 and 6 h.

3.4.2 Pharmacokinetic Parameters

Table 10 shows the pharmacokinetic parameters determined for the enantiomers. Since blood at each sampling time was collected from different animals, only mean pharmacokinetic parameters could be calculated. The systemic clearance (CL) of R(-)-mexiletine was 32% greater than that of the S(+)-enantiomer (161.8 ml/min/kg vs 122.9 ml/min/kg). Mexiletine is a restrictively cleared drug (Wilkinson and Shand, 1975), thus, its CL depends directly on both the intrinsic clearance (CL_{int}) and free fraction (f_f). Since the *in vivo* f_f of the enantiomers were not markedly different from each other (section 3.4.3), these results suggest that the CL_{int} of R(-)-mexiletine may be greater than that of the S(+)-enantiomer. A significantly greater glucuronidation of R(-)-mexiletine, relative to its opposite enantiomer, has been reported in man (Grech-Belanger *et al.*, 1986).

R(-)-Mexiletine was also found to have a 22% greater steady-state volume of distribution than the S(+)-enantiomer (9.0 L/kg vs 7.4 L/kg). Stereoselective differences in the volume of distribution of enantiomers

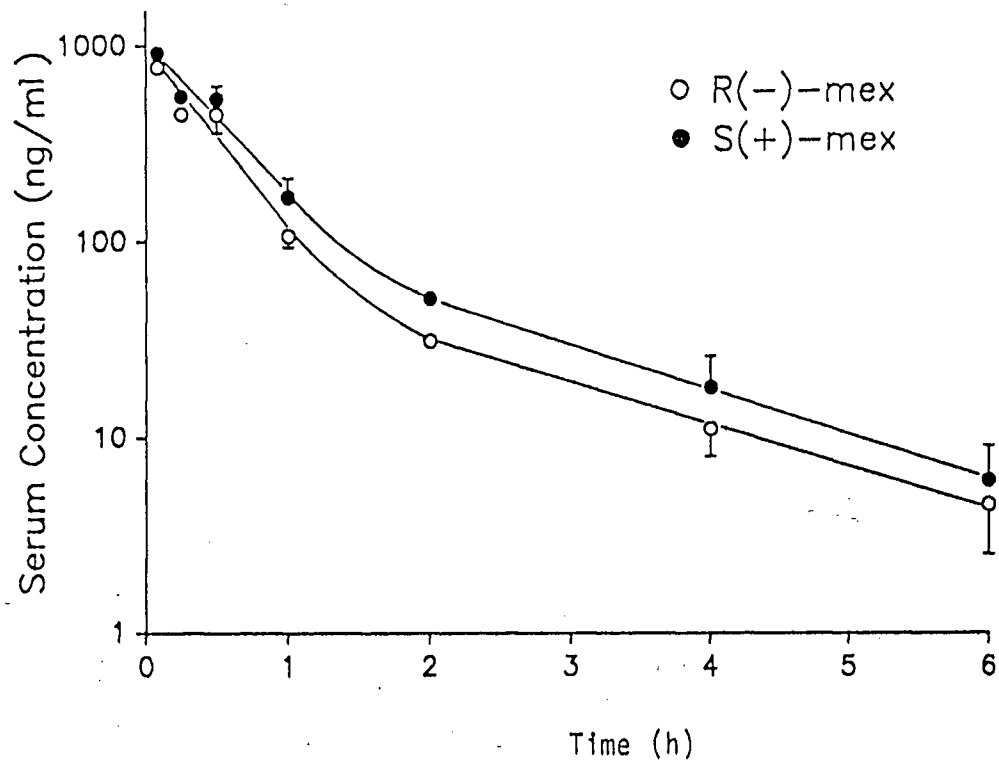


Figure 16. The semilogarithmic plot of serum concentration vs time for mexiletine enantiomers in rats following the administration of a single i.v. dose (10 mg/kg) of racemic mexiletine. Values are means \pm s.e.m, n = 4-5 rats.

Table 10. The pharmacokinetic parameters of mexiletine enantiomers calculated from serum concentration-time data after a single i.v. dose (10 mg/kg) of racemic mexiletine

Parameter	R(-)-mex	S(+)-mex
$t_{1/2}(\alpha)$ (min)	19.8	24.4
$t_{1/2}(\beta)$ (h)	1.4	1.3
AUC (ng.h.ml ⁻¹)	515.1	677.9
CL (ml/min/kg)	161.8	122.9
V_{ss} (l/kg)	9.0	7.4

can result from differences in their tissue and/or serum protein binding (Williams and Lee, 1985). Due to the small difference in the free fractions of the enantiomers, it is unlikely that serum protein binding would account for the observed stereoselective volume of distribution. The distribution half-life of S(+)-mexiletine, 24.4 min, was 24% greater than that of the R(-)-enantiomer, 19.8 min. Thus, the greater volume of distribution of R(-)-mexiletine appears to result from its greater tissue uptake. Stereoselective tissue uptake of other chiral drugs such as propranolol (Kawashima *et al.*, 1976) and timolol (Tocco *et al.*, 1976) have been reported. The terminal elimination half-lives of the enantiomers were 1.4 and 1.3 h for R(-)- and S(+)-mexiletine, respectively. These values were markedly shorter than those reported in humans (9.1 ± 2.9 and 11.0 ± 3.8 h for R(-)- and S(+)-mexiletine, respectively) (Igwemezie *et al.*, 1989). The expected shorter terminal elimination half-life for R(-)-mexiletine, due to its larger systemic clearance, was offset by its equally greater volume of distribution. This resulted in an apparent lack of a difference in the terminal elimination half-lives of both enantiomers.

3.4.3 Serum Protein Binding

Table 11 shows the *in vivo* percent free fractions of mexiletine enantiomers in the rats. The mean free fraction of R(-)-mexiletine, 49%, was significantly greater than that of the S(+)-enantiomer (44%). However, the difference in the free fractions is small and would not be expected to have any pharmacokinetic or pharmacodynamic consequences.

Table 11. The *in vivo* free serum concentrations (ng/ml) and the percent free fractions (f_f) of mexiletine enantiomers after a single i.v. dose (10 mg/kg) of racemic mexiletine

Time (h)	R(-)-mex	f_f	S(+)-mex	f_f
0.08	400.1 \pm 33.6	52	426.4 \pm 40.3	47
0.25	212.8 \pm 2.4	48	243.0 \pm 3.1	45
0.50	215.6 \pm 6.1	49	215.0 \pm 6.0	41
1.00	49.7 \pm 2.3	47	74.3 \pm 10.0	45
mean \pm s.e.m.		49 \pm 1		44 \pm 1*

Concentrations are means \pm s.e.m, n = 4-5 rats.

* significant ($p < 0.05$).

3.4.4 Recovery of the Enantiomers from the Tissues

Table 12 shows the recovery of the enantiomers from tissue homogenates. Recovery was approximately 80% or more in all the tissues studied.

3.4.5 Tissue Levels

Table 13 presents the concentration of the enantiomers in the various tissues studied. Maximum concentrations were observed at 5 min, which was the earliest sampling time. There were no significant differences in the maximum tissue concentrations except for the liver where the concentration of S(+)-mexiletine, $7.5 \pm 2.6 \mu\text{g/g}$, was 2.4 fold greater than that of the R(-)-enantiomer ($3.1 \pm 1.1 \mu\text{g/g}$). However, a significantly ($p < 0.05$) greater concentration for S(+)-mexiletine was evident at 1 hour and thereafter in all the tissues studied, reflecting the changes in the enantiomeric ratios in serum (section 3.4.1). These results indicated that there was a rapid uptake of mexiletine enantiomers into the highly perfused tissues (which included all the tissues studied except the fat), followed by a slower distribution, presumably into the deep tissue compartment. The distribution into the deep tissue compartment appeared to be significantly greater for the R(-)-enantiomer which resulted in the significantly lower serum concentrations observed for this enantiomer.

The pharmacokinetic parameters calculated for the enantiomers from the tissue concentration-time data are summarized in table 14. The elimination half-lives in the tissues studied were not significantly different from each other. The area under the tissue concentration-time

Table 12. Percent recovery of mexiletine enantiomers from tissue homogenates.

Enantiomer concentration (ng/ml)	liver	kidney	heart	fat	lung	brain
500 R(-)mex	79	83	86	92	82	85
S(+)mex	80	83	86	92	82	85
125 R(-)mex	87	87	87	94	90	90
S(+)mex	84	84	84	84	84	89

Values are the averages of duplicate determinations.
1 ml of tissue homogenate contained 100 mg of tissue.

Table 13. The time-dependent concentrations** ($\mu\text{g/g}$) of mexiletine enantiomers in the tissues of rats following a single i.v. dose (10 mg/kg) of racemic mexiletine

Time		Brain	Heart	Lung	Kidney	Liver	Fat
5 min	R(-)-mex	18.99 \pm 0.76	6.23 \pm 0.46	24.57 \pm 0.64	19.99 \pm 0.64	3.10 \pm 0.53*	1.03 \pm 0.04
	S(+)-mex	19.18 \pm 0.82	6.52 \pm 0.46	25.86 \pm 0.79	20.12 \pm 0.66	7.47 \pm 1.28	1.10 \pm 0.05
15 min	R(-)-mex	12.44 \pm 0.06	3.30 \pm 0.15	14.05 \pm 0.03	8.92 \pm 0.64	2.06 \pm 0.30*	1.04 \pm 0.04
	S(+)-mex	13.33 \pm 0.06	3.38 \pm 0.15	16.21 \pm 0.03	10.66 \pm 0.76	4.12 \pm 0.50	1.16 \pm 0.04
30 min	R(-)-mex	6.67 \pm 0.61	2.58 \pm 0.46	13.70 \pm 0.17	6.78 \pm 1.19	0.79 \pm 0.02*	0.78 \pm 0.14
	S(+)-mex	7.00 \pm 0.57	2.71 \pm 0.48	14.75 \pm 0.18	7.58 \pm 1.30	1.63 \pm 0.01	0.86 \pm 0.14
1 hr	R(-)-mex	3.3 \pm 0.18*	1.11 \pm 0.23*	6.48 \pm 1.09*	2.53 \pm 0.44*	0.65 \pm 0.09*	0.42 \pm 0.04*
	S(+)-mex	4.2 \pm 0.32	1.43 \pm 0.24	8.59 \pm 1.00	3.94 \pm 0.58	1.31 \pm 0.28	0.54 \pm 0.06
2 hr	R(-)-mex	0.84 \pm 0.09*	0.41 \pm 0.10*	2.68 \pm 0.66*	0.55 \pm 0.06*	0.23 \pm 0.02*	0.18 \pm 0.02*
	S(+)-mex	1.30 \pm 0.14	0.55 \pm 0.12	3.88 \pm 1.04	1.11 \pm 0.15	0.38 \pm 0.06	0.23 \pm 0.02
4 hr	R(-)-mex	0.18 \pm 0.02*	0.10 \pm 0.12*	0.47 \pm 0.08*	0.12 \pm 0.02*	0.03 \pm 0.01*	0.02 \pm 0.00*
	S(+)-mex	0.23 \pm 0.03	0.13 \pm 0.01	0.70 \pm 0.11	0.23 \pm 0.02	0.07 \pm 0.01	0.03 \pm 0.00
6 hr	R(-)-mex	0.05 \pm 0.01*	0.03 \pm 0.01*	0.14 \pm 0.02*	0.04 \pm 0.01*	0.02 \pm 0.01*	-
	S(+)-mex	0.07 \pm 0.01	0.04 \pm 0.01	0.22 \pm 0.02	0.07 \pm 0.01	0.04 \pm 0.00	-

* significant ($P < 0.05$)

** mean \pm s.e.m., n = 4-5 rats

Table 14. Pharmacokinetic parameters of mexiletine enantiomers from tissue data after a single i.v. dose (10 mg/kg) of racemic mexiletine

		$t_{1/2}$ (h)	AUC_T ($\mu\text{g}\cdot\text{h}\cdot\text{g}^{-1}$)	Tissue/Serum
Heart	R(-)-mex	1.1	4.4	8
	S(+)-mex	1.1	5.1	7
Brain	R(-)-mex	1.0	11.9	25
	S(+)-mex	1.0	14.0	21
Liver	R(-)-mex	1.1	2.0	4
	S(+)-mex	1.2	4.4*	8*
Lung	R(-)-mex	1.0	22.4	32
	S(+)-mex	1.0	27.1	28
Kidney	R(-)-mex	1.1	11.1	26
	S(+)-mex	1.0	13.6	22
Fat	R(-)-mex	0.7	1.3	1
	S(+)-mex	0.7	1.5	1

* Significant ($p < 0.05$), $n = 4-5$ rats.

Tissue/Serum ratios are values at the observed maximum tissue concentrations i.e. 5 min after drug administration.

AUC_T refers to area under the tissue concentration-time curve.

curve (AUC_T) was smaller for R(-)-mexiletine but the differences did not exceed 16%; except for the liver where the AUC_T was 120% greater for the S(+)-enantiomer. The tissue/serum ratio of the enantiomers (at the maximum observed tissue concentration) indicated an extensive tissue uptake. The highest ratios were found in the lungs which accumulated 32 and 28-fold the serum concentration of R(-)- and S(+)-mexiletine, respectively. The brain accumulated 25 and 21-fold; while the heart accumulated 8 and 7-fold the serum concentrations of R(-)- and S(+)-mexiletine, respectively. Similar high tissue/serum ratios have been reported for racemic mexiletine in rats (Barrigon *et al.*, 1983). The high tissue/serum ratios observed in the present study were also maintained over time, as shown for brain and heart tissues in figure 17. Except for the liver and fat, all the other tissues showed a 14-19% greater accumulation of R(-)-mexiletine relative to the S(+)-enantiomer. The liver tissue exhibited a 100 % greater accumulation of S(+)-mexiletine while the fat tissue did not accumulate the enantiomers. The high tissue/serum ratios of mexiletine enantiomers can be attributed to a number of mechanisms; two of which may be an active transport process and/or high affinity binding to sites in the tissues. The 2.4-fold greater liver concentration of S(+)-mexiletine relative to the R(-)-enantiomer may result from stereoselective active transport and/or tissue binding and/or metabolism. However, from the data obtained in this study, it was not possible to determine which mechanism(s) was in operation. The tissues studied exhibited redistribution at a rate greater for the R(-)-enantiomer, reflecting the changing ratio of the enantiomers in the serum. Thus, a significantly

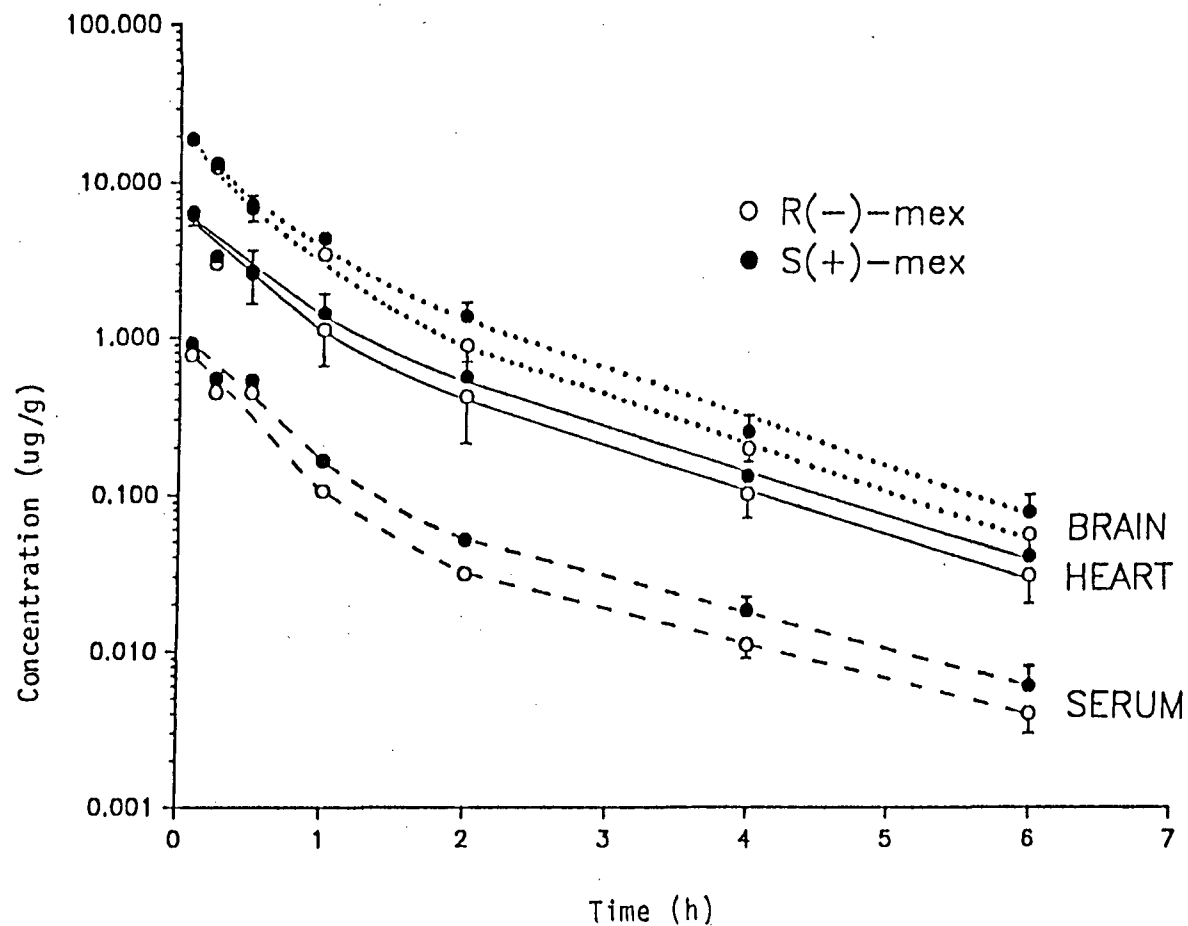


Figure 17. The concentration-time profile of mexiletine enantiomers in serum, heart and brain tissues following the administration of a single i.v. dose (10 mg/kg) of racemic mexiletine. Values are means \pm s.e.m. of results from 4-5 rats.

greater concentration of S(+)-mexiletine was evident in all the tissues between 1 and 6 hours but the enantiomeric ratio was constant as indicated by the comparable terminal elimination half-lives of the enantiomers in the tissues. However, much of the enantiomers were already cleared from the tissues at the time stereoselectivity became apparent, hence only small differences in the AUC_T of the enantiomers was evident in most of the tissues studied (table 14). This is not likely to have any pharmacokinetic consequences.

It can be concluded from the present results that the uptake of mexiletine enantiomers into the target tissue, i.e. the heart, as well as the brain, is not stereoselective. The high brain levels of the enantiomers are most likely responsible for the CNS side effects induced by mexiletine in both animals (section 3.5.2.5; Uprichard and Harron, 1989) and man (Palileo *et al.*, 1982, Waspe *et al.*, 1983).

3.5 Antiarrhythmic Effects of Racemic Mexiletine and its Enantiomers in Rats

3.5.1 Electrically-induced Arrhythmia in Pentobarbitone Anaesthetized Rats

The relative antiarrhythmic activity of racemic mexiletine and its enantiomers was investigated using electrical stimulation of the heart in pentobarbitone anaesthetized rats. To determine the true effects of the drugs on the measured variables, changes due to saline (control) were first subtracted. The cumulative dose-response curves generated were analysed by "Repeated Measures" ANOVA.

3.5.1.1 Dosage and Plasma Concentration

The doses of racemic mexiletine and its enantiomers used were 4, 8, 16 and 32 mg/kg, administered cumulatively. The observed plasma concentrations of racemic mexiletine and its enantiomers increased linearly ($r^2 > 0.99$) with dose as shown in figure 18. R,S- and S(+)-Mexiletine had significantly ($p < 0.05$) greater plasma concentrations than the R(-) enantiomer. The maximum concentrations achieved were 2.3 ± 0.2 , 2.3 ± 0.2 and 1.7 ± 0.1 $\mu\text{g/ml}$ for S(+)-, R,S-, and R(-)-mexiletine, respectively. Normally, the concentration of the racemate would be expected to be between that of the two enantiomers. Therefore, the comparable plasma concentrations of R,S- and S(+)-mexiletine indicated a possible interaction between the two enantiomers. This may occur at the serum binding, tissue binding and/or metabolism level. The *in vivo* free fractions determined for racemic mexiletine and its enantiomers are shown in table 15. There were no significant differences in the free fraction values. Thus, it is unlikely that serum binding interactions would have been responsible for the observed plasma concentrations. These results suggest that there may have existed an interaction between the enantiomers of mexiletine at the tissue binding and/or metabolism level which resulted in greater than expected plasma concentrations of the racemate. Such an interaction affecting metabolism has been reported for levomethorphan where increased plasma levels and analgesic activity were observed when the drug was co-administered with its opposite enantiomer, dextromethorphan (Cooper and Anders, 1974). However, based on data obtained from the present study, it was not possible to determine

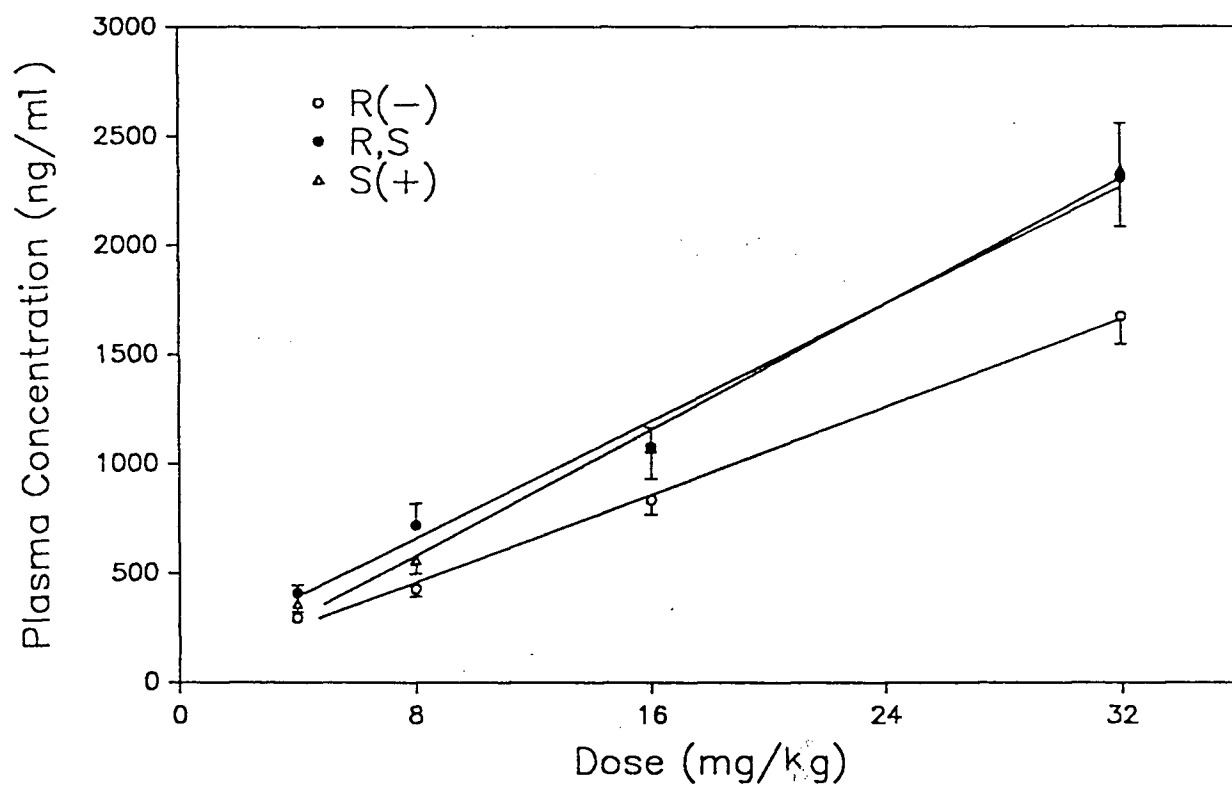


Figure 18. The relationship between dose (cumulative) and the plasma concentration of racemic mexiletine and its enantiomers in Pentobarbitone anaesthetized rats. Blood samples were collected 10 min after each dose. Values are means \pm s.e.m. of results from 7-8 rats.

Table 15. The *in vivo* percent free fractions of racemic mexiletine and its enantiomers in pentobarbitone anaesthetized rats

Rat	R(-)-mex	R,S-mex	S(+)-mex
1	51	57	36
2	34	47	47
3	35	43	61
4	41	31	42
5	34	48	56
6	35	-	-
mean	39	45	48
± s.e.m.	3	4	4

whether tissue binding and/or metabolism was responsible for the greater than expected plasma concentrations of racemic mexiletine.

3.5.1.2 Antiarrhythmic Effects

3.5.1.2.1 Ventricular Fibrillation Threshold (VFT)

Many experimental studies have used VFT as a quantitative measure of cardiac vulnerability to fibrillation (section 1.1.2.5.1). The underlying assumption is that physiologic or pharmacologic interventions that alter VFT will produce a similar directional change in the vulnerability to spontaneous fibrillation (Moore and Spear, 1975). It has been suggested that electrically induced VF is a result of local re-entry (Marshall *et al.*, 1983). The properties of a pharmacological agent required to break or prevent a re-entrant circuit include a slowing of conduction, an increase in membrane threshold and an increase in the effective refractory period (Marshall *et al.*, 1983). These properties are the same as those possessed by class I antiarrhythmic agents (section 1.1.2.4) and several studies have reported elevation of VFT by many class I antiarrhythmic drugs (Wiggers and Wegria, 1940; Yoon *et al.*, 1974; Gerstenblith *et al.*, 1972; Hodess *et al.*, 1979). The determination of VFT was therefore considered to be a suitable method for a comparative study of the antiarrhythmic activity of racemic mexiletine and its enantiomers.

In the present study, the average VFT values observed before each drug treatment are listed in table 16. These pre-drug values were not significantly different in any of the groups. The relative increases in

Table 16. Pre-drug values for the variables for ventricular fibrillo-flutter in pentobarbitone anaesthetized rats

	VFT (μ A)	MFF (Hz)	ERP (msec)
R(-)-mex	136 \pm 26	13.7 \pm 0.4	48 \pm 3
R,S-mex	110 \pm 25	14.4 \pm 0.6	50 \pm 3
S(+)-mex	105 \pm 22	15.8 \pm 0.7	46 \pm 3

Values are means \pm s.e.m., n = 7-8 rats

VFT (% change from pre-drug) for each drug are shown graphically in figure 19. Racemic mexiletine and the two enantiomers caused a dose-dependent significant ($p < 0.05$) increase in VFT. However, the differences between the drug treatments did not reach statistical significance. It was not possible to compare the results of this study with those of others since the effects of mexiletine enantiomers on VFT have not been reported. Racemic mexiletine, however, has been shown to increase VFT in anaesthetized rats (Marshall *et al.*, 1981) and dogs (Allen *et al.*, 1977).

3.5.1.2.2 Cardiac Refractory Period

The refractory period of the cardiac tissue is an important determinant of the initiation of arrhythmias (section 1.1.2.2). Most methods used to determine cardiac refractoriness measure the effective refractory period (ERP) directly or indirectly. ERP is dependent on MRD, the MP at which the inward sodium current is reactivated, the duration of the repolarization phase and the time course of the reactivation process (Winslow, 1984). Singh (1978) reported that the decrease in MRD evoked by class I antiarrhythmic drugs was associated with an increase in the ERP. Thus, ERP is a further index of the antiarrhythmic actions of class I drugs (Vaughan Williams, 1984). In this study, ERP was measured directly by the extra-stimulus method and indirectly by determining the maximum frequency at which cardiac muscle can be induced to follow strong electrical stimulus (MFF). This parameter has an inverse relationship with ERP. MFF has been used to

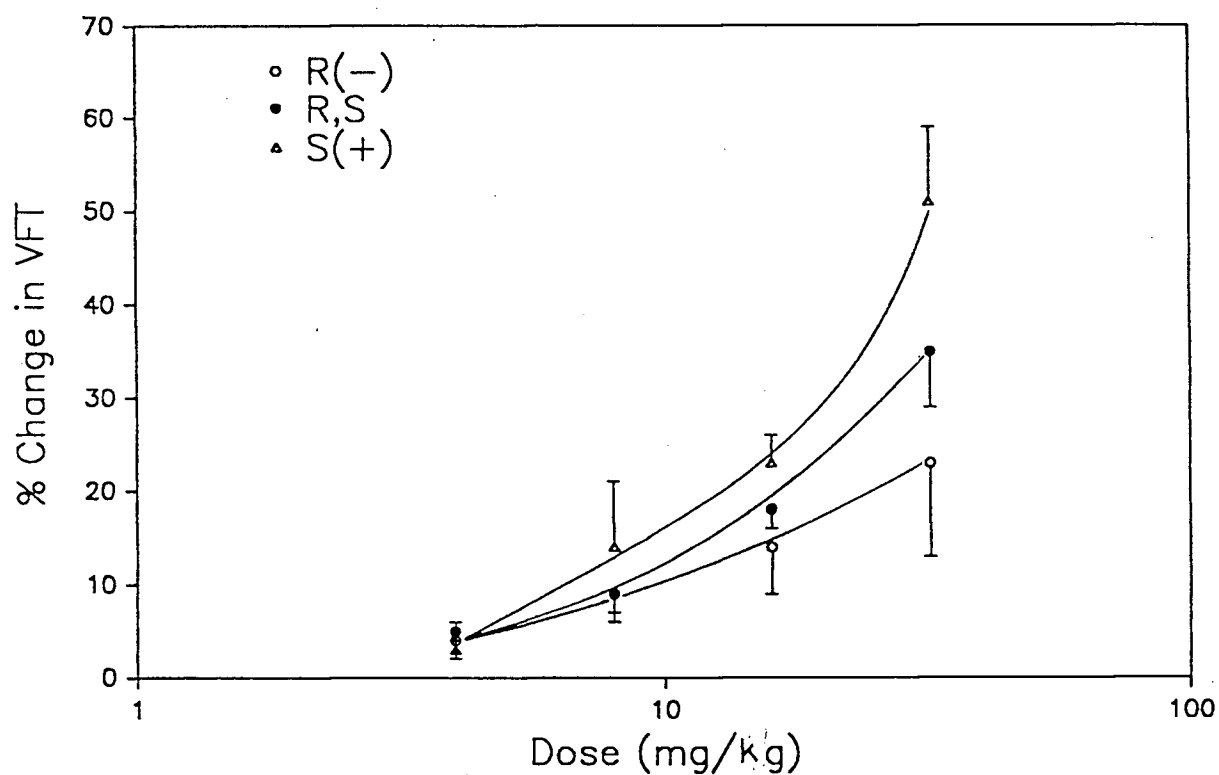


Figure 19. Cumulative dose-response curves of the effects of racemic mexiletine and its enantiomers on ventricular fibrillation threshold (VFT) in pentobarbitone anaesthetized rats. Values are means \pm s.e.m. of results from 7-8 rats. The ordinate refers to change from pre-drug values.

quantitatively study the antiarrhythmic actions of other class I agents (Vaughan Williams and Szekeres, 1961).

The pre-drug values for ERP and MFF are shown in table 16. There were no significant differences between the drug treatment groups. Using the extra-stimulus method, racemic mexiletine and the enantiomers were found to increase the ERP significantly ($P < 0.05$) and dose-dependently (fig. 20). The differences between the drug treatments did not reach statistical significance. A similar lengthening of ERP to that obtained in this study was reported in isolated canine ventricular tissue (Arita *et al.*, 1979). As expected, MFF was significantly ($P < 0.05$) decreased by the drugs (fig. 21) and this was also dose-dependent. However, R,S- and S(+)-Mexiletine were significantly ($P < 0.05$) more potent than the R(-)-enantiomer but R,S- and S(+)-mexiletine did not differ significantly from each other. The significantly greater effects of R,S- and S(+)-mexiletine on MFF relative to the R(-)-enantiomer were not consistent with their effects on ERP, where significant differences could not be detected. It has been reported that MFF is a less precise method of determining ERP than the extra-stimulus method (Winslow, 1984). This is because ERP is affected by factors such as the fundamental driving frequency (Vaughan Williams and Szekeres, 1961; Winslow, 1984). Furthermore, the effects of racemic mexiletine and its enantiomers on VFT did not differ significantly in this study. Therefore, the conclusion drawn from the present results was that the effects of racemic mexiletine and its enantiomers on ERP were not markedly different from each other.

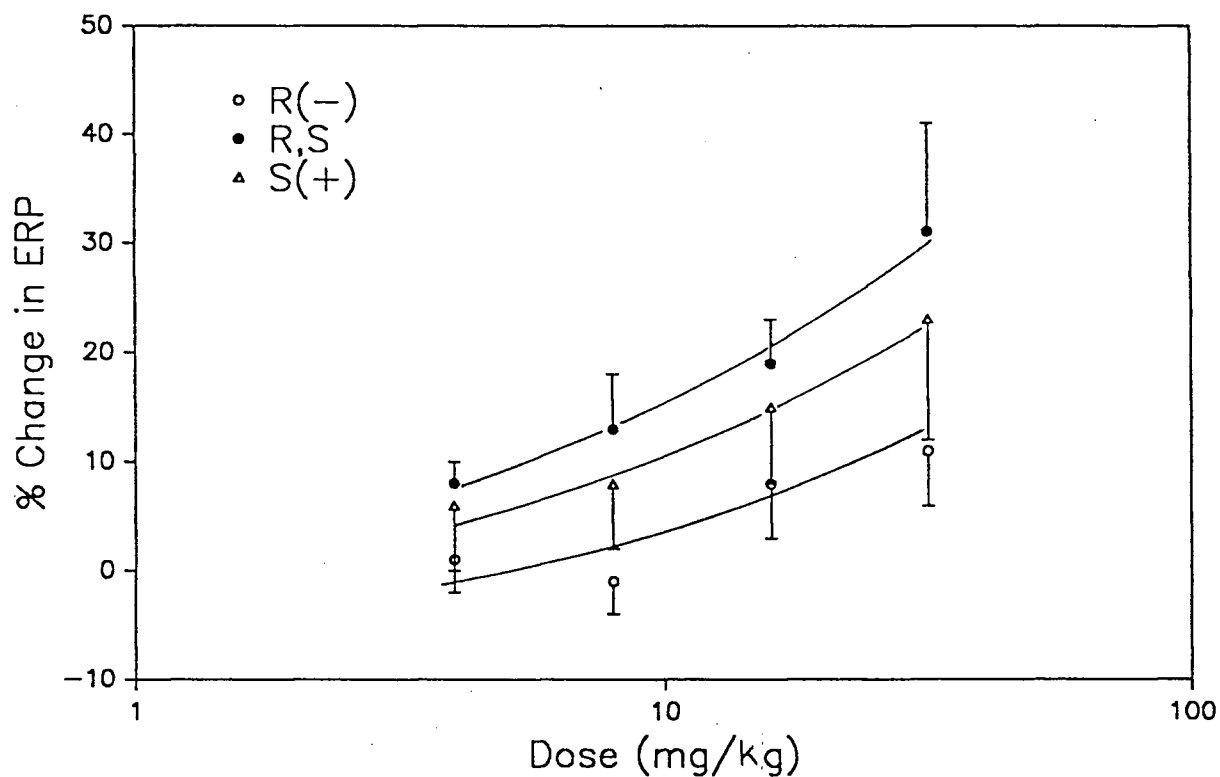


Figure 20. Cumulative dose-response curves of the effects of racemic mexiletine and its enantiomers on the effective refractory period (ERP) in pentobarbitone anaesthetized rats. Values are the means \pm s.e.m. of results from 7-8 rats. The ordinate refers to change from pre-drug values.

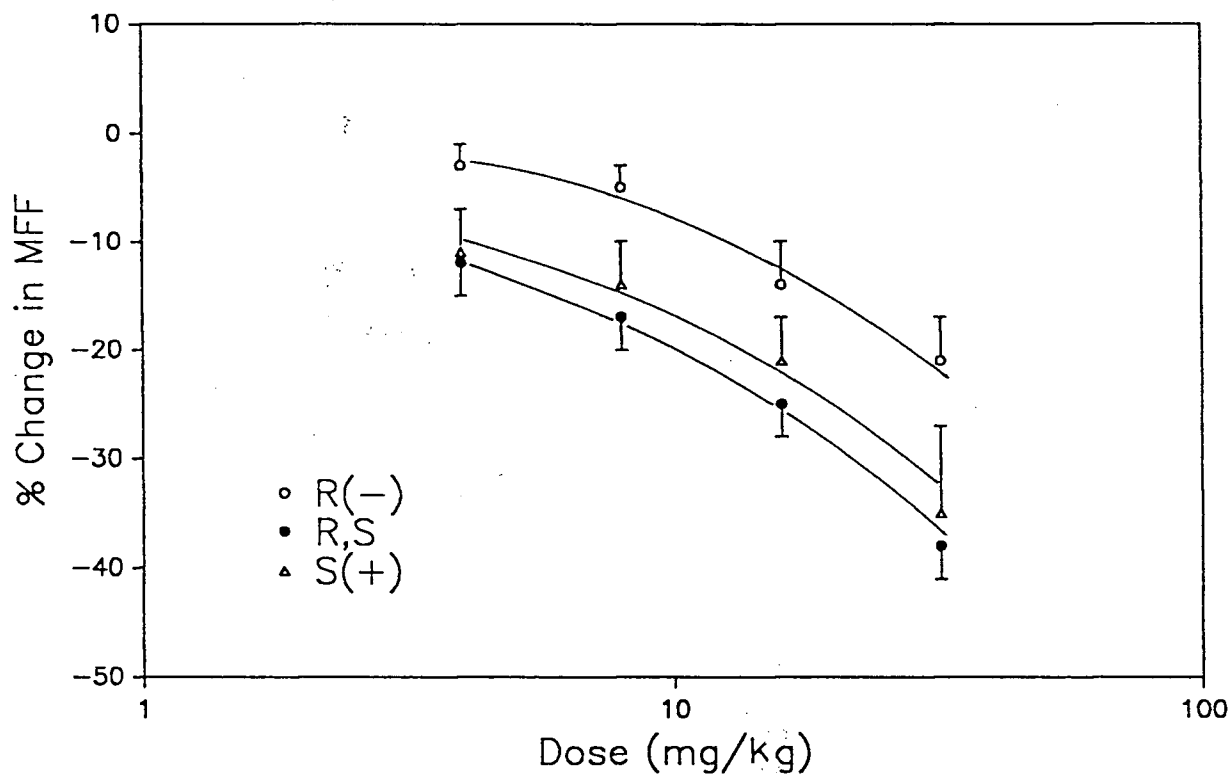


Figure 21. Cumulative dose-response curves of the effects of racemic mexiletine and its enantiomers on the maximum following frequency (MFF) in pentobarbitone anaesthetized rats. Values are means \pm s.e.m. of results from 7-8 rats. The ordinate refers to change from pre-drug values.

3.5.1.3 Cardiovascular Effects

The electrocardiographic effects of racemic mexiletine and its enantiomers are shown in table 17. The QRS and QT_c intervals were not significantly affected by drug treatment. The PR interval was significantly increased but only at the highest cumulative dose (32 mg/kg) compared to pre-drug values. There were no significant differences in the PR prolongation induced by the drugs. The significant increase in the PR interval indicated a slowing of AV conduction. Since it has been reported that racemic mexiletine does not possess any calcium antagonist properties nor affect the β -adrenergic responses (Singh *et al.*, 1980), it is most likely that the observed PR prolongation is a result of sodium channel blockade in the peripheral AV nodal tissue.

Table 18 shows the pre-drug values for the haemodynamic variables (heart rate and blood pressure). There were no significant differences between the drug treatment groups. Racemic mexiletine and the enantiomers caused significant ($P < 0.05$) bradycardia (fig. 22) and hypotension (fig. 23) in the rats. However, these effects did not differ significantly between the drug treatment groups. The observed decrease in heart rate may have resulted from a depression of phase 4 slope of pacemaker potentials which has been reported for class 1 antiarrhythmic agents (Singh and Hauswirth, 1974). Prolongation of PR interval and decrease in heart rate has been reported to be a common feature of class 1 antiarrhythmic agents (Marshall *et al.*, 1981). The hypotensive effects of the racemate and the individual enantiomers of

Table 17. The effects of racemic mexiletine and its enantiomers on the ECG parameters in pentobarbitone anaesthetized rats

		PR	QRS	QT _c
		(msec)	(msec)	(msec)
R(-)mex	pre-drug	45 ± 2	30 ± 0.8	190 ± 9
	4	45 ± 2	30 ± 0.8	190 ± 10
	8	46 ± 1	30 ± 0.8	190 ± 10
	16	45 ± 1	31 ± 0.8	182 ± 10
	32	47 ± 1	31 ± 0.8	182 ± 10
R,S-mex	pre-drug	47 ± 1	30 ± 0.7	195 ± 4
	4	48 ± 1	31 ± 0.4	189 ± 4
	8	48 ± 1	31 ± 0.4	182 ± 3
	16	48 ± 2	32 ± 0.7	184 ± 10
	32	51 ± 2	31 ± 1.2	181 ± 11
S(+)mex	pre-drug	44 ± 1	32 ± 0.7	189 ± 7
	4	45 ± 2	32 ± 0.8	185 ± 7
	8	46 ± 2	33 ± 0.7	187 ± 8
	16	47 ± 2	33 ± 1.1	180 ± 7
	32	49 ± 3	34 ± 1.2	188 ± 8

Values are means ± s.e.m., n = 7-8 rats

Table 18. Pre-drug values for the haemodynamic parameters in pentobarbitone anaesthetized rats

	HR (beats/min)	BP (mm Hg)
R(-)-mex	401 \pm 10	123 \pm 6
R,S-mex	388 \pm 14	122 \pm 5
S(+)-mex	389 \pm 16	106 \pm 6

Values are means \pm s.e.m., n = 7-8 rats

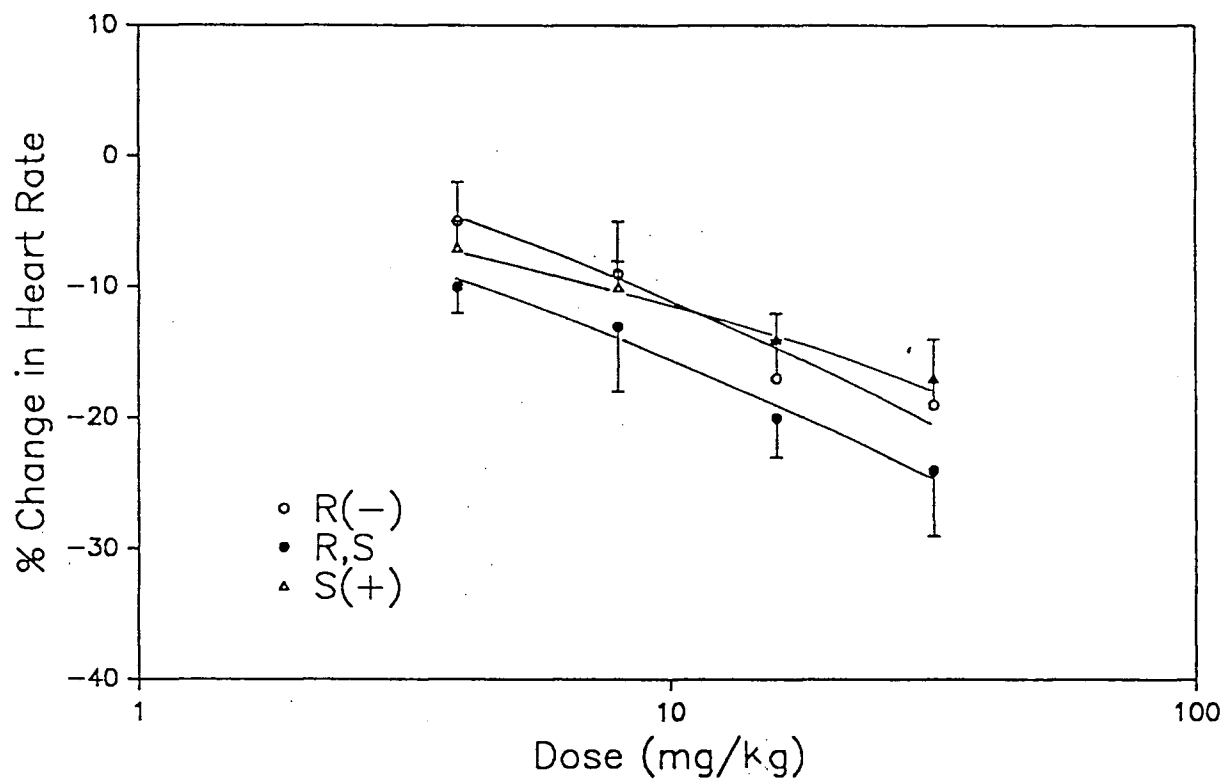


Figure 22. Cumulative dose-response curves of the effects of racemic mexiletine and its enantiomers on the heart rate in pentobarbitone anaesthetized rats. Values are means \pm s.e.m. of results from 7-8 rats. The ordinate refers to change from pre-drug values.

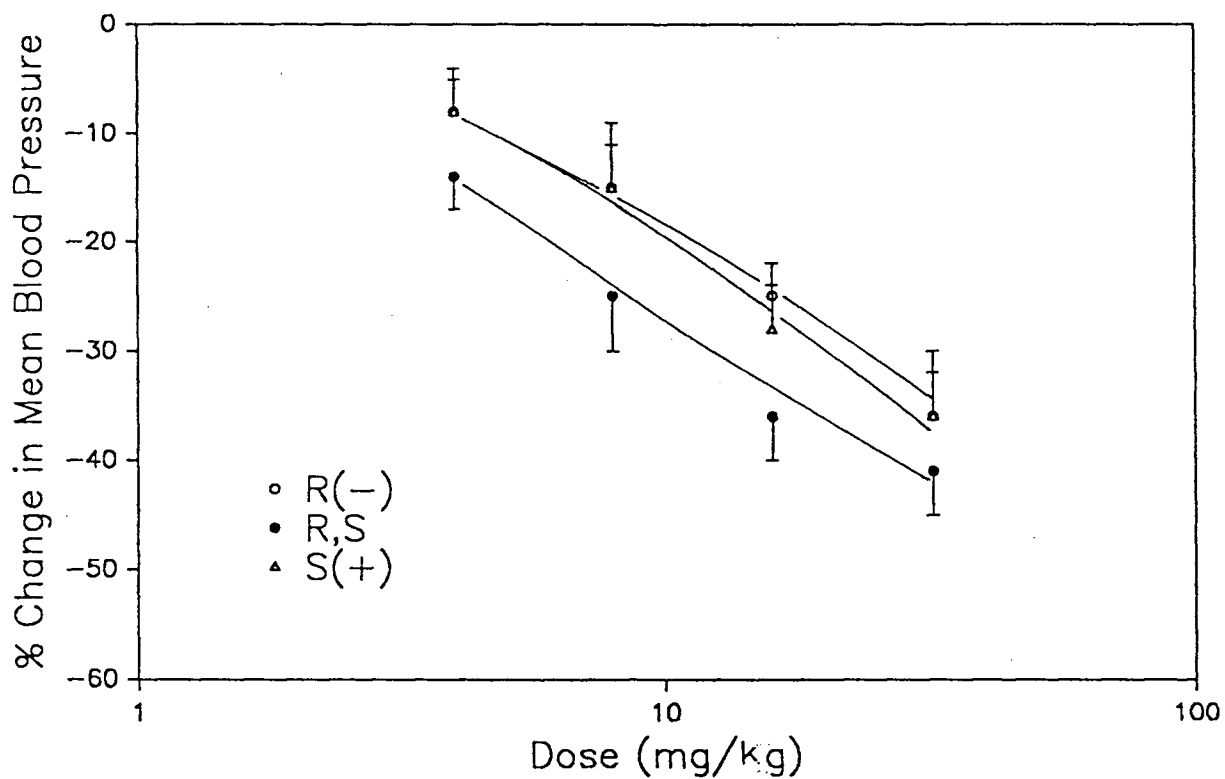


Figure 23. Cumulative dose-response curves of the effects of racemic mexiletine and its enantiomers on the mean arterial blood pressure in pentobarbitone anaesthetized rats. Values are means \pm s.e.m. of results from 7-8 rats. The ordinate refers to change from pre-drug values.

mexiletine were not consistent with results obtained in the conscious rats (section 3.5.2.4) where no significant decrease in mean arterial blood pressure was produced by the drugs. It can be speculated that as a result of recent surgery and the presence of anaesthesia in these animals, their cardiovascular status may be less satisfactory than that of the conscious rats.

In summary, the results of the present study have shown a lack of distinct differences in the effects of racemic mexiletine and its enantiomers on VFT and ERP, as well as heart rate, blood pressure and PR interval. Although a significantly greater MFF was observed for S(+)- and R,S-mexiletine relative to the R(-)-enantiomer, the data obtained for all the other variables do not support the presence of significant differences in the antiarrhythmic and cardiovascular actions of racemic mexiletine and its enantiomers.

3.5.2 Coronary Artery Occlusion-Induced Arrhythmia in Conscious Rats

The antiarrhythmic and cardiovascular effects of racemic mexiletine and its enantiomers were further investigated using coronary artery occlusion-induced arrhythmia in conscious rats. The choice of this model was based on reports in the literature which indicated that the sodium channel blocking action of racemic mexiletine, *in vitro*, was potentiated in ischaemic and hypoxic conditions (Hohnloser *et al.*, 1982; Frame *et al.*, 1982). Similar results have been observed with lidocaine (Kuppersmith *et al.*, 1975; Lazarra *et al.*, 1978). The use of conscious animals circumvented the influence of anaesthesia and recent surgery on the effects of racemic mexiletine and the enantiomers.

3.5.2.1 Dosage and Plasma Concentration

A single dose of racemic mexiletine and its enantiomers (20 mg/kg) was used for the study. The dose was infused over 10 min and it produced overt CNS toxic effects (ataxia) in almost all the rats at the end of the infusion and convulsions developed in a few of the animals. The dose of drug used made it possible to measure the plasma concentrations responsible for these CNS effects in the rats. The toxic effects were transient, lasting usually less than 5 min. Higher doses produced severe CNS toxicity and death in the rats. Due to the rapid distribution and elimination of mexiletine in the rats (section 3.4) and the relatively long monitoring period (4 h) following coronary artery occlusion, the administration of a second dose of drug (20 mg/kg) was necessary. This was initiated 1.5 h post-occlusion and was infused over 30 min. The plasma concentration-time relationships of racemic mexiletine and the enantiomers that were observed are shown in figure 24. R,S- and S(+)-Mexiletine had significantly ($p < 0.05$) greater mean plasma concentrations than R(-)-mexiletine at each sampling time. These results are consistent with those obtained in the pentobarbitone anaesthetized rats (see section 3.5.1.1 for explanation of plasma concentrations).

3.5.2.2 Occluded Zone (OZ)

The extent of myocardial ischaemia is known to influence the incidence of arrhythmias (Janse, 1987). In general, the larger the ischaemic area (occluded zone), the more frequent the arrhythmias. Austin *et al.* (1982) reported that the incidence of arrhythmias

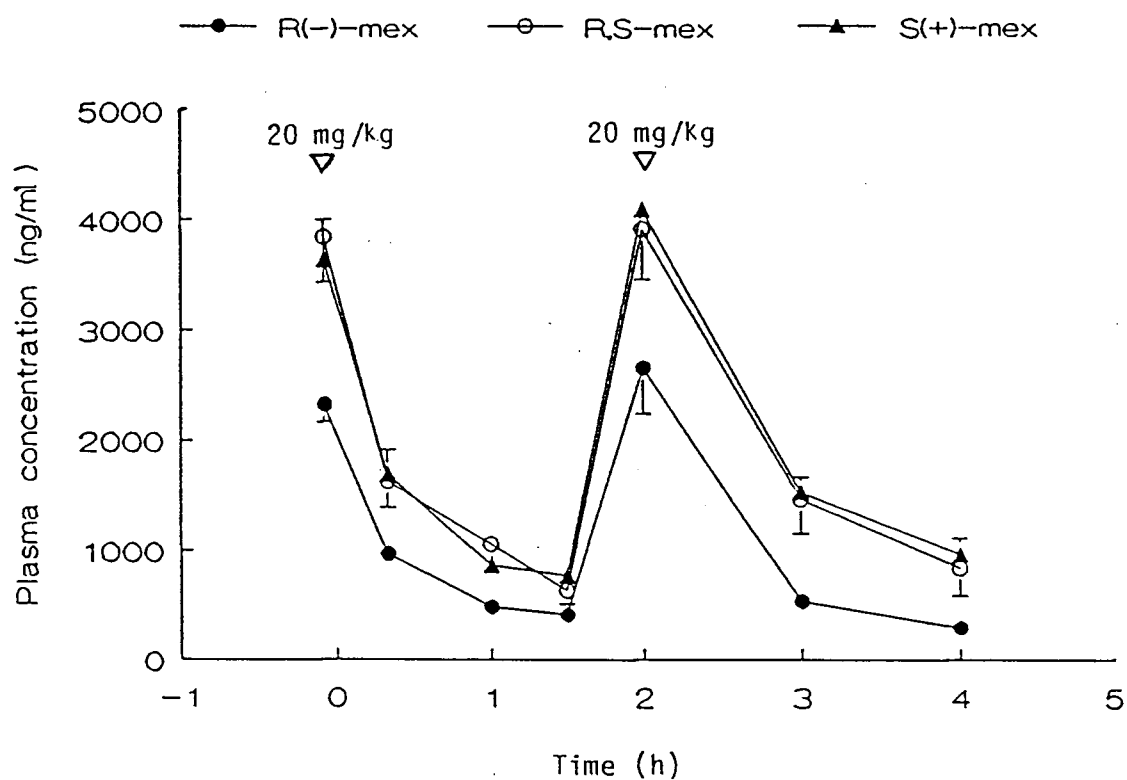


Figure 24. The plasma concentration-time profile of racemic mexiletine and the individual enantiomers in ischemic conscious rats after the administration of a dose of 20 mg/kg, followed 1.5 h later by a second dose (20 mg/kg). Values are means \pm s.e.m of results from 9 rats.

following coronary artery occlusion in mongrel dogs was critically dependent on OZ size. Johnston *et al.* (1983) in their experiments in conscious rats, showed that the "arrhythmia score" (a number scale which summarizes arrhythmias in terms of incidence and duration) was linearly related to the square root of OZ. Thus, variability in OZ size among the treatment groups could be a potential source of error in the outcome of coronary artery occlusion. Table 19 shows the percent OZ observed in rats in the (R)-, S(+)- and R,S-mexiletine and saline (control) treatment groups. No significant differences in OZ size were observed between the groups.

3.5.2.3 Antiarrhythmic Effects

The occlusion of the left anterior descending (LAD) coronary artery evoked reproducible arrhythmias in the conscious rats. Figure 25 shows the occlusion snare around the LAD coronary artery while figure 26 shows the resultant arrhythmias which included ventricular fibrillation (VF), ventricular tachycardia (VT) and premature ventricular contractions (PVCs). The arrhythmias occurred in two distinct phases consistent with the report of Johnston *et al.* (1983). The early phase comprised of the period 0-30 min post-occlusion, while the late phase arrhythmias occurred mainly between 1.5 and 4 h. The arrhythmias observed in this study are presented as early and over-all (0-4 h) arrhythmias. The data given in table 20 show the incidence of the major arrhythmias (VT & VF) in the conscious rats. Racemic mexiletine and its enantiomers did not significantly reduce the incidence of these arrhythmias. The effects of the different treatments on the number of

Table 19. The mean (\pm s.e.m.) weight and occluded zone (OZ) in the different treatment groups in conscious ischaemic rats

	Weight* (g)	OZ (%)
Saline	337 \pm 17	34 \pm 1
R(-)-mex	352 \pm 20	33 \pm 2
R,S-mex	339 \pm 14	37 \pm 1
S(+)-mex	324 \pm 17	36 \pm 1

* weight of rats in each group.

OZ refers to the percentage of the ventricle that was occluded.

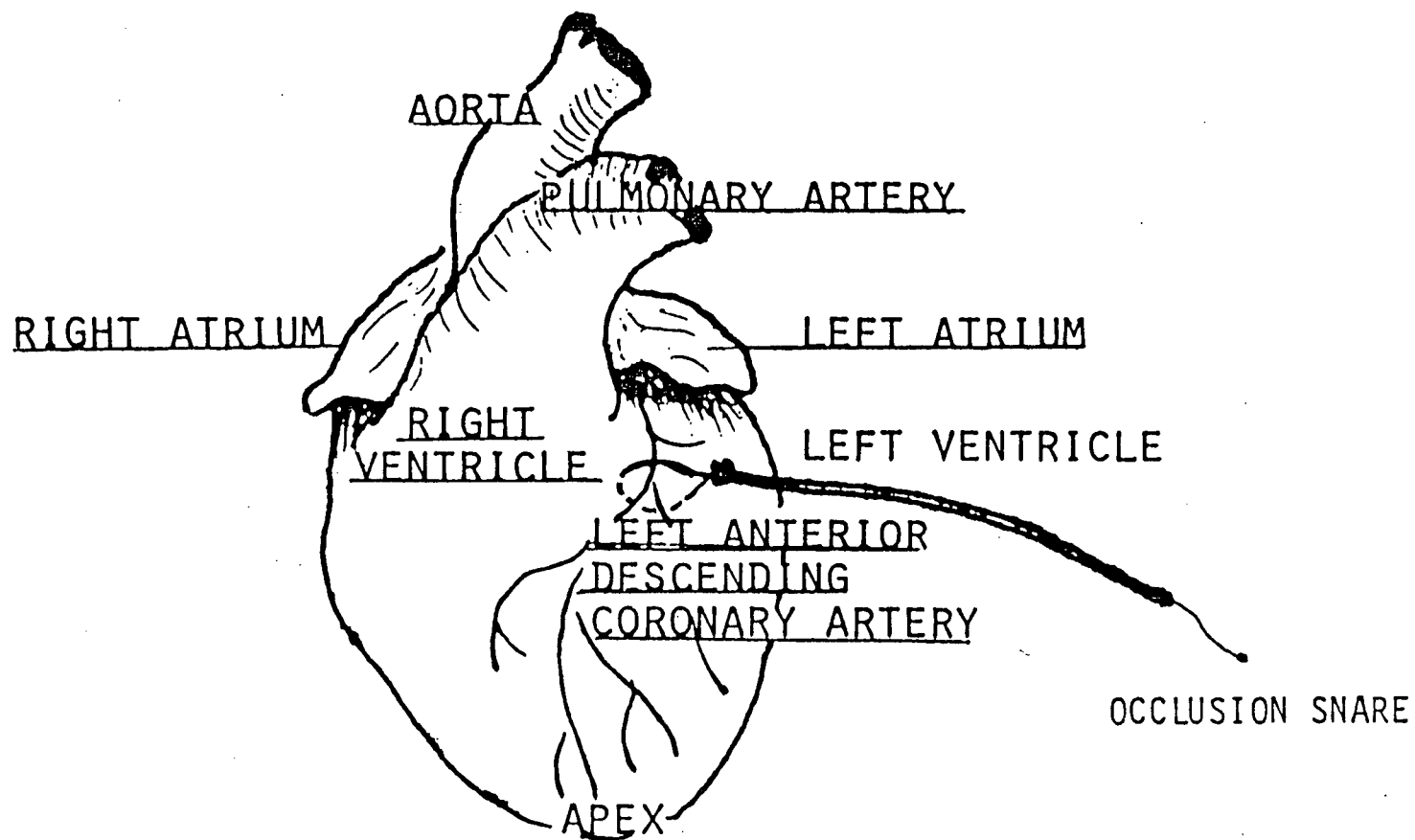


Figure 25. The rat heart showing the occlusion snare around the left anterior descending coronary artery.

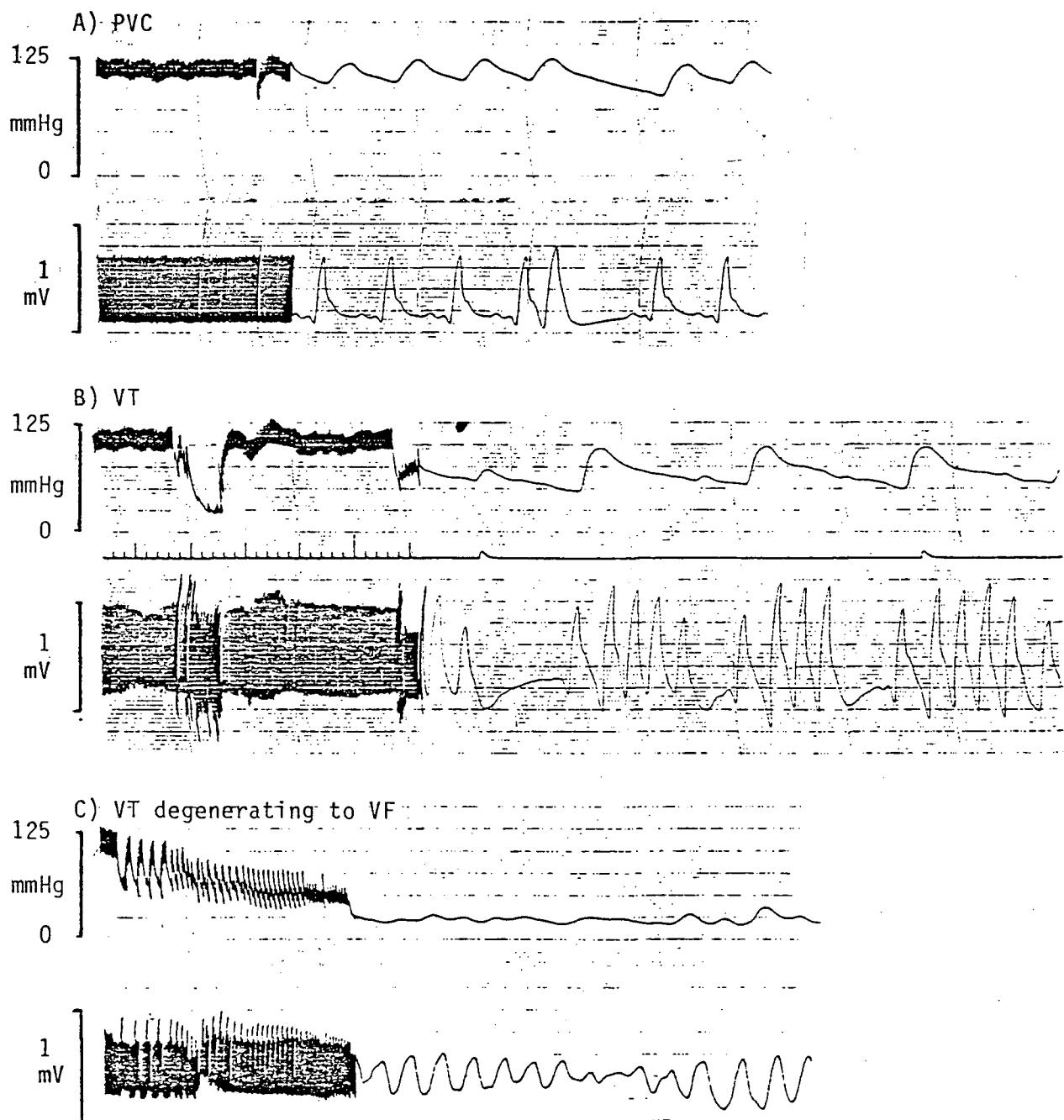


Figure 26. Typical arrhythmias resulting from occlusion of the left anterior descending coronary artery. Premature ventricular contractions (PVCs) are shown in panel A, spontaneously reverting ventricular tachycardia (VT) in B, and spontaneously reverting VT degenerating to ventricular fibrillation (VF) in C. The top and bottom traces in each panel represent blood pressure and ECG, respectively.

Table 20. The effects of racemic mexiletine and its enantiomers on the incidence of arrhythmias following coronary artery occlusion in conscious rats

	VT		VF		SVF		NSVF	
	0-0.5h	0-4h	0-0.5h	0-4h	0-0.5h	0-4h	0-0.5h	0-4h
Saline	6/9	8/9	6/9	8/9	2/9	4/9	5/9	8/9
R(-)-mex	3/9	9/9	2/9	7/9	0/9	8/9	2/9	6/9
R,S-mex	7/9	9/9	4/9	7/9	4/9	5/9	2/9	6/9
S(+)-mex	9/9	9/9	8/9	8/9	5/9	6/9	7/9	8/9

VT = ventricular tachycardia, VF = ventricular fibrillation,
 SVF = spontaneously reverting ventricular fibrillation,
 NSVF = non-spontaneously reverting ventricular fibrillation.

PVCs are shown in table 21. As was the case with VT and VF, no significant differences could be detected when the drug treatment groups were compared to the saline group. Figures 27 and 28 show the "arrhythmia scores" calculated for the enantiomers. There were no significant differences between the drug treated rats and those that received saline. In general, during the early phase, there was a tendency towards lower incidence and number of arrhythmias in the R(-)-mexiletine group relative to saline (Fig. 27). In contrast, the S(+)-mexiletine group showed a higher incidence and number of arrhythmias during the same time period.

The occlusion of the LAD coronary artery in conscious rats is an established and relatively simple model for the production of experimental cardiac arrhythmias. This model has been used to test the effectiveness of many class I antiarrhythmic drugs, including ORG 6001 (Kane *et al.*, 1980), lidocaine and quinidine (Johnston *et al.*, 1983). In the present study, racemic mexiletine and its enantiomers failed to protect conscious rats from ischaemia-induced arrhythmias. It was not possible to compare the results of this study with those of others since the effects of mexiletine enantiomers on ischaemic arrhythmias in rats have not been previously reported. However, Marshall *et al.* (1981), using a similar model in anaesthetized Wistar rats, investigated the antiarrhythmic actions of racemic mexiletine. These authors reported that 1 mg/kg of racemic mexiletine administered 15 min before coronary artery occlusion completely abolished VF in the 30 min period post-occlusion. This is in disagreement with results obtained in the present study. Based on the rapid distribution and plasma clearance of

Table 21. The effects of racemic mexiletine and its enantiomers on the number of premature ventricular contractions (\log_{10} PVCs) following coronary artery occlusion in conscious rats

	\log_{10} PVCs	
	0-1/2 h	0-4 h
saline	1.63 \pm 0.35	2.97 \pm 0.12
R(-)-mex	1.24 \pm 0.41	2.64 \pm 0.15
R,S-mex	1.90 \pm 0.23	2.80 \pm 0.13
S(+)-mex	2.02 \pm 0.12	3.00 \pm 0.10

Values are means \pm s.e.m, n = 9 rats.

Premature ventricular contractions are expressed as \log_{10} PVCs since the number of PVCs is a \log_{10} normally distributed variable.

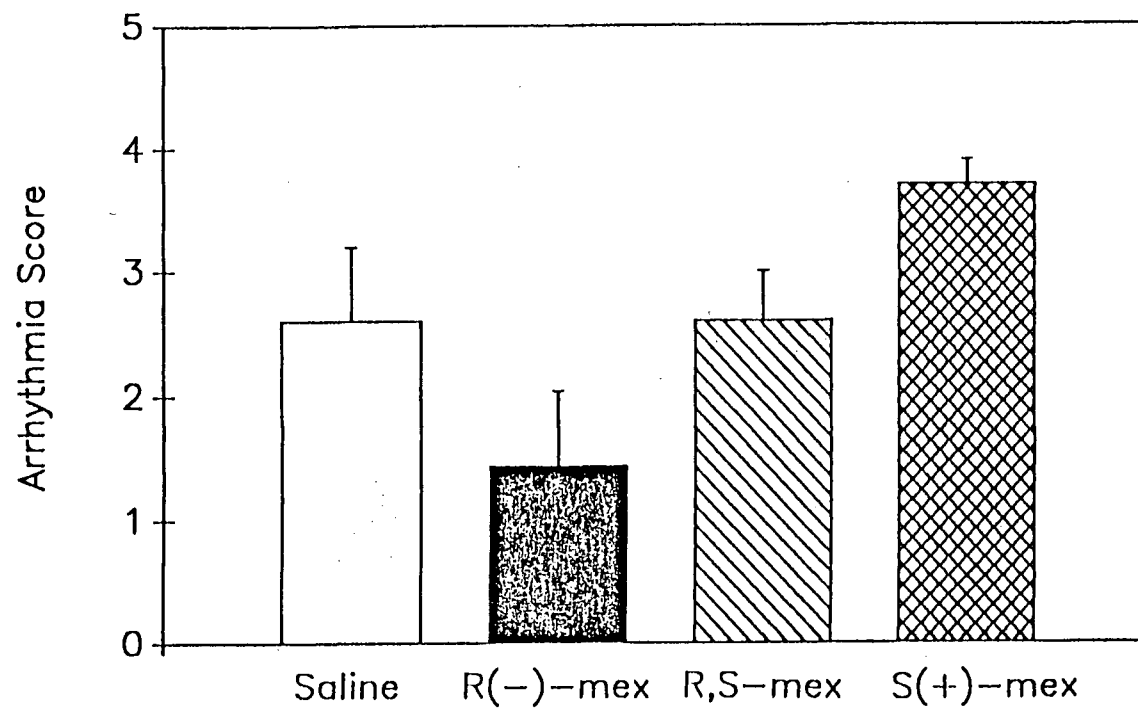


Figure 27. The effects of (R)-, R,S- and S(+)-mexiletine and saline on "arrhythmia score" for the 0-30 min period following coronary artery occlusion in conscious rats.

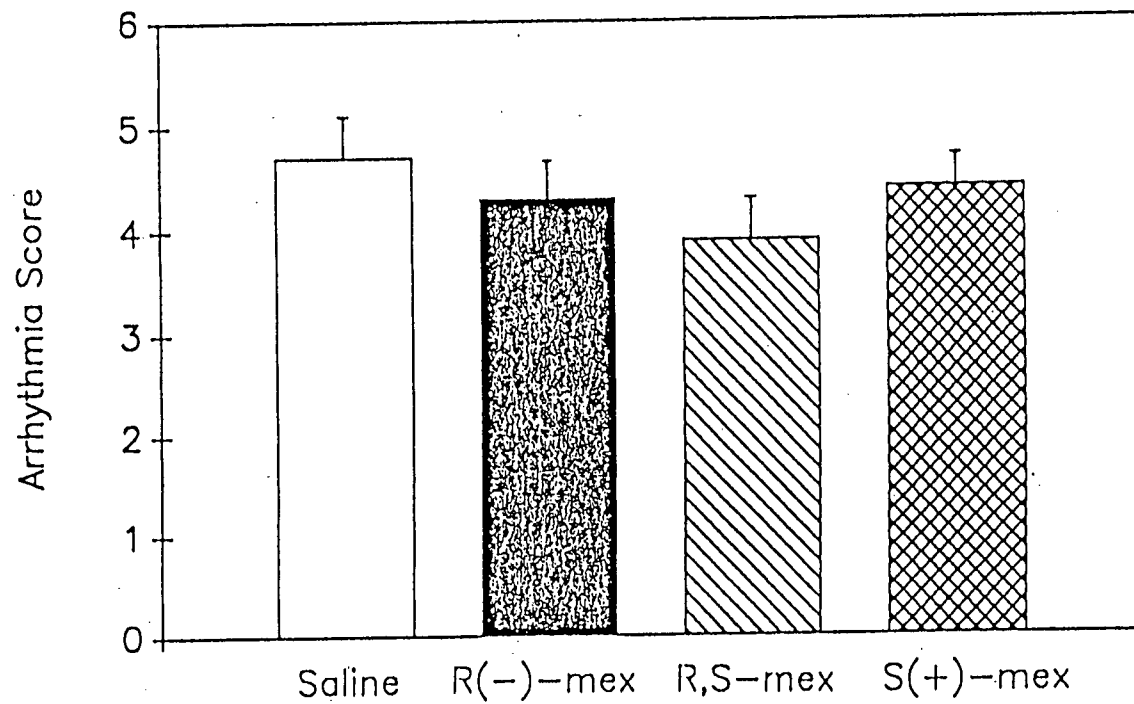


Figure 28. The effects of (R)-, R,S- and S(+)-mexiletine and saline on "arrhythmia score" for the 0-4 h period following coronary artery occlusion in conscious rats.

mexiletine enantiomers in the rat (section 3.4.2), it is unlikely that therapeutic concentrations of racemic mexiletine would be achieved and maintained for the 30 min period post-occlusion from a 1 mg/kg dose administered 15 min before occlusion. Other studies on the antiarrhythmic actions of racemic mexiletine have been carried out in dogs using the Harris 2-stage coronary artery occlusion procedure (Allen *et al.*, 1977; Mertz and Steffe, 1980; Hashimoto *et al.*, 1984).

Hashimoto *et al.* (1984) reported that a mean plasma concentration of 1.9 $\mu\text{g/ml}$ significantly reduced "arrhythmia ratios" (number of PVCs divided by the total heart rate). However, the maximum reduction in "arrhythmia ratio" was less than 50%. Their results were consistent with those of Mertz and Steffe (1980) who used the same dose of racemic mexiletine as Hashimoto *et al.*, (1980) (5 mg/kg). The latter authors pointed out that the administration of a higher dose of mexiletine was limited by CNS toxic effects which included tremors and convulsions. Allen *et al.* (1977), however, reported that at a mean plasma concentration of $5.33 \pm 0.34 \mu\text{g/ml}$, sinus rhythm was completely returned in conscious ischaemic dogs. This study did not report any CNS toxic effects at this plasma concentration in the dogs. From the results of these 3 studies, it may be speculated that greater plasma concentrations than those achieved in the present study would be required for the antiarrhythmic effects of racemic mexiletine and its enantiomers to be observed in the rat. This speculation was confirmed with a higher dose of racemic mexiletine in ischaemic pentobarbitone anaesthetized rats (section 3.5.3.1). Plasma concentrations of mexiletine and its enantiomers, such as those reported by Allen *et al.* (1977), would

produce severe CNS toxicity in the conscious rats (see toxic plasma concentrations in table 23).

3.5.2.4 Cardiovascular Effects

Table 22 shows the effects of the different treatments on the ECG parameters in conscious rats. Racemic mexiletine and the enantiomers caused a significant ($p < 0.05$) increase in the PR interval from pre-drug values relative to saline. There were no significant differences between the drug treatment groups. Neither the drugs nor saline affected the QRS interval of the rat heart.

The effects of racemic mexiletine and the enantiomers on heart rate and blood pressure are presented in figures 29 and 30, respectively. The drugs significantly ($p < 0.05$) decreased the heart rate from pre-drug values when compared to saline (fig. 29) (heart rate at 1 min before coronary artery occlusion were compared by one-way ANOVA). There were no significant differences between the drug treatment groups. When the heart rate over the 4 h observation period was analysed by "Repeated Measures" ANOVA, no statistically significant differences could be detected between the drug and saline treatment groups. A similar decrease in heart rate following the administration of racemic mexiletine has been reported in dogs (Hashimoto et al., 1984). The mean arterial blood pressure was not significantly changed by either drug or saline treatment (fig. 30).

The cardiovascular effects of racemic mexiletine and its enantiomers in the conscious rats are consistent with those observed in

Table 22. The effect of drug treatment on the ECG parameters in conscious ischaemic rats

	PR (msec)		QRS (msec)	
	pre-drug	post-drug	pre-drug	post-drug
saline	44.5 \pm 1.4	44.0 \pm 1.4	24.0 \pm 1.4	24.0 \pm 1.3
R(-)-mex	39.9 \pm 1.1	48.0 \pm 1.8*	22.8 \pm 1.0	22.8 \pm 0.7
RS-mex	43.0 \pm 2.0	49.3 \pm 1.3*	22.0 \pm 0.8	22.0 \pm 1.0
S(+)-mex	44.0 \pm 2.3	53.0 \pm 1.4*	22.0 \pm 1.4	23.0 \pm 1.0

Values are means \pm s.e.m, n = 9 rats.

* Significant (p<0.05) when compared to saline with respect to increase from pre-drug.

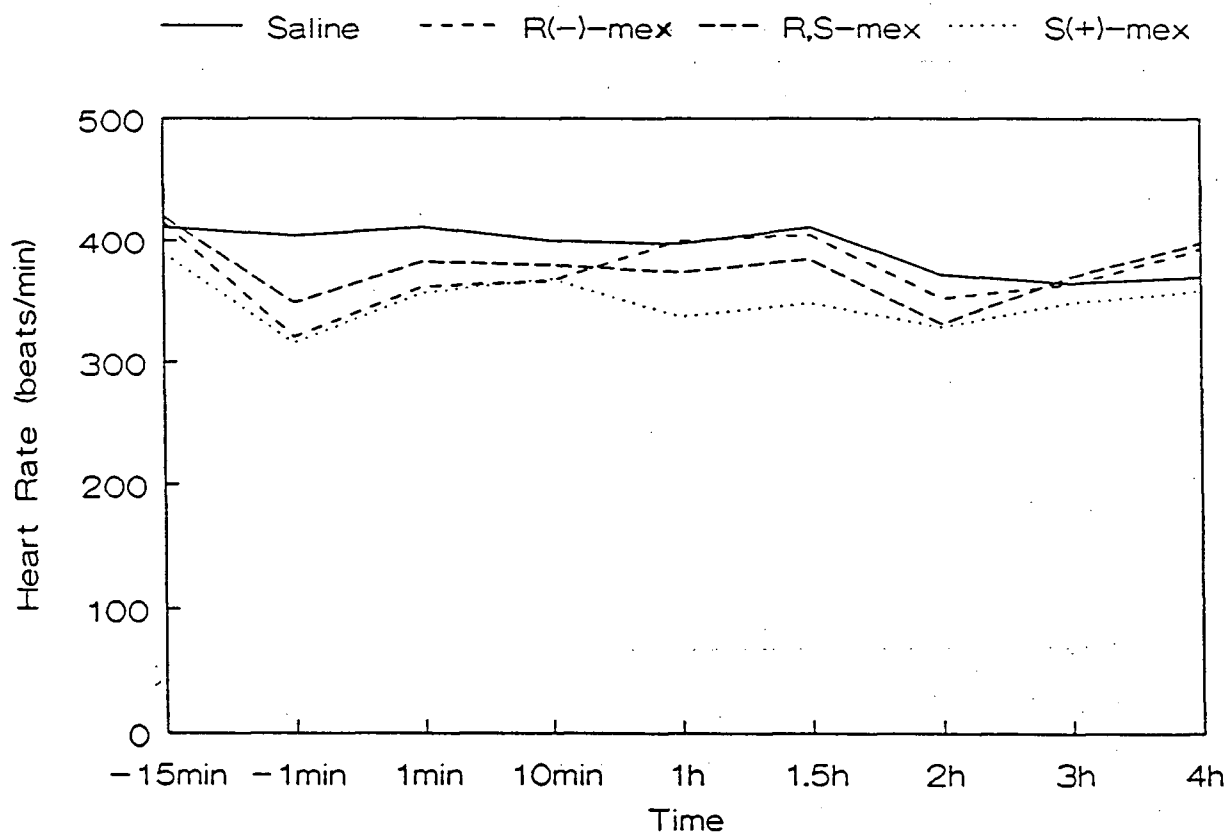


Figure 29. The effects of (R)-, R,S- and S(+)-mexiletine and saline on heart rate in ischaemic conscious rats. The s.e.m are omitted for clarity of presentation.

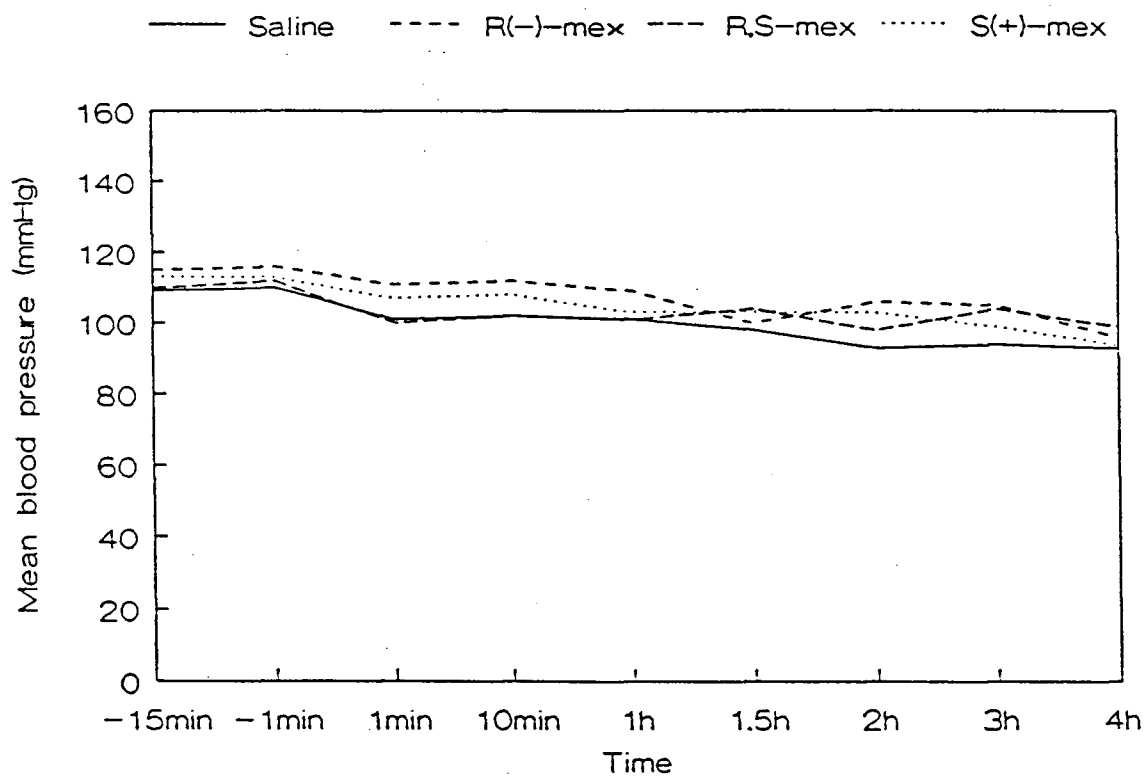


Figure 30. The effects of (R)-, R,S- and S(+)-mexiletine and saline on mean arterial blood pressure in ischaemic conscious rats. The s.e.m are omitted for clarity of presentation.

the pentobarbitone anaesthetized rats, except for their effect on blood pressure (see section 3.5.1.3).

3.5.2.5 CNS Side Effects

Table 23 shows the incidence of overt CNS toxic effects (ataxia and convulsions) in the conscious rats. The corresponding plasma concentrations at which these signs of toxicity appeared are also presented. The incidence of CNS toxic effects were significantly ($p < 0.05$) greater in the drug treated rats compared to those that received saline. However, there were no significant differences between the drug treatment groups. The CNS toxic effects were presumed to be due to the high brain uptake of mexiletine enantiomers in the rat (section 3.4.5).

In summary, racemic mexiletine and its enantiomers did not protect conscious rats from coronary artery occlusion-induced arrhythmias. The bradycardic and PR prolongation effects of racemic mexiletine and its enantiomers were significant but did not differ from each other. The drugs produced comparable CNS side effects in conscious rats.

3.5.3 Coronary Artery Occlusion-Induced Arrhythmia in in Pentobarbitone Anaesthetized Ischaemic Rats

To explain the ineffectiveness of racemic mexiletine and its enantiomers against ischaemia-induced arrhythmias in the conscious rats, the antiarrhythmic effects of a higher dose of racemic mexiletine (40 mg/kg), which could not be tolerated by conscious rats, was investigated. The effects of the same dose of the racemate used in conscious rats (20 mg/kg) and saline (control) were also evaluated. The

Table 23. The incidence of CNS toxic effects and the corresponding plasma concentrations in conscious rats following the administration of racemic mexiletine and its enantiomers (20 mg/kg)

	CNS toxic effects**	Plasma concentration ($\mu\text{g/ml}$)
Saline	0/9	-
R(-)-mex	8/9*	2.3 ± 0.16
R,S-mex	7/9*	3.8 ± 0.41
S(+)-mex	8/9*	3.6 ± 0.36

* Significant ($p < 0.05$) when compared to saline.

** CNS toxic effects observed were ataxia and convulsions.

Plasma concentrations are means \pm s.e.m. R,S- and S(+)-Mexiletine had significantly ($p < 0.05$) greater plasma concentrations than the R(-)-enantiomer.

animals were monitored for 30 min after coronary artery occlusion (which covered the first phase of arrhythmias). This investigation was carried out in pentobarbitone anaesthetized rats. The arrhythmias resulting from coronary artery occlusion in pentobarbitone anaesthetized rats have been shown to correspond approximately with those seen in conscious rats (Johnston *et al.*, 1983).

3.5.3.1 Antiarrhythmic Effects of Racemic Mexiletine

Table 24 shows the percent OZ in the 3 treatment groups. There were no significant differences in this parameter between the groups. The incidence of VT and VF and the number of PVCs following the 2 doses of racemic mexiletine (20 and 40 mg/kg) and saline are shown in tables 25 and 26, respectively. At the dose of 20 mg/kg, R,S-mexiletine did not significantly reduce the incidence of VT and VF nor the number of PVCs when compared with the saline control group (tables 25 & 26). These results were similar to those obtained for the early arrhythmias in conscious rats. At the higher dose of 40 mg/kg, R,S-mexiletine significantly ($p < 0.05$) reduced the incidence of VT and the number of PVCs (tables 25 & 26) and completely suppressed VF in all the rats (table 25). The "arrhythmia scores" obtained for the 3 treatment groups are shown in figure 31. A significantly ($p < 0.05$) lower "arrhythmia score" was observed in the 40 mg/kg group when compared to the 20 mg/kg and saline groups. The mean "arrhythmia score" for the 20 mg/kg group, however, was not significantly different from that of the saline group. The plasma concentration-time relationship following the administration

Table 24. The mean (\pm s.e.m.) weight and occluded zone (OZ) in the different treatment groups in pentobarbitone anaesthetized rats

	Weight* (g)	OZ (%)
Saline	402 \pm 18	34 \pm 1
R,S-mex (20 mg/kg)	410 \pm 6	36 \pm 1
R,S-mex (40 mg/kg)	410 \pm 10	34 \pm 1

* Weight of rats in each group.

OZ refers to the percentage of the ventricle that was occluded.

Table 25. The effect of R,S-mexiletine on the incidence of arrhythmias following coronary artery occlusion in pentobarbitone anaesthetized rats

	VT	VF	SVF	NSVF
Saline	9/9	8/9	4/9	6/9
R,S-mex (20 mg/kg)	8/9	4/9	4/9	2/9
R,S-mex (40 mg/kg)	3/9*	0/9*	0/9*	0/9*

* significant ($P < 0.05$) when compared to saline.

VT, VF, SVF and NSVF have the same meaning as in table 20.
Rats were monitored over a 30 min period.

Table 26. The effects of racemic mexiletine on the number of premature ventricular contractions (\log_{10} PVCs) following coronary artery occlusion in pentobarbitone anaesthetized rats

	\log_{10} PVCs
saline	2.42 ± 0.16
R,S-mex (20 mg/kg)	2.80 ± 0.06
R,S-mex (40 mg/kg)	$1.68 \pm 0.30^*$

Values are the means \pm s.e.m., n = 9 rats.

* Significant ($p < 0.05$) when compared to saline.

Premature ventricular contractions are expressed as \log_{10} PVCs since the number of PVCs is a \log_{10} normally distributed variable.

Rats were monitored over a 30 min period.

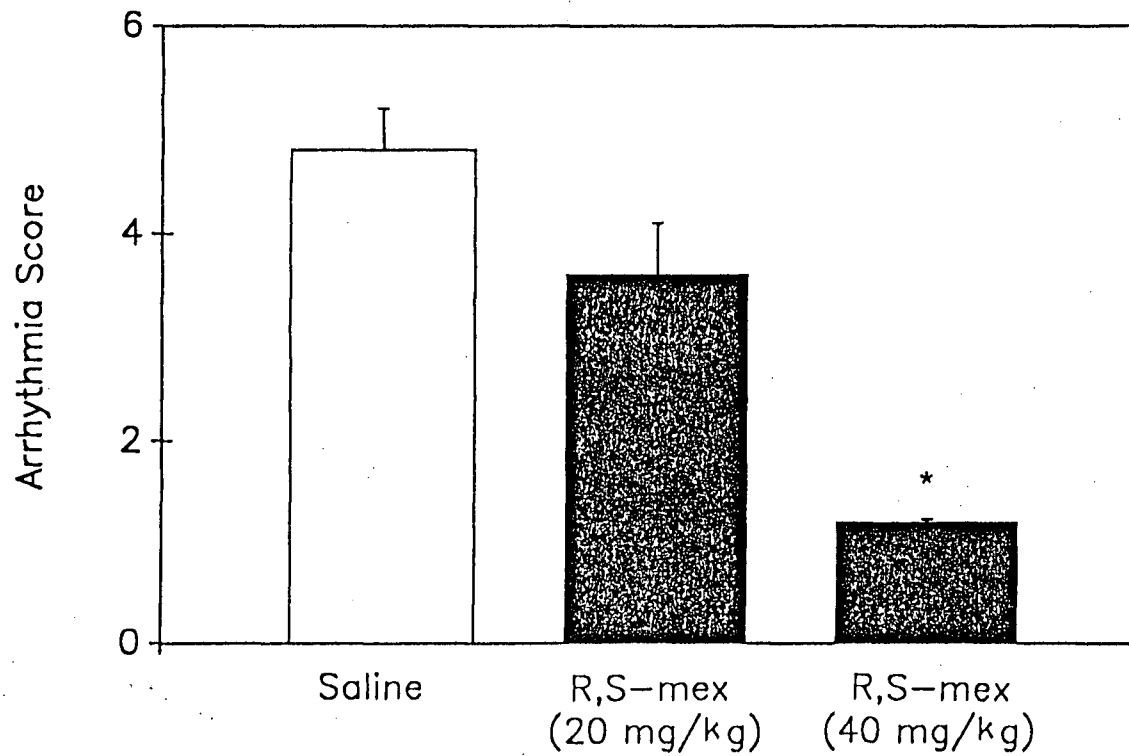


Figure 31. The effects of racemic mexiletine (20 and 40 mg/kg) and saline on "arrhythmia score" for the 0-30 min period following coronary artery occlusion in pentobarbitone anaesthetized rats.

* Significant ($p < 0.05$) when compared to saline.

of 20 mg/kg of R,S-mexiletine and the distribution of major arrhythmias (VT + VF) during the 30 min after coronary occlusion are shown in figure 32. The mean plasma concentrations ranged from $5.0 \pm 0.3 \mu\text{g/ml}$ at the end of drug infusion (5 min before coronary artery occlusion) to $1.6 \pm 0.2 \mu\text{g/ml}$ 30 min post-occlusion (fig. 32A). There was a significantly ($p < 0.05$) lower incidence of major arrhythmias during the 0-5 min period post-occlusion (Fig. 32B) when compared to the saline group (Fig. 33). The corresponding mean plasma concentration, 5 min post-occlusion, was approximately $3.5 \mu\text{g/ml}$ (Fig. 32A). In the group of rats that received 40 mg/kg of R,S-mexiletine, where significant ($p < 0.05$) suppression of arrhythmias was observed throughout the 30 min period post-occlusion (Fig. 34B), plasma concentrations ranged from $10.3 \pm 0.5 \mu\text{g/ml}$ at the end of drug infusion to $3.3 \pm 0.3 \mu\text{g/ml}$ 30 min post-occlusion (fig. 34A).

These results confirmed that the antiarrhythmic effects of racemic mexiletine are seen at higher plasma concentrations than those achieved in the conscious rats. However, these concentrations are comparable to, or greater than those associated with CNS toxic effects in conscious rats (table 25). The results obtained from both the electrical and ischaemic arrhythmia studies indicated that the antiarrhythmic, cardiovascular and CNS toxic effects of racemic mexiletine and its enantiomers are not markedly different from each other.

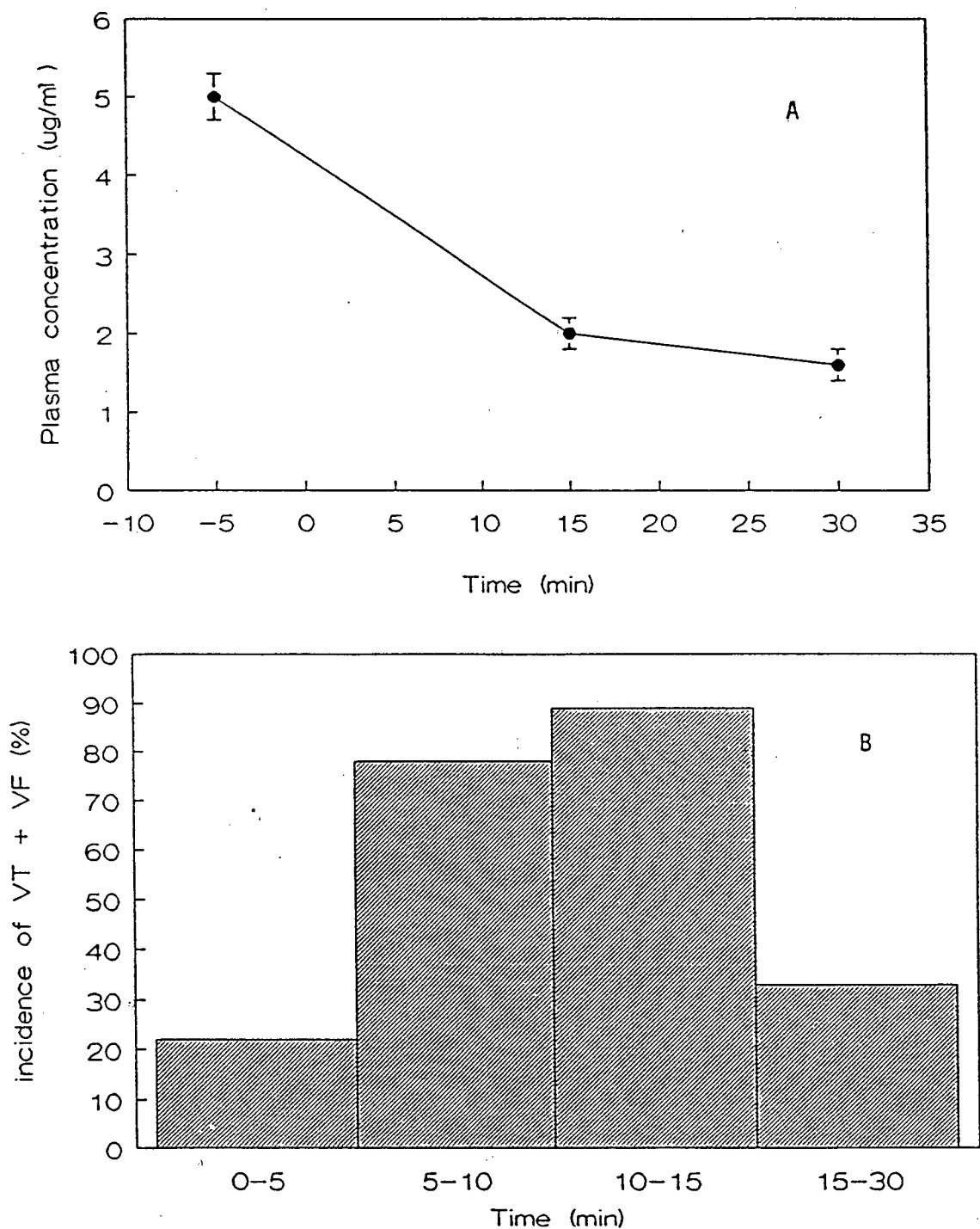


Figure 32. The plasma concentration-time relationship following the administration of racemic mexiletine (20 mg/kg) (A) and the distribution of major arrhythmias (VT + VF) in ischaemic pentobarbitone anaesthetized rats (B). The abscissa indicates time (min) with respect to coronary artery occlusion. The plasma concentrations are means \pm s.e.m. of results from 9 rats.

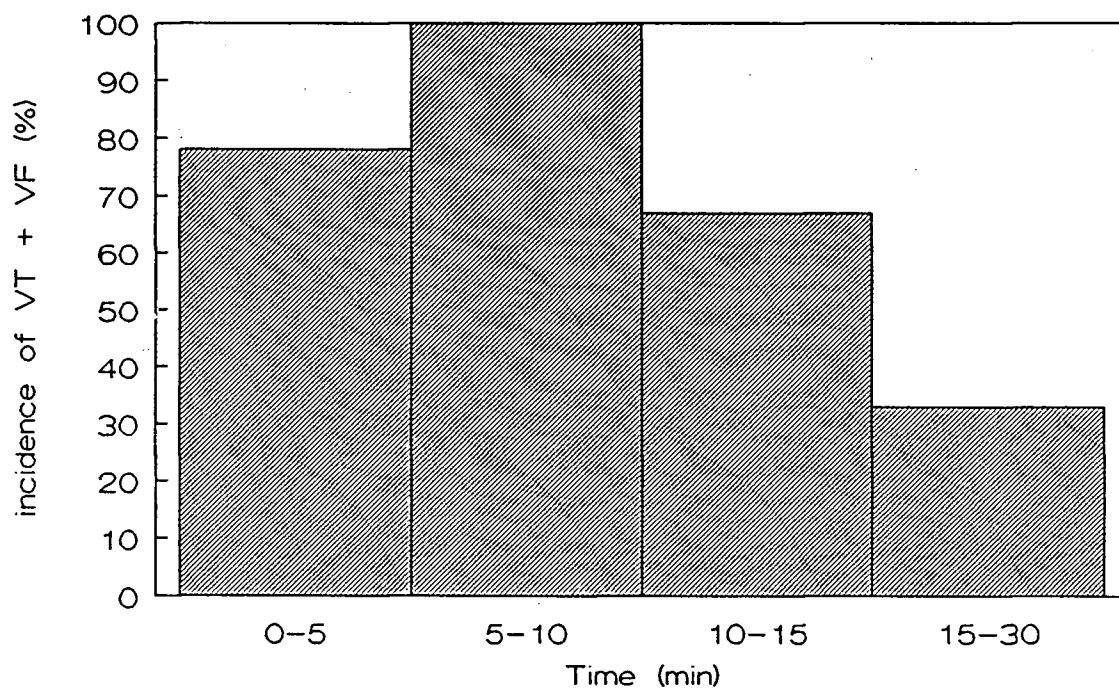


Figure 33. The distribution of major arrhythmias (VT + VF) in ischaemic pentobarbitone anaesthetized rats following the administration of saline (control). The abscissa indicates time (min) with respect to coronary artery occlusion

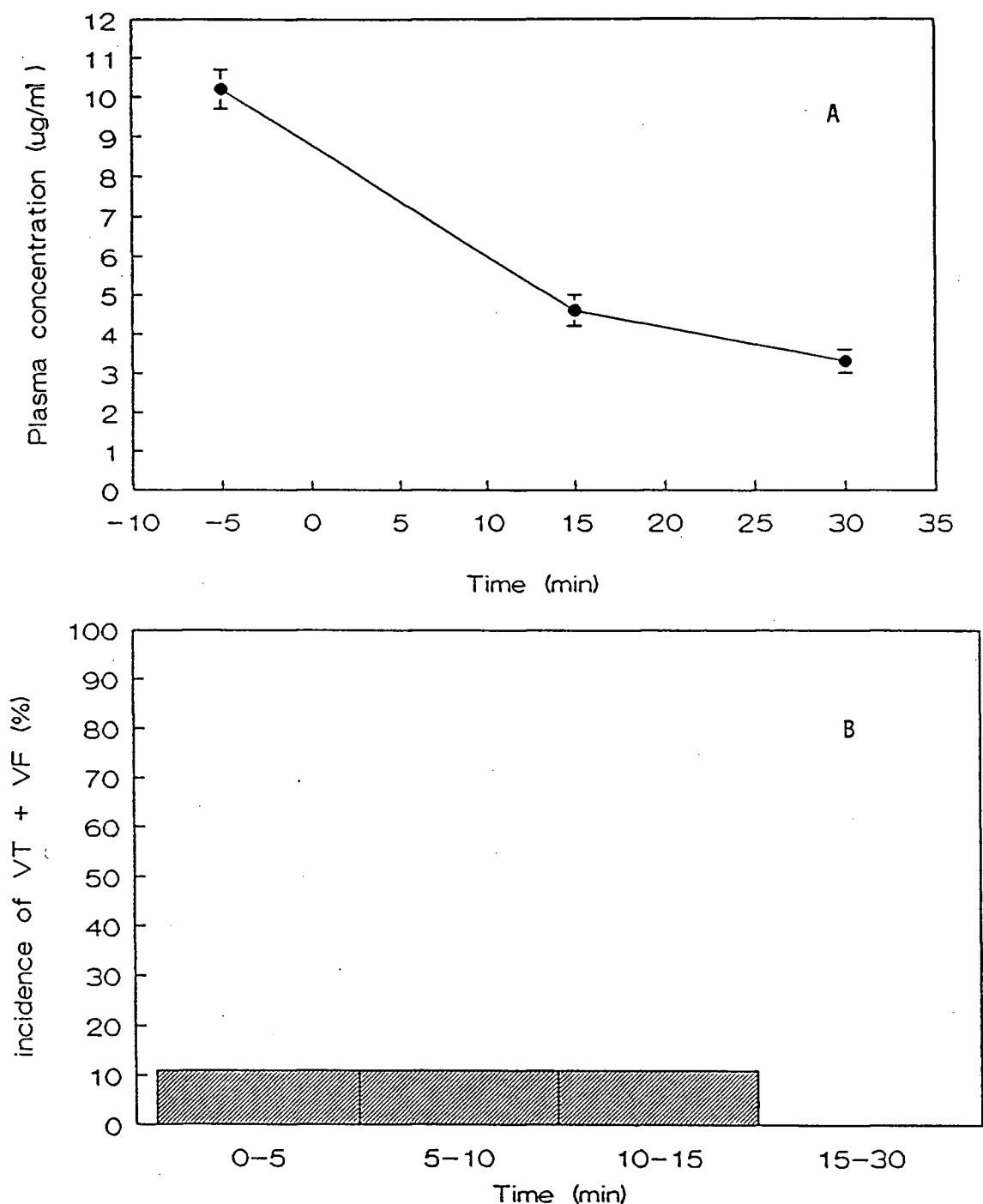


Figure 34. The plasma concentration-time relationship following the administration of racemic mexiletine (40 mg/kg) (A) and the distribution of major arrhythmias (VT + VF) in ischaemic pentobarbitone anaesthetized rats (B). The abscissa indicates time (min) with respect to coronary artery occlusion. The plasma concentrations are means \pm s.e.m. of results from 9 rats.

4. SUMMARY and CONCLUSIONS

The synthesis and characterization of 2-anthroyl chloride was successfully accomplished. Derivatization of mexiletine enantiomers with the acid chloride was rapid and the enantiomer derivatives formed were resolved on a Pirkle^R ionic (phenyl glycine) chiral column by HPLC. 2-Anthroyl chloride was found to be a highly sensitive fluorescence derivatization reagent. Attempts at using this derivatization reagent to quantitate mexiletine enantiomers were unsuccessful due to the presence of an interfering peak co-eluting with S(+)-mexiletine which could not be resolved. A previously developed, stereoselective HPLC assay, with 2-naphthoyl chloride as derivatization reagent, was subsequently used for the pharmacokinetic and pharmacodynamic studies.

The serum protein binding studies in humans revealed that the binding of mexiletine enantiomers was pH-dependent; with free fractions decreasing significantly as the pH was increased from 7.0 to 8.0. These results have important implications since when pH was maintained at physiological values (≈ 7.4), the observed binding of mexiletine was moderate and significantly lower than that reported in the literature (Talbot *et al.*, 1973; McErlane *et al.*, 1987). More importantly, the binding of the enantiomers was not stereoselective at physiological pH. These changes in free fractions are most likely due to pH-dependent changes in the ionization of the binding protein(s). The binding studies did not reveal any significant interactions between the enantiomers affecting binding nor any concentration-dependent binding within the therapeutic range of racemic mexiletine. The enantiomers were determined to bind mainly to AAG and HSA. Binding to serum and HSA

indicated the presence of two classes of binding sites. These were a high affinity, low capacity and a low affinity, high capacity class of binding sites. In contrast, binding to AAG showed only one class of binding sites which was a high affinity, low capacity site.

Pharmacokinetic and tissue distribution studies in rats indicated very extensive tissue uptake and rapid elimination of the enantiomers. R(-)-Mexiletine had a markedly greater systemic clearance and steady-state volume of distribution than the S(+)-enantiomer. The terminal elimination half-lives of the enantiomers were not different. The uptake of the enantiomers into the tissues studied was not stereoselective, except for the liver tissue that showed a much greater uptake of the S(+)-enantiomer. High tissue/serum ratios (>20) were observed in brain, lungs and kidneys. The brain accumulated 3-fold the heart concentrations of the enantiomers. This relatively high uptake of the enantiomers into the brain tissue could explain the CNS toxic effects often observed with mexiletine therapy.

The pharmacodynamic studies using electrical-induced arrhythmias in pentobarbitone anaesthetized rats showed that racemic mexiletine and its enantiomers significantly increased VFT. However, no significant differences in this effect was found between the drugs. MFF was significantly decreased and R,S- and S(+)-mexiletine showed significantly greater effects than the R(-)-enantiomer. However, the increase in ERP induced by racemic mexiletine and its enantiomers did not show significant differences. The prolongation of PR interval and decrease in heart rate due to the drugs did not differ significantly from each other. Based on these results, it is unlikely that the

antiarrhythmic effects of racemic mexiletine and its enantiomers are markedly different from each other. The 3 drugs produced comparable hypotension in pentobarbitone anaesthetized rats, although, this effect may have been due to the presence of anaesthesia and/or recent surgery in these animals. This conclusion is based on the fact that similar effects were not present in the conscious rats.

In the conscious ischaemic rats, racemic mexiletine and the enantiomers did not significantly reduce the incidence and number of arrhythmias nor the "arrhythmia score" when compared to saline. As was the case in the pentobarbitone anaesthetized rats, the significant bradycardic and PR prolongation effects of the drugs were not stereoselective. Comparable CNS toxicity was induced by racemic mexiletine and the enantiomers in the conscious rats. Studies in the pentobarbitone anaesthetized ischaemic rats using a higher dose of racemic mexiletine (which could not be tolerated by conscious rats) revealed that the effective plasma concentrations of racemic mexiletine were within the toxic range in rats. The results in the anaesthetized rats explained the ineffectiveness of racemic mexiletine and its enantiomers in conscious rats. The data obtained from both the electrical and occlusion-induced arrhythmia studies suggested that there were no marked differences in the antiarrhythmic, cardiovascular and CNS toxic effects of racemic mexiletine and its enantiomers.

The important question raised by the results of this study is whether a truly effective antiarrhythmic plasma concentration of racemic mexiletine can be achieved and maintained in man without the manifestation of severe CNS toxic effects. Although the results of many

studies carried out in animals (mainly dogs) have shown the antiarrhythmic effectiveness of racemic mexiletine, these studies have used the Harris 2-stage coronary artery occlusion procedure. It is well known that VF does not occur in these dogs (section 1.1.2.5.3). Thus, this model would not provide information on the effect of racemic mexiletine on VF, which is the primary cause of sudden death. Many investigators who have studied the beneficial effects of racemic mexiletine with respect to prevention of sudden death in MI patients have not found any significant reduction in death rate in these patients when compared to control patients (section 1.2.5). It is plausible that the ineffectiveness of racemic mexiletine was due to the maintenance of sub-therapeutic plasma concentrations in these patients.

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