A pharmacological comparison of methanesulfonamide class III antiarrhythmic agents in various species.

by E.S. Hayes

B.Sc. University of British Columbia, British Columbia

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Department of Pharmacy and Therapeutics

The University of British Columbia
Vancouver, Canada

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ABSTRACT.

In the search for new and more effective antiarrhythmic agents a great deal of effort has been expended on the development of new Class III drugs. This class of antiarrhythmics act by prolonging action potential duration and thereby increasing refractoriness. For most of these new Class III drugs the mechanism by which they produce their effects on action potential duration is blockade of one or more cardiac potassium channels. A large number of potassium channels have been described with at least six different types occurring in cardiac tissue. The full pharmacological profile of new class III drugs has not been investigated since they have only recently been developed. We therefore considered it appropriate to compare the pharmacological actions of three sotalol derivatives (sematilide, ibutilide, and E4031) and a structurally dissimilar compound (almokalant), in a number of species, using a variety of cardiovascular models. Each of the chosen drugs was administered over a wide range of doses (except almokalant) in order to fully explore their pharmacological actions. The species used included primates, guinea pigs and rats. Most of the studies were performed in intact animals in which blood pressure, heart rate, and ECG responses were recorded together with electrical stimulation of the left ventricle and, in some cases, recording of monophasic action potentials. In addition an *in vitro* preparation of guinea pig vas deferens was studied as being representative of nerve/smooth muscle preparations. At low doses the responses expected of blockers of the cardiac delayed rectifier potassium current were observed in all species excluding rats. There was a selective dose-dependent widening of the Q-Tc interval of the ECG and increased refractoriness in species with delayed rectifier currents in their ventricles (primates & guinea pigs). In the rat, a species which lacks a delayed rectifier current, no such effects were observed. The profile of action of low doses of such drugs was best exemplified by sematilide and almokalant in baboons. Sematilide and almokalant produced a dose-related widening of the monophasic action potential (MAP), as recorded from the right ventricle, and this correlated with widening of the Q-Tc interval and increased refractoriness. Furthermore, such effects were dependent upon heart rate such that at higher heart rates effects...
on action potential duration were less ("negative frequency dependence"). Ibutilide and E4031 had similar cardiovascular profiles. Results in primates were then compared with those obtained in guinea pigs using a wide range of doses, from low to very high. Similar studies were also performed in the rat. In the guinea pig, at low doses, the pattern of ECG and cardiovascular changes were similar to those seen in primates. However, at higher doses signs of possible sodium channel blockade were seen in terms of ECG changes and responses to electrical stimulation. Signs of sodium channel blockade were seen in rats and these occurred only after very high doses. In none of the tests was ibutilide different from E 4031. This was somewhat unexpected in view of the fact that the actions of ibutilide may not be due to blockade of the delayed rectifier, but to activation of an inward sodium current. Thus, in terms of cardiovascular actions in intact animals, the class III drugs tested were very similar to each other. In addition to in vivo studies, the effects of the drugs on a field stimulated vas deferens preparation from guinea pigs were studied. The pattern of response of the vas deferens was similar in the presence of ibutilide and E4031. Both ibutilide and E4031 produced bell shaped concentration response curves, enhancing the field stimulation induced contractions at low concentrations but inhibiting them at higher concentrations.

In conclusion, the above studies helped establish the pharmacological profile of a group of new Class III antiarrhythmics. The drugs were remarkably similar to each other, even in terms of the possible sodium channel blocking actions at high doses and were very potent in producing Q-Tc widening in species other than the rat. In terms of in vitro effects ibutilide and E4031 had very similar actions.
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LIST OF ABBREVIATIONS

α-level  level of significance
B.P.  blood pressure
°C  degree Celsius
ECG  electrocardiogram
g  gram
Hz  hertz
i.p.  intraperitoneally
i.v.  intravenous
kg  kilogram
<  less than
μM  micromolar
ml  milliliter
mmHg  millimetres of mercury
mM  millimolar
min  minute
%  percentage
SD  standard deviation of the mean
SEM  standard error of the mean
sec  second
1 INTRODUCTION

1.1 Molecular structure of potassium channels

Potassium (K\(^{+}\)) channels play an important role in the function of almost every cell and tissue type. Many potassium channels with different structures and electrophysiological properties have been identified in a wide variety of tissues (Palotta and Wagoner, 1992). However, molecular biology techniques have been used to classify potassium channels, based on amino acid sequence, into three groups (Hoshi and Zagotta, 1993). These channels maintain structural characteristics which allow them to be grouped into either shaker, mini or inward rectifier/ATP sensitive potassium channel super families (Jan and Jan, 1992).

1.1.1 Shaker potassium channels

The gene family that comprises the shaker potassium channel group is large and has been subdivided further into a number of subfamilies of shaker-like channels (i.e. shaw, shab & shal) (Palotta and Wagoner, 1992). The genes from these subfamilies produce the protein subunits necessary for assembly of a variety of delayed rectifier (cardiac) and A-type (neuronal) potassium channels (Pongs, 1992; Yao and Tseng, 1994). Studies of wild type and toxin-insensitive mutant shaker K\(^{+}\) channels, when expressed in *Xenopus* oocytes, suggest that these channels exist as homotetramers and that each monomer consists of six transmembrane sequences (S1-S6) (MacKinnon, 1991). Both the amino and carboxyl terminals reside on the intracellular surface of the membrane. Each monomer has a pore forming segment between S5 and S6 and a voltage sensitive sequence in S4 (Liman et al., 1991; MacKinnon et al., 1988). Point mutation experiments with shaker H4 K\(^{+}\) channels expressed in *Xenopus* oocytes have shown that an important part of the pore forming region is an eight amino-acid sequence which is the most highly conserved amongst all of the K\(^{+}\) channels which have been cloned (MacKinnon et al.,
1988). Similar experiments using the same signature sequence have suggested that this region acts as a selectivity filter and that hydroxyl groups at positions 2 and 3 and an aromatic group at position 7 are not important in determining K⁺ selectivity (Heginbotham et al., 1994). These results suggest that the pore forming region acts not only to facilitate passage of ions but also as a selectivity filter for the appropriate species of ion (Yool and Schwarz, 1991; Hartman et al., 1991).

A number of different studies using analysis of single channel recordings have been used in an attempt to measure the activation gating charge in shaker K⁺ channels (Liman et al., 1991; Schoppa et al., 1992; Benzanilla et al., 1991). A range of values (from 5-12 residues) has been proposed by various authors as the number of residues involved in shaker K⁺ channel activation gating. This discrepancy is thought to be the result of differences in the numerous analytical methods used to determine charge. There is also considerable controversy as to whether or not activation gating in K⁺ channels is cooperative in nature or is a series of independently linked steps (Tytgat and Hess, 1992; Hurst et al., 1992). These differences may once again be due to the different analytical methods used.

Hypotheses concerning the inactivation of shaker K⁺ channels are less controversial. Inactivation of most shaker channels is believed to be a "ball and chain" or "N-type" open channel block of the pore by the amino terminus (Demo and Yellen, 1991). This type of inactivation is thought to be modulated by a non-channel β protein (Rettig et al., 1994). Association of α subunits (conducting portion) of non-inactivating delayed rectifier K⁺ channels with β proteins gives channels the ability to completely inactivate (Rettig et al., 1994). Shaker and other K⁺ channels are also thought to develop "C-type" inactivation. This process is not voltage dependent and can be modulated by external monovalent ions. It may involve an external site closely associated with the TEA binding site (Heginbotham and MacKinnon, 1992). Shaker K⁺ channels are voltage dependent and open and close in response to depolarization.

1.1.2 Mini potassium channels
The minimal (mini; $I_{sk}$) potassium channel is also a voltage operated channel but its primary and tertiary structure are quite distinct from shaker potassium channels. The mini-$K^+$ channel consists of a single transmembrane segment with the amino and carboxyl terminals at the extracellular and intracellular face, respectively (Wilson et al., 1994). This potassium channel consists of a linear sequence of only 130 amino acids and an estimated molecular weight of 15 kilodaltons (Takumi et al., 1988). The channel has 1 hydrophobic sequence and has N-linked glycosylation and phosphorylation sites (Attali et al., 1993). At this time it is not known how many of these individual subunits are required to form a functional channel. Goldstein and Miller (1991) have suggested that a minimum of four monomers are required for an active channel. Blumenthal and Kaczmarek (1994) suggest that single monomers do not conduct current themselves but simply modulate existing membrane channels. This idea is based on the observation that current amplitude does not increase in a linear fashion with increasing protein content.

Mini-$K^+$ channels, cloned from rat kidney cDNA's and expressed in *Xenopus* oocytes, show voltage dependence of activation but fail to show steady state current development even after depolarization pulses lasting as long as three minutes (Ben-Efraim et al., 1994). Inactivation is not apparent even after removal of the depolarizing stimulus (Herzer et al., 1994). Wilson et al. (1994) have mutated the transmembrane sequence of rat kidney $I_{sk}$ over a five amino acid segment, and discovered that substitutions of phenylalanine (F) for cysteine (C) at sites F55C and F58C shifts the current-voltage (I-V) relationship in hyperpolarizing and depolarizing directions respectively. These results suggest that these residues may play an important role in the activation gating of this channel. There appears to be functional and sensitive amino acids at every third position within the monomer thus suggesting that the channel exists in an $\alpha$-helical configuration (Salkoff et al., 1992).

There is some speculation that the mini $K^+$ channel may be responsible for the slow component of the delayed rectifier potassium current (Blumenthal and Kaczmarek, 1992). This
speculation is based on the similarities in the slow time course of activation and the mutual modulation of the two channels by cAMP dependent protein kinase A and protein kinase C (Walsh and Kass, 1988; Huang et al., 1994). Mini-K⁺ channels have been identified, using PCR techniques, in human atria and ventricular myocytes (Hoshi and Zagotta, 1993).

1.1.3 Inward rectifier/ATP sensitive potassium channels

Inward rectifier and ATP-sensitive potassium channels have recently been identified using molecular biology techniques (Morishige et al., 1994; Stanfield et al., 1994) and share a subunit stoichiometry similar to, but not identical with, the shaker channels described above. These channels are also tetramers assembled from monomers which consist of two transmembrane segments separated by a pore forming region (i.e., similar to shaker channels without S1-S4) (Tytgat et al., 1994). Both the pore forming region and the M1 and M2 transmembrane sequences show a remarkable sequence homology to the same segments (S5 and S6) in the voltage gated shaker K⁺ channels (Kerr and Sansom, 1995).

Although the two inward rectifier channels so far identified (IRK1 & ROMK1) are structurally similar, they have functional properties which put them at opposite ends of the spectrum for channels which show inward rectification (Nichols, 1993). ROMK1 channels have an asparagine (N) (uncharged) residue at position 172 whereas the IRK1 channels have an aspartate (D) (negative charge) residue at this position (Stanfield et al., 1994). The negative charge provided by the aspartate (D) group in the IRK1 channels makes them much more susceptible to rectification by internal magnesium ions (Ficker et al., 1994). This result suggests that these channels will be subject to stronger inward rectification due to synergism between voltage and external K⁺ dependent mechanisms, and Mg²⁺ mechanisms (Wible et al., 1994). However the lack of an S4 region in the sequence of both explains the limited voltage dependence of these channels which ensures that the channels are open at negative membrane potentials close to the potassium reversal potential, E_K. The rectification properties of I_k1 and I_kATP channels are
similar and suggests that a high degree of homology exists for sequences which determine the ion conducting components of the channels (Perney and Kaczmarek, 1991). It is clearly evident that alternative splicing of just a few potassium channel genes could produce a large number of \( K^+ \) channels with an enormous capacity for diversity of current production and modulation.

1.2 Diversity and distribution of potassium currents

Physical determination of \( K^+ \) channel structure combined with electrophysiological characterization of channel behavior has allowed scientists to locate specific potassium currents in a wide variety of tissues including brain (Triggle, 1990), skeletal muscle (Davies et al., 1991), heart (Lederer and Nichols, 1989) and pancreas (Ashcroft et al., 1984). Since the development of the patch clamp technique (Hamill et al., 1981) the understanding of the currents carried by these channels in various tissues under physiological and pathophysiological conditions has advanced dramatically. Currents arising from the opening of membrane \( K^+ \) channels are involved in functions as diverse as repolarization of the action potential in excitable cells, volume regulation in neuronal tissue, slow wave activity in smooth muscle, and cellular secretion (Triggle, 1990). Not only does \( K^+ \) channel diversity occur between species and tissues, but also within different regions of the same tissue (Jahnel et al., 1994; Wettwer et al., 1994). In view of complicated nature of \( K^+ \) channel classification within and between tissues and species, the following overview will be restricted to a description of cardiac \( K^+ \) currents. For a comprehensive overview of non-cardiac \( K^+ \) currents please refer to pages 31-33 of the Receptor Nomenclature Supplement (Tips, January 1991).

1.2.1 Myocardial potassium currents

At least six different cardiac \( K^+ \) currents have been described as occurring in wide range of species (Rudy, 1988). Inward rectifier (\( I_{k1} \)), delayed rectifier (\( I_K \)), transient outward (\( I_{to} \))
pacemaker (\(I_f\)) and plateau (\(I_{K_p}\)) \(K^+\) currents are grouped together on the basis of their time and voltage dependent kinetics (Carmeliet, 1989). Cardiac \(K^+\) currents whose activation and inactivation is chemically modulated include sodium and calcium activated \(K^+\) currents (\(I_{K(\text{Na})}\) & \(I_{K(\text{Ca})}\)), acetylcholine and adenosine sensitive \(K^+\) currents (\(I_{K(\text{Ach})}\) & \(I_{K(\text{Ado})}\)) and ATP regulated \(K^+\) currents (\(I_{K(\text{ATP})}\)) (Carmeliet, 1989). The time and voltage dependent currents appear to play a major role in normal repolarization and maintenance of resting potential, whereas chemically sensitive currents appear to be more active during metabolic and pathophysiological processes (Wilde and Janse, 1994; Edwards and Weston, 1994).

1.2.2 Inward rectifier potassium currents

Inward rectifier potassium currents (\(I_{K_1}\)) have been described in atrial and ventricular myocytes from guinea pig, rabbit and cow (Sakmann and Trube, 1984a; Kameyama et al., 1983; Tseng et al., 1987). Single cell recordings from rabbit sino-atrial (S.A.) and atrio-ventricular (A.V.) nodal cells have shown that the \(I_{K_1}\) current is relatively sparse in this tissue and thus may explain the low resting potential seen in these cells (Noma et al., 1984). Although both atria and ventricles have \(I_{K_1}\) currents it has been shown, using single cell techniques, that the steady state \(I-V\) relationships for this current differ between these tissues despite similarities in overall conductance (Hume and Uehara, 1985). \(I_{K_1}\) has also been described in sheep (DiFrancesco, 1984), calf (Carmeliet et al., 1982) and canine Purkinje fibers (Tseng et al., 1987).

1.2.3 Delayed rectifier potassium currents

Delayed rectifier (\(I_K\)) potassium currents, initially labeled \(I_{x1}\) and \(I_{x2}\), were first described in sheep Purkinje fibers by Noble and Tsien (1969). These \(I_K\) currents have also been identified in the ventricle of the same species. It was originally suggested that the ventricular current was completely different from the Purkinje current based on the differences in reversal potential
(McDonald and Trautwein, 1978). However this difference was later ascribed to K+ accumulation in the clefts of multicellular preparations. With the advent of single cell recording techniques the term $I_k$ was adopted to describe a single current (Brown et al., 1980). Nakayama et al. (1984) have investigated $I_k$ in S.A. and A.V. nodal cells from rabbit hearts and found that not only is the current present but it is more prominent than that found in ventricular myocytes. Delayed rectifier currents appear to be similar in the various regions of the heart and have been described in detail in ventricular myocytes of most species (Cohen et al., 1986). $I_k$ appears to be absent in rat hearts (Josephson et al., 1984).

1.2.4 Transient outward potassium currents

Transient outward ($I_{to}$) potassium currents have been known to exist in Purkinje fibers of most species for a long time (Josephson et al., 1984; Siegelbaum and Tsien, 1980). $I_{to}$ has been studied in depth in crista terminalis cells of rabbit hearts (Giles and van Ginneken, 1985). This preparation lacks $I_k$ and has only weak $I_{k1}$ properties and thus allows for a relatively uncontaminated investigation of current behavior. $I_{to}$ has also been described in A.V. nodal cells of the same species but shows marked differences in single channel properties (Trube and Hescheler, 1983; Nakayama et al., 1985). $I_{to}$ has been described in ventricular myocytes from most experimental species (Siegelbaum and Tsien, 1980; Josephson et al., 1984; Coraboeuf and Carmeliet, 1982). $I_{to}$ currents in human atrial cells have been described as being composed of two different components (Escande et al., 1987) and show important electrophysiological differences between subendocardial and epicardial cells (Wettwer et al., 1994; Ravens et al., 1994).

1.2.5 Pacemaker and plateau potassium currents
The potassium current responsible for pacemaker activity (I_p) in nodal cells was originally described in cells isolated from frog sinus venosus (Brown et al., 1977). This hyperpolarization activated current has also been described in some detail in rat, guinea pig and rabbit nodal cells as well as rabbit and calf Purkinje fibers (DiFrancesco, 1980; DiFrancesco, 1985; DiFrancesco, 1981; Seyama, 1976). At present it is unclear whether the current is actually responsible for generation of phase four diastolic depolarization, or if it merely serves a modulatory role for pacemaker activity. The depolarization dependent plateau potassium current (I_{k_p}) was originally described in guinea pig ventricular cells (Yue and Marban, 1988). Reports of potassium currents with properties similar to I_{k_p} have also been presented for cells isolated from rat ventricles (Apkon and Nerbonne, 1988) and isolated chick embryonic heart cells (Mazzanti and DeFelice, 1990). Physical determination of I_{k_p} using molecular biological techniques should help to confirm or deny the presence of this current in the myocardium.

1.2.6 Sodium and calcium sensitive potassium currents

Sodium activated potassium currents have not been characterized in the majority of cardiac tissues from a wide variety of species. Kameyama et al. (1984) and Luk (1990) have demonstrated the presence of such a current in guinea pig ventricular myocytes. Single cell studies in calf and sheep Purkinje fibers have shown activity of potassium currents sensitive to internal calcium concentration (Callewaert et al., 1986; Coraboeuf and Carmeliet, 1982). Calcium-dependent potassium currents have also been documented in guinea pig and human atria (Baro and Escande, 1989; Escande et al., 1987).

1.2.7 Acetylcholine and adenosine sensitive potassium currents

Potassium currents sensitive to acetylcholine and adenosine are found in both S.A. and A.V. nodal cells of rabbit and guinea pig hearts (Noma et al., 1983; Sakmann et al., 1983).
Demonstration of $I_{K(Ach/Ado)}$ channels in these tissues is expected since it is known that these G-protein linked currents are responsible for the vagally mediated bradycardiac actions of acetylcholine and adenosine. Both of these currents are known to share a common intracellular G-protein pathway (Brown, 1990). These currents have also been described in atrial cells from guinea pigs and humans (Iijima and Taira, 1987; Escande et al., 1987) and ventricular cells from rats (Heidbuchel et al., 1989). $I_{K(Ach/Ado)}$ has also been demonstrated in Purkinje fibers from sheep and rabbit hearts (Carmeliet and Ramon, 1980; Mubagwa and Carmeliet, 1983).

1.2.8 ATP sensitive potassium currents

Single cell and single channel studies concerning ATP sensitive potassium currents ($I_{KATP}$) were originally plagued by problems with channel run down under experimental conditions. Despite this early setback researchers have recently been able to characterize this current using bath and pipette solutions which limit or abolish channel run down (Nichols and Lederer, 1991). $I_{KATP}$ was first described from single channel studies of guinea pig ventricular myocytes (Noma, 1983) and has since been demonstrated in cat (Vleugels et al., 1980), rat (Findlay, 1987) and rabbit (Trube et al., 1986) ventricular myocytes. The presence of $I_{KATP}$ had been eluded to much earlier based on the observations that metabolic inhibition and hypoxia both reduced the action potential duration in canine Purkinje fibers (Noble and Tsien, 1969).

1.3 Electrophysiology of potassium currents

1.3.1 Electrophysiological description of potassium currents

Electrophysiological identification and study of potassium currents is made simple in most cases due to the voltage and time-dependence of channel activation and inactivation. Thus most potassium currents under investigation can be separated with appropriate holding potentials and
step depolarization/hyperpolarization protocols (Coraboeuf and Nargeot, 1993). Channel selectivity can be determined from membrane potentials at which current amplitude is zero, referred to as the reversal potential ($E_{rev}$). Thus the closer the reversal potential of a given channel is with respect to the Nernst equation for potassium, sodium and calcium, the more selective the channel is for conducting of either of these ions respectively. Current-voltage relationships (I-V) and voltages for half maximal activation of peak current amplitude ($V_{1/2}$), as determined from the Boltzman equation, are also important for distinguishing differences in currents conducted by various potassium channels (Hille, 1984). Slope conductance derived from the slope of the I-V relationship is also a useful variable for the electrophysiological identification and isolation of biological currents in vitro (Hamill et al., 1981). Voltage dependence and ion selective rectification of ion channel currents may also be useful in describing potassium currents evoked from single channels (Sakmann and Trube, 1984b).

### 1.3.2 Inward rectifier potassium currents

The inward rectifier is rapidly activated and inactivated and passes no outward current at depolarized potentials (i.e., it has a negative slope conductance in the steady state I-V relationship) (Carmeliet, 1982). This channel passes outward $K^+$ current at hyperpolarized potentials positive to $E_K$ and inward $K^+$ current at potentials more negative than $E_K$; however the current is rapidly inactivated at potentials much more positive or negative than $E_K$ (Matsuda et al., 1987). Carmeliet et al. (1987) has shown, using voltage clamped canine Purkinje fibers, that this channel exhibits a region of negative slope conductance at potentials negative to -140 mV thus suggesting that the activation and inactivation kinetics of the current cannot be accounted for by accumulation-depletion of $K^+$ ions alone, but must also involve a gating mechanism. Single channel studies from Purkinje fibers have indicated that the activation of the channel is dependent upon voltage dependent binding of potassium to a site controlling the channel and the external $K^+$ concentration (Carmeliet et al., 1987). Slope conductance in rabbit ventricular myocytes was
found to be 31 pS with a mean open time of 230 msec (Trube, 1988). Hyperpolarization of patch membranes can cause an increase in mean closed time as well as a reduction of both mean open time and the number of opening transitions per unit time (Carmeliet et al., 1987). These results would indicate a voltage dependent gating mechanism for inactivation. Matsuda et al. (1987) & Vandenberg (1987) suggest that rectification of this current involves voltage dependent block of the outward currents by physiological concentrations of intracellular Mg\(^{2+}\). However Carmeliet (1989) has suggested that rectification by Mg\(^{2+}\) and Ca\(^{2+}\) is not voltage dependent and that inactivation proceeds via a voltage and extracellular K\(^{+}\) dependent mechanism involving Na\(^{+}\). The reversal potential of I\(_{K1}\) is very negative (close to E\(_K\)) in most preparations studied thus indicating a high degree of selectivity for K\(^{+}\) (Trube, 1988).

1.3.3 Delayed rectifier potassium currents

The reversal potential for delayed rectifier currents in isolated human atrial myocytes is also very close to E\(_K\) but exhibits no dependence on external [K\(^{+}\)] (Wang et al., 1993). Isenberg et al. (1983) have shown that the reversal potential for the rapidly activating component of I\(_K\) (I\(_{Kr}\)) and the slowly activating component (I\(_{Kr}\)) in guinea pig ventricular myocytes are different. The reversal potential for I\(_{Kr}\) was -94 mV while that for I\(_{Ks}\) was -77 mV. Tail currents elicited from cat right atrial cells, after repolarization from 1 second depolarization steps, also show a reversal potential of -78 mV for (I\(_{Ks}\)) (Zhou and Lipsius, 1994). The rapidly activating component, I\(_{Kr}\), activates with a time constant between 15-25 msec and has an inactivation time constant of approximately 125 msec (Baskin and Lynch, 1994; Wang et al., 1993). Single channel conductance in human atrial myocytes was 17 pS for this component with a current density of 4 pA/pF. The half maximal activation voltage (V1/2) was -6 mV. Kinetic properties and time constants for activation and inactivation are similar in other species and preparations (Trube, 1988). Steady state current in whole cell voltage clamp studies is achieved in approximately 125 msec (Baskin and Lynch, 1994). Single channel conductance for I\(_{Kr}\) is large in most preparations.
and is usually 10 times that of $I_{Ks}$ (Sanguinetti and Jurkiewicz, 1990; Katritsis and Camm, 1993). Steady state current for the slowly activating component occurs within 1-6 seconds and exhibits a decay time constant of 750 msec (Baskin and Lynch, 1994; Katritsis and Camm, 1993). Unlike $I_{Ks}$, the time constants for activation and inactivation of $I_{Ks}$ are best fit by 2-3 exponentials thus suggesting multiple open and closed states prior to channel opening and closing (Sanguinetti and Jurkiewicz, 1990). In guinea pig ventricular myocytes $I_{Ks}$ is activated only at potentials positive to 0 mV (Trube, 1988).

1.3.4 Transient outward potassium currents

The transient outward potassium current is composed of rapidly ($I_{Tof}$) and slowly ($I_{Tos}$) inactivating components. In rat ventricular myocytes $I_{Tof}$ current shows an inactivation time constant of 35 msec with a $V_{1/2}$ of -58 mV. $I_{Tos}$, on the other hand, is described by an inactivation time constant of 1.5-2 seconds with a $V_{1/2}$ of -88 mV (Weis et al., 1993). These results suggest that there is a significant difference in the voltage dependence of inactivation between the two components of this current in this preparation. Whole cell voltage clamp experiments with human and rabbit atrial myocytes have also shown a species difference in that the rapid component of $I_{To}$ appears to be rate independent in humans but not in rabbits (Ravens et al., 1994). This same group also demonstrated a bi-exponential process for current inactivation in rabbit as opposed to a mono-exponential inactivation in humans. Reversal potential for both $I_{Tof}$ and $I_{Tos}$, calculated from tail currents in human atrial myocytes, is approximately -60 mV thus indicating that this current is not as selective for $K^+$ as other $K^+$ currents (Escande et al., 1987). In human ventricular myocytes the current density of $I_{Tos}$ varies considerably between the subepicardium (SEPC) where it is 7.9 pA/pF and the subendocardium (SEDC) where it is 2.3 pA/pF (Wettwer et al., 1994). The $V_{1/2}$ for activation and recovery from inactivation also vary between SEPC and SEDC in this preparation. $V_{1/2}$ for activation was shown to be +26 mV for cells in the endocardium and +9 mV for cells in the epicardium. SEPC recovery from inactivation was best fit with a single exponential
component with a time constant of 24 msec whereas inactivation in the SEDC was best fit by two exponential components with time constants of 25 msec and 328 msec respectively (Wettwer et al., 1994). Inactivation of the slow component was incomplete after 6 seconds. In ferret ventricular myocytes $I_{t0}$ is described by a single component rapid activation process which varies between 5-15 msec (Campbell et al., 1993). Like $I_{t0}$ in other species activation was voltage-dependent but was independent of Na$^+$ or Ca$^{2+}$. V1/2 for $I_{t0}$ in this preparation was shown to be $-13.5$ mV. In contrast to $I_{t0}$ described in other cells, this current was shown to have an inactivation process which developed and recovered in a mono-exponential fashion.

1.3.5 Pacemaker and plateau potassium currents

Single channel studies of the pacemaker K$^+$ current in rabbit S.A. nodal cells show a single channel conductance of only 1 pS and a reversal potential of $-48$ mV (Ho et al., 1994). The time courses for activation and inactivation of this current have a sigmoidal character and are best fit by Hodgkin-Huxley models involving multiple open and closed states (DiFrancesco, 1984). Activation of this current is slow at potentials more negative than $-80$ mV with peak current developing slowly and reaching a maximum 2 seconds after step depolarization (Earm et al., 1983). The small conductance and slow time course of activation may preclude this current from having any effect on diastolic depolarization under normal conditions. However the sensitivity of this current to noradrenaline may implicate this current as a modulator of spontaneous depolarization in nodal cells (DiFrancesco, 1985). The plateau K$^+$ current is difficult to demonstrate in single channel or whole cell studies due to contamination by larger currents. However, Yue and Marban (1988) have demonstrated an Ohmic I-V relationship with no rectification for this current in rabbit ventricular cells. Kakei et al. (1986) have also demonstrated a lack of inward rectification in guinea pig ventricular myocytes.

1.3.6 Sodium and calcium sensitive potassium currents
Large conductance sodium activated $K^+$ currents have been demonstrated in a limited number of different preparations. For example, in human ventricle, activation of $I_{K(Na)}$ by increasing intracellular $Na^+$ (> 20 mM) causes a large slope conductance of 207 pS (Kameyama et al., 1984). In guinea pig ventricular myocytes a slope conductance of 150 pS is observed with the open channel probability ($P_o$) increasing sigmoidally with increasing intracellular $Na^+$ (Sanguinetti, 1990). This channel shows a marked lack of voltage dependence at potentials positive to -80 mV. Inward rectification of this channel has been demonstrated in the presence of $Na^+$ and $Mg^{2+}$ ions. A weak rectification of this current has also been found with $Ba^{2+}$. It is interesting to note that $Li^+$ ions which are often used as an $Na^+$ substitute in electrophysiological studies can not be used for the activation of $I_{K(Na)}$ (Dryer, 1994).

Calcium activated $K^+$ currents in guinea pig atria and calf Purkinje cells show linear I-V relationships with single channel conductance of 120 pS. In both of these preparations the activation time course is rapid (10-20 msec) whereas the inactivation time course is relatively slow (30-100 msec) (Callewaert et al., 1986).

1.3.7 Acetylcholine and adenosine sensitive potassium currents

Acetylcholine and adenosine sensitive $K^+$ currents in rabbit atrial cells are small conductance currents, compared to ATP-sensitive currents, which are very dependent on raised external $[K^+]$ for activation (Soejima and Noma, 1984). A single channel conductance of 13 pS and rapid gating kinetics can be used to distinguish this channel from the electrophysiologically similar inward rectifier current. Inward rectification resulting from large depolarizations is voltage dependent and may be the result of block of outward currents by intracellular $Mg^{2+}$ and/or $Na^+$ (Noma et al., 1984). Reversal potentials similar to that of $I_{K1}$ have also been demonstrated and suggest a high degree of selectivity for potassium ions for these currents.
1.3.8 ATP sensitive potassium currents

The ATP-sensitive potassium current like the acetylcholine and adenosine sensitive currents is very dependent on external K+ for activation. When the external potassium ion concentration is varied between 150 mM to 5mM, single channel conductance can vary 4-5 times in magnitude (Kakei et al., 1985). Single channel studies in isolated heart cells suggest an activation/inactivation model consistent with one open state and one short and one long closed state (Trube and Hescheler, 1983). In the region of the reversal potential for this current the inactivation time constant for open channels is maximal and that for closed channels is minimal suggesting that the open probability of this channel is decreased upon hyperpolarization and may not contribute to maintenance of the resting potential (Kakei et al., 1985; Trube and Hescheler, 1983).

1.4 Pharmacology of potassium currents

1.4.1 Pharmacological description of potassium channels

Pharmacological manipulation of cardiac potassium currents can be confusing as a result of the large number of chemically dissimilar compounds which affect them. Substances originally investigated for their ability to modify potassium currents were bivalent ions such as barium and cesium (Ehara et al., 1980; Isenberg, 1976) and several quaternary ammonium compounds including 4-aminopyridine (4-AP), tetraethyl ammonium (TEA) and tetramethyl ammonium (Coraboeuf and Vasort, 1968). However the actions of these compounds are rather non-specific and their use as agents for the treatment of a variety disorders involving potassium channel modulation is limited compared to newer selective agents. A number of toxic proteins derived from natural sources have also been invaluable in the elucidation of structure and function of specific potassium currents. These include Gaboon viper venom, α-dendrotoxin and apamin
(Triggle, 1990; Koumi et al., 1994). Unlike the aminopyridines and ammonium compounds these agents are very selective for potassium channels and are toxic at very low doses as a result of their affinity for these channels.

1.4.2 Inward rectifier potassium currents

Pharmacological blockade of $I_{k1}$ in various preparations has been demonstrated with TEA and 4-AP (Arena et al., 1990), class 1 antiarrhythmic agents (Martin et al., 1994), viper venom, and a wide variety of monovalent and divalent cations (dv2). $I_{k1}$ is very sensitive to a group of compounds related to RP 62,719, a benzopyran structure (Beregi et al., 1994; Rees and Curtis, 1993b). Tertiary analogs of a potent delayed rectifier blocker, clofilium, are also potent $I_{k1}$ blockers (Arena and Kass, 1988). At present it is not clear whether or not $I_{k1}$ is subjected to intracellular regulation in a manner similar to $I_k$. However recent evidence concerning the inhibition of $I_{k1}$ in endothelial cells by endothelin-1, and negative chronotropic effects of the same compound on guinea pig atrial myocytes, suggests that endothelin may inhibit this current through stimulation of intracellular G-proteins (He et al, 1994; Ono et al., 1994). Fakler et al. (1994) have shown independent regulation of this current by protein kinases and ATP. Whether or not this type of regulation resembles that observed for $I_k$ remains to be determined.

1.4.3 Delayed rectifier potassium currents

Pharmacological blockade of $I_k$ has been demonstrated for a wide variety of compounds including angiotensin converting enzyme inhibitors (Rake et al., 1994), antihistamines (Rampe et al., 1993; Salata et al., 1995), toad venom (Cruz and Matsuda, 1994), class I and class IV antiarrhythmic agents (Benz and Kohlhardt, 1994; Follmer et al., 1992b), TEA and 4-AP (Benz and Kohlhardt, 1994), activated protein kinases (Walsh and Kass, 1988; Huang et al., 1994), clofilium (Rees and Curtis, 1993a; Yool 1994), 5-HT (Zhang et al., 1994), angiotensin II and
triametrene (Daleau and Turgeon, 1994a; Daleau and Turgeon, 1994b) and a variety of monovalent and divalent cations (Follmer et al., 1992a; Huang et al., 1994). As mentioned above the cardiac delayed rectifier current can be pharmacologically separated into two components. The rapidly activating component of $I_k$ is selectively inhibited by E4031, dofetilide and sematilide (Martin and Chinn, 1992) whereas the slowly activating component is inhibited by NE10064 and amiodarone (Busch et al., 1994; Balser et al., 1991; Sanguinetti and Jurkiewicz, 1990). All of the selective blockers can block their respective components at micromolar concentrations in vitro.

1.4.4 Transient outward potassium currents

$I_{to}$ is inhibited by 4-AP and by a variety of class I and class IV antiarrhythmic agents (Jahnel et al., 1994; Berger et al., 1991). Inhibition of the rapidly inactivating component of $I_{to}$ is achieved with tedisamil whereas the, tedisamil insensitive, slowly inactivating component is inhibited by caffeine (Zygmunt, 1994). Anoxia decreases both components of $I_{to}$ in mouse ventricular cells (Thierfelder et al., 1994). Hypertrophy of rat ventricular myocytes also causes a reduction in the rapidly inactivating component whereas the slowly inactivating component remains unaffected (Coulombe et al., 1994).

1.4.5 Pacemaker and plateau potassium currents

Pharmacological manipulation of $I_f$ has not been studied in detail but Yanagihara and Irisawa (1980) and Ho et al. (1994) have shown that this current is sensitive to a number of divalent cations. More recently DiFrancesco (1994) has described the selective blockade of the pacemaker current in rabbit S.A. node cells by UL-FS 49. The conditions of $I_f$ block by UL-FS 49, a substituted benzazepine, were such that a model describing the action of this compounds was one of an open channel block occurring from the cytoplasmic side of the myocyte membrane. An interesting aspect of $I_f$ block produced by this compound was the relief of block when the cell
was hyperpolarized. Since this channel is activated upon hyperpolarization relief of block upon hyperpolarization would give such compounds a bizarre form of frequency-dependent action. DiFrancesco and Mangoni (1994) have also described a modulatory role for cAMP on this current. Chang et al (1994) demonstrated an inhibitory effect of neuropeptide Y, and a stimulatory effect of vasoactive intestinal peptide, on this current in canine Purkinje fibers. Specific modulation of plateau potassium currents has not been described.

1.4.6 Sodium and calcium sensitive potassium currents

\( I_{K(Na)} \) is inhibited by 4-AP and TEA (Kameyama et al., 1984) and can also be reduced through reduction of \( Na^+ \) entry into cells by using compounds such as tetrodotoxin (Bader et al., 1985). In contrast to \( I_{K(Na)} \), \( I_{K(Ca)} \) is not blocked by TEA but is susceptible to block by 4-AP and quinine (TiPS, January, 1991). \( I_{K(Ca)} \) is also blocked by kaliotoxin, a peptide toxin isolated from scorpions of the genus \textit{Androctonus} (Fernandez et al., 1994). Wisgirda and Dryer (1994) have demonstrated a functional and spatial dependence of \( I_{K(Ca)} \) on L-type \( Ca^{2+} \) currents in a non-cardiac \textit{in vitro} model involving chick neurons. It has not been determined if the same relationship is present in cardiac cells.

1.4.7 Acetylcholine and adenosine sensitive potassium currents

These currents are sensitive to agents which antagonize the effects of acetylcholine and adenosine at their respective receptors (Belardinelli and Isenberg., 1983) and also to agents, such as pertussis toxin and non-hydrolysable GTP analogues, which disrupt the function of the commonly used intracellular G-protein signal (Brown et al., 1991; Kirsch et al., 1990).

1.4.8 ATP sensitive potassium currents
$I_{\text{KATP}}$ is potently inhibited by the sulfonylurea drugs such as tolbutamide and glybenclamide (Kakei et al., 1985), amide local anaesthetics (Stanfield and Standen, 1987) and weakly by TEA (Castle and Haylett, 1987). $I_{\text{KATP}}$ can be activated by a number of compounds from seven distinct chemical families of potassium channel openers (i.e. benzopyrans (cromakalim) and nicotinamides (nicorandil), see Quast (1992) for a complete description of chemical structures).

1.5 Potassium currents and myocardial repolarization

1.5.1 Action potential morphology

The physiological role of $K^+$ currents in the heart is of particular importance due to the special electrical requirements imposed upon cardiac cells. The electrical properties of the heart must change with changes in rate, rhythm and sympathetic tone (Weirich et al., 1992). Action potential morphology in any given tissue is the result of the net balance between inward and outward currents (Kass et al., 1990). Therefore the action potentials of different tissues will have different shapes depending on the diversity and density of inward and outward currents. Cells of the S.A. and A.V. node and the ventricle are characterized by a relatively long action potential duration, and hence a slow terminal repolarization, with a very positive plateau potential (Carmeliet, 1993a). In contrast the cells of atrial fibers have an abbreviated plateau and a more rapid repolarization phase. Purkinje fibers, like ventricular fibers, have a prolonged plateau phase that exists at more negative potentials and like atrial fibers have a rapid initial repolarization phase. There are also key differences in action potential morphology between epicardial and endocardial cells of the same tissue. Epicardial cells are characterized by a prominent "spike and dome" component of early repolarization which is not as prominent in endocardial cells. Repolarization of the cardiac action potential is often divided into three phases with phases 1, 2 and 3 corresponding to a rapid initial, voltage and time-dependent plateau and terminal repolarization, respectively (Vinet and Roberge, 1994). In a simplistic sense the initiation of
repolarization can be thought of as the time and voltage dependent decrease and increase of \( g_{Ca^{2+}} \) and \( g_{K^+} \) respectively. However when considering repolarization on a tissue to tissue basis the role of individual K\(^+\) currents becomes important.

1.5.2 Inward rectifier potassium currents

The potassium current evoked by activation of the inward rectifier potassium channel is responsible for maintenance of the resting membrane potential and for repolarization of the terminal portion of the action potential (late phase 3) (Ibarra et al., 1991). The negative slope conductance and inward rectifying properties of this potassium current have important implications for action potential configuration. The lack of outward conductance at very depolarized potentials allows for a prolonged depolarization in cells which express a high density of this current relative to delayed rectifier current (Carmeliet, 1993b). On the other hand the large inward component of this current at potentials negative to \( E_K \) may prevent excessive repolarization associated with \( Na^+-K^+ \) electrogenic pump activity at the end of action potential repolarization. The slight outward conductance at potentials just positive to \( E_K \) is essential for the maintenance of the resting potential in cells which do not have a potential dependent phase 4 diastolic depolarization (Ibarra et al., 1991).

1.5.3 Delayed rectifier potassium currents

The delayed rectifier potassium currents are activated upon depolarization, after a brief delay, and are largely responsible for initiating repolarization at the end of the plateau of the action potential (phase 2). Although the inward currents responsible for the depolarization of various cardiac tissue may vary, the initiation of repolarization in most cardiac tissue is carried out by the delayed rectifier current (Morad and Tung, 1982). The dual component nature and time and voltage dependent inactivation properties of this current are of particular importance in this
respect. Based on the relative sizes of the two components of this current, one might expect that the rapidly activating component would have little to do with the repolarization when compared to the slowly activating component. However, the differences in the time course of the activation of each component means that by the end of the plateau phase of the action potential the relative contribution of each component to overall repolarization is nearly the same (Sanguinetti and Jurkiewicz, 1990; Katritsis and Camm, 1993). The time- and potential-dependent inactivation of this current has important implications during high rates of stimulation. $I_k$ currents activated during depolarization of the previous pulse in canine ventricular cells inactivate only upon full repolarization (Carmeliet, 1989). Therefore at high rates of stimulation inactivation becomes incomplete leading to an accumulation of $I_k$ and an abbreviation of the action potential duration. Modulation of this current by $\beta$-adrenoceptor activation and internal $Ca^{2+}$ (Heath and Terrar, 1994), whether by direct actions or secondary phosphorylation reactions, may have important consequences in the repolarization of nodal cells during periods of increased sympathetic tone (Carmeliet, 1993a).

1.5.4 Transient outward potassium currents

The transient outward potassium current is largely responsible for the initial rapid repolarization during phase 1 of the cardiac action potential in atrial, ventricular and Purkinje fibers (Antzelevitch et al., 1990). The rapid activation of this current during phase 0 depolarization is followed by a voltage dependent inactivation. The fact that current inactivation is a voltage-dependent process may underlie this channels control of action potential configuration during changes in rate and rhythm (i.e., inactivation develops quickly and decays slowly) (Carmeliet, 1977; Ruiz-Petrich and Leblanc, 1989). In canine Purkinje fibers extra stimuli applied during the period when $I_{to}$ has not completely recovered from inactivation produces an extrasystole with a less prominent spike and a more prominent plateau (Carmeliet, 1993b). The consequences of this is an enhanced inactivation of slow inward $Ca^{2+}$ current and increased
activation of delayed rectifier outward currents to produce an abbreviation of action potential duration. The greater relative density of $I_{to}$ in the epicardium compared to the endocardium also has important implications in rate dependent changes in action potential duration between these two tissues. In canine ventricular fibers the differential effects of steady-state stimulation rate have been documented in epicardial and endocardial preparations (Carmeliet, 1977). At increased cycle lengths the changes in total duration of the action potential are enhanced more in epicardial cells and are related to changes in the early phase of repolarization carried out by $I_{to}$. These differences between epicardial and endocardial cells may also be manifest in the electrocardiogram as changes in T-wave form and polarity (Hiraoka and Kawano, 1987).

1.5.5 Pacemaker and plateau potassium currents

Activation of $I_f$ by β-adrenergic stimulation results in an increase in inward current during phase four depolarization in nodal and Purkinje fibers, thus increasing cardiac rate. Acetylcholine has been shown to antagonize the actions of β-adrenergic stimulation on $I_f$ (DiFrancesco et al., 1988) thus suggesting that this current may play an important role in the modulation of action potential morphology in conditions when the balance of autonomic tone has shifted in favor of one or the other divisions (DiFrancesco and Tortora, 1991). Since the S.A. node is normally under parasympathetic tone one would expect that this current would have little to do with normal resting action potential morphology. Furthermore, the slow time course of activation of this current, in the absence of β-stimulation, in vitro suggests that the activity of this channel only becomes apparent in action potentials at high heart rates (DiFrancesco, 1994). Plateau potassium currents offer slight resistance to the depolarizing Na$^+$ and Ca$^{2+}$ currents during the plateau phase of the action potential. Thus this current plays an important role in maintaining the action potential plateau within a voltage range that will allow for both inactivation of inward currents and activation of outward currents at the end of the action potential plateau (Carmeliet, 1993b).
1.5.6 Sodium and calcium sensitive potassium currents

Under normal conditions $I_{K(Na)}$ and $I_{K(Ca)}$ do not contribute significantly to the repolarization of the action potential. This is largely the result of dependence of these currents on intracellular concentrations of $Na^+$ and $Ca^{2+}$ which are not maintained during normal cardiac function, and the slow time course involved in channel inactivation. A problem which is often encountered in trying to determine the role of these channels in repolarization is their sensitivity to changes in $Na^+/K^+$ and $Na^+/Ca^{2+}$ pump function as well as changes in intracellular pH (Veldkamp et al., 1994).

1.5.7 Acetylcholine and adenosine sensitive potassium currents

The presence of these currents within S.A. node and atria means that activation of either will result in differential effects depending upon the tissue under investigation. Activation of these currents in the S.A. node will tend to cause a hyperpolarization of the nodal myocyte membranes thus decreasing the slope of phase four depolarization and causing a slowing of sinus rate (Narula et al., 1971). However, in the atria, spontaneous diastolic depolarization does not take place and the main effect of activation of these currents in this tissue will cause a shortening of action potential duration without the strong hyperpolarizing effect (Engelstein et al., 1994). Therefore activation of one or both of these currents should result in a bradycardia with increased intra-atrial conduction velocity.

1.5.8 ATP sensitive potassium currents

Under physiological conditions the ATP/ADP ratio in cardiac cells is sufficiently high enough to enable ATP-dependent block of $I_{KATP}$ (Wilde and Janse, 1994). Thus under normal
conditions $I_{KATP}$ has little effect on the repolarization of the action potential and drugs which block $I_{KATP}$ have little effect on action potential configuration (de Weille, 1992). The effects of sulphonylureas on spontaneous rate in isolated Purkinje fiber preparations is thought to be the result of non-specific actions not related to $I_{KATP}$ channel blockade (Edwards and Weston, 1994). Drugs which open ATP sensitive $K^+$ channels, such as cromakalim and pinacidil, can shorten the action potential under normal conditions. However this effect is diminished when $K^+$ conductance is maximized as a result of activation of other $K^+$ currents or under conditions of increased extracellular $K^+$ (Wilde and Janse, 1994).

1.6 Potassium current modulators as antiarrhythmics

1.6.1 Potassium currents as targets for antiarrhythmic agents

A direct physiological consequence of the selective inhibition of one or more of these potassium currents is lengthening of the action potential duration and increasing cellular refractoriness to excitation by delaying the voltage dependent recovery of Na$^+$ channels (Hondeghem and Snyders, 1990). If an increase in refractoriness is the ultimate beneficial aspect of the class III agent then blockade of one or more of the inward rectifier, delayed rectifier and/or transient outward potassium currents would be expected to be a useful antiarrhythmic action (Roden et al., 1985; Tamkun, 1994).

Blockade of the inward rectifier would be expected to delay the rapid repolarization of the terminal portion of the action potential thus slowing the recovery from inactivation of myocardial sodium currents (Janse, 1992). If recovery of sodium channel availability is required for maintenance of re-entrant arrhythmias (Singh and Courtney, 1990; Rees and Curtis, 1994) then in this setting block of $I_{K1}$ would be useful. However excessive block of $I_{K1}$ may lead to proarrhythmia (Opthof, 1994). This idea stems from the fact that this channel controls terminal repolarization and resting membrane potential and depolarization with prolongation of action
potential duration would be expected to favor the development of early and/or late afterdepolarizations (Hondeghem, 1992).

Blockade of the delayed rectifier current would also be expected to prolong the action potential by decreasing the initial phase of repolarization at the end of the plateau phase (Borchard et al., 1989). This effect might consist of two components, the first being a prolongation of the active plateau inward currents and the second due to an increased time to activation of $I_{K1}$ at the terminal phase of the action potential. Prolongation of action potential duration by this means would be expected to terminate reentry arrhythmias by reducing the diastolic window available for reentrant excitation (Hondeghem, 1991). However excessive inhibition of the delayed rectifier may lead to early afterdepolarizations associated with the recycling of time-dependent inward plateau currents, and thus may be arrhythmogenic (Tsuchihashi et al., 1991; O'rourke et al., 1994).

Blockade of the transient outward current would also be expected to confer antiarrhythmic protection through lack of reduction of positive plateau potentials and delay in the inactivation and activation of inward and outward currents respectively, thus producing a prolonged action potential (Adaikan et al., 1992; Hondeghem, 1992). However the temporal relationship of this current with respect to the delayed rectifier means that excessive inhibition of this current would lead to membrane potentials that favor the enhanced activation of $I_{Ks}$. Thus in cardiac cells having a strong delayed rectifier component, action potential duration may in fact be reduced as a result of the blockade of the transient outward current (Carmeliet, 1989; Hondeghem, 1994).

Potassium channels activated under pathophysiological conditions would be an important target for the development of antiarrhythmic agents. However as yet there is little evidence for specific modulators of these currents and it is not known what, if any, beneficial antiarrhythmic effects would result from modulation of such potassium currents. Specific blockers of the pacemaker current would be useful in prolonging nodal action potential duration and hence limiting reentry arrhythmias involving the S.A. node (Hondeghem, 1994). Inhibition of the
plateau potassium conductance would be expected to increase the action potential duration in those tissues which have prominent positive plateaus. Due to the brief nature of the plateau in atrial cells one would expect that $I_{k_p}$ block would be of greater benefit in suppressing ventricular arrhythmias. Based on the differential effects of adenosine in nodal and atrial tissue one would expect that the activation of these currents would be of benefit in nodal reentry but not for other supraventricular arrhythmias (Engelstein et al., 1994). Blockade of $I_{k_{ATP}}$ currents would be expected to antagonize the action potential shortening that is observed during episodes of ischaemia. This action may confer antiarrhythmic protection in cells which are sensitive to the effects of decreased ATP during ischaemia. In the case of $I_{k_{ATP}}$ activation the end result is a shortening of action potential duration whereas refractoriness may not be altered (Edwards and Weston, 1994; Quast, 1992). Activation of these currents may provide antiarrhythmic protection by subjecting tissue at risk to a period of ischaemic preconditioning, a phenomenon which is antiarrhythmic (Murray et al., 1986; Cole et al., 1991).

1.6.2 Potassium channel openers vs. potassium channel blockers

Alteration of the cardiac action potential and refractoriness has become a very important area for research in the development of new antiarrhythmic agents. More recently it has been suggested that the activation of potassium currents, rather than blockade, would confer beneficial antiarrhythmic activity (Escande and Cavero, 1992; Tosaki et al., 1993). This hypothesis is based on the idea that the shortening of the action potential, in response to ischaemia, is an inborn protective measure and that chemical simulation of these conditions would be protective. Proponents of potassium channel opening as an antiarrhythmic principle suggest that rapid hyperpolarization of the membrane will protect against the triggered activity that may be proarrhythmic in the setting of ischaemia (Yao and Gross, 1994; Bril, 1994). D'Alonzo and Grover (1994) suggest that potassium channel openers will have beneficial or harmful effects depending on the arrhythmogenic stimulus, but these comments are speculative in nature. In
experimental situations the drug pinacidil can induce ventricular fibrillation that is antagonized by the potassium blocker glibenclamide (Black and Lucchesi, 1994). Some researchers even use potassium channel opening drugs to induce arrhythmias in attempts to study the antiarrhythmic potential of other potential class III agents (Spinelli and Colatsky, 1994). There is still a great deal of controversy as to the beneficial effects of potassium channel openers in the treatment of arrhythmias (Black et al., 1993). Compounds which act by blockade of potassium channels on the other hand have been reported to be successful in reducing or eliminating arrhythmias in a number of experimental and clinical situations (Colatsky et al., 1990). Furthermore, beneficial antiarrhythmic activity may be produced by specific blockade of only one of a number of cardiac potassium currents (Sanguinetti, 1992). However there is also a risk of deleterious actions with the use of these compounds which may stem from their specific chemistry and actions (see below).

1.6.3 Class III antiarrhythmic agents

Increasing action potential duration and refractoriness as a means to combat arrhythmias was first introduced by Vaughan Williams in 1969 and was given the designation "class III" in his original antiarrhythmic classification system (Singh and Vaughan Williams, 1970). This theory was based on observations concerning the incidence of mortality from sudden cardiac death in patients with hypothyroidism and thyrotoxicosis (Freeberg et al., 1969). The first agents to be studied for class III activity were amiodarone and racemic sotalol. These agents have proven efficacy in the treatment of life threatening arrhythmias which is largely attributed to APD prolongation (Nademanee, 1992; Taggart et al., 1985; Nademanee et al., 1985). Johnston et al. (1985) showed that both (±) sotalol and (+) sotalol dose dependently increased the Q-Tc interval in healthy male volunteers and that the repolarization effects were completely independent of β adrenoceptor activity. Amiodarone also produces a dose dependent increase in the Q-Tc interval of the ECG which is well correlated with termination of clinical arrhythmias (Roden and Woosley,
1985; Vaughan Williams, 1985). However the exact mechanism which produces this beneficial effect is still a source for debate since the action potential takes shape as a result of the net movement of charge during the action potential, and both of these compounds have a number of different actions on one or more of the currents involved in the generation of the action potential (Lynch et al., 1984; Singh et al., 1987; Sager et al., 1993). Just as the acute beneficial effects of these compounds may be the result of class III action so may the detrimental effects. Excessive Q-T prolongation caused by both compounds has been associated with an increased incidence of tacharrhythmias even though the incidence of Torsade des Pointes, a morphologically distinct ventricular arrhythmia, is not increased in patients receiving amiodarone (Cobbe, 1988; Laakso et al., 1981). This may be due to the fact that amiodarone has multiple ion channel blocking properties and retains its class III actions to a greater extent than sotalol in ischaemic myocardium (Cobbe, 1988).

1.6.4 Novel Class III antiarrhythmic agents

The search for novel class III antiarrhythmic agents (Diagram 1) lacking the non-specific actions of amiodarone and sotalol is an area of intense investigation. A number of the newer class III agents are chemical variants of the methanesulfonamide structure of sotalol. Sematilide, E4031 and ibutilide are such derivatives which are currently under investigation in pre-clinical and clinical settings (Sager et al., 1994; Fujiki et al., 1994). Almokalant is a new class III agent which lacks the methanesulfonamide moiety that is characteristic of the sotalol derivatives (Ohler and Ravens, 1994). Sematilide and almokalant produce class III actions as a result of selective inhibition of the rapidly activating component of the delayed rectifier current (Carmeliet, 1991). Class III actions of E4031 are also believed to result from inhibition of this component of the delayed rectifier as well (Hiraoka et al., 1994). Although ibutilide is structurally similar to both sematilide and E4031 it is believed to have class III actions as a result of activation of a slow inward Na\(^+\) current during the plateau phase of the cardiac action potential (Lee et al., 1993;
Wesley et al., 1993). In rabbits and dogs, all of these compounds have demonstrated protection against arrhythmias due to electrical stimulation in the presence of a previous infarct but have not been able to suppress the generation of spontaneous rhythm disorders (Buchanan et al., 1993a; Hiraoka et al., 1994; Singh and Courtney, 1990). At present, there is no conclusive evidence which suggests that these compounds have protective activity in animal models of ischaemia induced arrhythmias (Lad and Mackenzie, 1991; Nemeth and Papp, 1992).

1.6.5 Limitations in the use of class III antiarrhythmics

A common problem with the newer class III agents as a whole is reverse use-dependence of action (Hafner et al., 1988; Hondeghem and Snyders, 1990). This action limits class III effects at high heart rates (most often associated with life threatening tacharrhythmias) and may precipitate Torsades de Pointes at slow heart rates and/or after long diastolic pauses (commonly occurring in patients with decreased myocardial function or sick sinus syndrome) (Sanguinetti, 1992; Singh, 1993). Sematilide and E4031 are believed to exhibit reverse use-dependence in their class III action whereas ibutilide apparently does not. Demonstration of negative rate dependence is often limited to studies in vitro and the mechanisms involved in both in vitro and in vivo settings are not clearly understood. The antiarrhythmic and arrhythmogenic actions of these new class III agents are extremely similar despite proposed differences in mechanism of action and further study, both in vitro and in vivo, is warranted.

1.7 Aims of studies

1.7.1 Overall aim

We felt it was important to study these sotalol derivatives in a variety of species in an attempt to demonstrate the proposed differences in mechanism in terms of differential effects on
various cardiovascular variables. To further define the pharmacological actions of some new class III antiarrhythmics studies were undertaken which included the following:

1.7.2 Studies in primates *in vivo*

These studies were used a) to assess the *in vivo* electrophysiological and toxic actions of sematilide and almokalant (putative class III antiarrhythmic agents) in a species similar to man and b) to assess the cardiovascular and toxic actions of ibutilide and E4031, compounds structurally related to sematilide, in a primate model not involving electrophysiological testing.

1.7.3 Studies in small animals *in vivo*

These studies were used a) to document the cardiovascular actions of ibutilide and E4031, over a large dose range, in a species lacking $I_k$ (rat) in an attempt to distinguish the proposed differences in their mechanisms of action *in vivo* and b) to characterize the cardiovascular effects of ibutilide and E4031 in a species having mostly $I_k$ and very little $I_{to}$ (guinea pigs). In addition, an attempt was made, in this species to determine whether or not either of these compounds demonstrated reverse use dependence using a novel *in vivo* preparation.

1.7.4 Studies *in vitro*

The aim of the *in vitro* studies was to determine whether or not the proposed electrophysiological differences of ibutilide and E4031 could be distinguished by their actions on a nerve/smooth muscle preparation.

2 METHODS
Group comparisons for tests of significance were made using repeated measures ANOVA at $\alpha=0.05$ and Duncan's test for multiple comparisons. Pairwise comparisons were made using a students t-test at $\alpha=0.05$.

2.1 Studies in primates.

2.1.1 Sematilide and almokalant

Male and female baboons (Papio sp. 15-25 kg) were sedated with ketamine (15 mg/kg, i.m.) and anaesthetized with halothane (1 ± .5 %, n=6). Blood pressure was recorded from a transcutaneous catheter implanted in the right femoral artery. ECG was recorded on both a computer and Gould chart recorder (model TA 2200) from electrodes in a lead II configuration. Drugs were administered as cumulative i.v. doses through the internal jugular vein or femoral vein. Monophasic action potential duration at 75 % repolarization (MAPD75) was recorded at various cycle lengths from an Ag/AgCl pacing/recording electrode (6 French, 100 cm EP technologies Inc.) implanted in the right ventricle. Ventricular effective refractory periods (VERP) were also determined at the various cycle lengths by measuring the pause necessary for conduction of an extrastimulus after eight preceding stimuli at the given rate under investigation. Ventricular accommodation and restitution were determined from multiple step pacing at various cycle lengths for 1 minute. Action potential duration of a single extra stimulus interposed during periods of steady state pacing were used to construct restitution curves. A plot of the linear regression of APD75 on log cycle length was then used to calculate accommodation and restitution values for comparison with increasing doses of sematilide. Serum drug levels were measured from plasma samples by S Laganiere at the University of Ottawa Heart Health Institute, Ottawa, Ontario, using high performance liquid chromatography analysis. If Torsades des Pointes occurred and persisted for more than twenty seconds overdrive pacing of the right ventricle was initiated in an attempt to suppress the arrhythmia. Cardioversion was initiated in cases when
Torsades des Pointes was refractory to overdrive pacing. All primates were monitored at the end of experiments until full recovery from anaesthetic was observed.

2.1.2 Ibutilide and E4031

Male and female longtail and pigtail monkeys (n=3) were sedated with ketamine (15 mg/kg, i.m.) and anesthetized with pentobarbitone (15 mg/kg, i.v.). All animals were intubated with endotracheal tubes of the appropriate size. Pentobarbitone and test compounds were administered through a 21 gauge butterfly catheter inserted into a superficial forearm vein. Pentobarbitone was administered as i.v. injections (2-5 mg/kg) until a final cumulative dose of 30-45 mg/kg was achieved. ECG variables were obtained from external limb leads in a Lead II configuration and recorded on paper traces from a Gould (TA 2200) chart recorder. ECG signals were also monitored on an oscilloscope. Blood pressure was recorded from a transcutaneous catheterization of the right femoral artery. Temperature was maintained above 36°C with the use of a coupled rectal probe and electric heating blanket. Ibutilide and E4031 were given as i.v. bolus doses at 15 minute intervals. Trace recordings were taken at 0.5, 1, 2, 5, 10, and 15 minutes after each dose. Drug administration was halted if arrhythmias occurred. All primates were monitored at the end of experiments until full recovery from anesthetic was observed.

2.2 Dose response studies in guinea pigs and rats.

2.2.1 Studies in guinea pigs

Male Hartley guinea pigs (350-500 g, n=5) anaesthetized with a combination of 15 mg/kg diazepam, 20 μg/kg fentanyl and 20 mg/kg pentobarbitone (i.p.) were subjected to left carotid artery and right external jugular venous cannulation under halothane (1%) delivered from a humidified vaporizer via a nasal mask. An endotracheal tube (14 gauge Jelco i.v. catheter) was
used for airway support. Once preparatory surgery was complete, halothane was removed and animals were given 20 μg/kg (i.v.) fentanyl. External limb leads, in a lead II configuration, were used to record ECG variables. ECG and blood pressure were recorded at 0.5, 1, 2 and 5 minutes after drug administration on a Grass polygraph (model 79) while the ECG was monitored simultaneously on an oscilloscope (E for M Honeywell). Spontaneously breathing guinea pigs were given either ibutilide, E4031 or saline as equal volume bolus doses, in a blind and random manner, at 5 minute intervals. Drug was administered until signs of toxicity were apparent. Serum K$^+$ was measured, from heparinized 0.2 ml arterial blood samples (K$^+$ sensitive electrodes), before drug administration and at the 5 minute interval of the highest dose.

2.2.2 Studies in rats

Male Sprague-Dawley rats (250-350 g, n=5), anaesthetized with pentobarbitone (65 mg/kg, i.p.), were subjected to cannulation of the left carotid artery and right external jugular vein. An endotracheal tube (14 gauge Jelco i.v. catheter) was used for airway support. External limb leads, in a lead II configuration, were used to record ECG variables. Spontaneously breathing rats were given either ibutilide, E4031 or saline as equal volume bolus doses, in a blind and random manner, at 5 minute intervals. ECG and blood pressure were recorded at 0.5, 1, 2 and 5 minutes after drug administration on a Grass polygraph (model 79) while ECG was monitored simultaneously on an oscilloscope (E for M Honeywell). Drug was administered until signs of toxicity were apparent. Serum K$^+$ was measured as described above.

2.3 Electrical stimulation in rats and guinea pigs

Male Sprague-Dawley rats (250-350 g, n=5) and male Hartley guinea pigs (350-500g, n=5) were prepared as previously described and fitted with externalized intracardiac sliver wire electrodes (Teflon coated silver wire, Ag3 Lieco Industries) by trans-thoracic placement. Animals
were ventilated with air at 10 ml/kg at a rate of 60 strokes/min. Electrical stimulation variables ($I_t$, $T_t$, ERP, MFF and VF$t$), described in detail by Beatch (1991), were quantified by pacing the heart with two Grass stimulators (model S9), connected in series, to the externalized silver wire electrodes. Control responses to electrical stimulation were performed until three stable readings could be obtained at three minute intervals. Drugs and saline control were administered as bolus injections in a blind and random manner at 5 minute intervals. ECG and blood pressure were recorded at 0.5, 1, 2 and 5 minutes after drug administration. Responses to electrical stimulation were recorded after the two minute chart recording was taken. Serum $K^+$ was also measured as described above.

2.4 **Assessment of rate dependent effects in guinea pigs and rats.**

Guinea pigs and rats of similar size, gender and number were acutely prepared as described above for electrical stimulation studies. In both species the right vagus was dissected free from the internal carotid artery and was tied off proximal to the heart. The portion of the nerve proximal to the tie was crushed with fine forceps to prevent retrograde stimulation of the CNS through the vagus. The portion of the nerve distal to the tie was bathed in saline and coated with paraffin wax. The nerve was then placed onto bipolar stimulating electrodes attached to a Grass stimulator (model S9). Stimulation frequency, pulse duration and voltage were adjusted so as to obtain heart rates of approximately 100 beats per minute that were consistent over 3 minute intervals. Drugs and control solutions were administered blindly and randomly at 5 minute intervals. Trace recordings for ECG and blood pressure were taken at 0.5, 1, 2, 2', 5 and 5' minutes after drug administration (2' and 5' being rate controlled periods). Determination of electrical stimulation variables was carried out at 2 minute and 2' minute time intervals.

2.5 **In vitro experiments.**
2.5.1 Studies in isolated field stimulated guinea pig vas deferens

Male guinea pigs were exanguinated with CO₂ and the vasa deferentia were removed. Dissected tissues were placed into a carbogen bubbled (95% O₂, 5% CO₂), carbonated Krebs-Henseleit buffer of the following composition in mM; NaCl (118), KCl (4.6), CaCl₂ (2.4), MgSO₄ (1.2), KH₂PO₄ (1.0), NaHCO₃ (25) and Glucose (11.1). The sample was cleared of surrounding connective tissue and suspended in oxygenated 15 ml or 25 ml organ baths and allowed to equilibrate for 1 hour under an isometric tension of 1 g. Tissues were field stimulated by bipolar platinum electrodes attached to a Grass stimulator (model s8800). Stimulation protocols were based on variables (voltage, pulse width, pulse duration and frequency) which gave substantial submaximal contractions consistently over three 15 minute intervals. Tissues were washed twice, once at 5 minutes and once at 10 minutes, between successive stimulation’s. Ibutilide and E4031 were applied to the bath 2 minutes prior to stimulation. Prostaglandin E₁, an inhibitor of neurotransmitter release, and minoxidil, a non-specific potassium channel opener, were added 2 minutes prior to the addition of ibutilide or E4031 in studies involving the antagonism of ibutilide and E4031 action.

3 RESULTS

Chemical formulae for all test compounds are given in diagram 1.

3.1 Effects sematilide and almokalant in large primates.

3.1.1 Cardiovascular effects of sematilide

The actions of sematilide in large primates were those expected for a class III compound. Blood pressure and heart rate were not significantly depressed over the dose range
examined (0.075-1.2 mg/kg; Figure 1a). PR and QRS intervals of the ECG were not affected over the dose range which produced significant increases in APD75, VERP and Q-Tc (Figure 1b). Plasma concentrations of sematilide increased linearly over the dose range examined and correlated well with increases in action potential duration (Table I).

Table I  Serum concentrations of sematilide in primates at 15 minutes after dosing.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Serum Levels (ng/ml)</th>
<th>Increase in APD75 (600 msec CL; msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Time (+15' after dose)</td>
<td></td>
</tr>
<tr>
<td>Pre-drug</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.075</td>
<td>75±10</td>
<td>35±8</td>
</tr>
<tr>
<td>.15</td>
<td>120±20</td>
<td>60±5</td>
</tr>
<tr>
<td>.3</td>
<td>250±50</td>
<td>80±10</td>
</tr>
<tr>
<td>.6</td>
<td>560±100</td>
<td>95±13</td>
</tr>
<tr>
<td>1.2</td>
<td>1190±190</td>
<td>115±7</td>
</tr>
</tbody>
</table>

Table I shows the increases in serum concentration of sematilide with increasing dose in halothane anaesthetized primates. Values are expressed as ng of sematilide per ml of plasma taken from 3 ml blood samples (mean ± SEM, n=6). \( r = .83 (.773-.987) \) for correlation between serum concentration sematilide and increase in APD75 at 600 msec cycle length (95% confidence interval).

3.1.2 Electrophysiological effects of sematilide

Right ventricular effective refractory period (VERP) (Figure 2a) and monophasic action potential duration at 75 % repolarization (MAPD75) (Figure 2b) were prolonged in a dose dependent manner. These increases correlated well with increases in the Q-Tc interval of the surface ECG. Prolongation of MAPD and increases in VERP were greatest at longer cycle
lengths. MAPD75 was prolonged significantly from pre-drug values at all pacing cycle lengths and at all doses above 0.075 mg/kg. However MAPD75 at 600 msec cycle length was not significantly different from MAPD75 at 400 and 500 msec cycle lengths at doses below 1.2 mg/kg. MAPD75 at 600 msec cycle length was significantly larger than MAPD75 at 340 msec cycle length at doses above 0.15 mg/kg (Figure 2b). Ventricular accommodation was significantly increased at doses above 0.15 mg/kg while restitution was significantly altered at doses above 0.3 mg/kg (Figure 3).

3.1.3 Cardiovascular and electrophysiological effects of almokalant

Although almokalant is not structurally related to sematilide the cardiovascular and electrophysiological actions of this compound, at the dose tested, were qualitatively similar in this species (Table II). Blood pressure was not affected by this dose of almokalant. Heart rate was reduced, but not significantly, while PR and QRS intervals remained unaffected. The Q-Tc interval of the ECG and right ventricular monophasic action potential duration at 75 % repolarization (MAPD75) were the only variables that showed significant changes from pre-drug values. Almokalant also demonstrated a negative frequency-dependent action with MAPD75 increasing more with an increase in cycle length above 400 msec (Figure 4).
Table II shows the effects of a single 0.003 mg/kg bolus dose of almokalant on primate haemodynamic, ECG and electrophysiological variables. Data are expressed as mean ± SD (n=4). 1 min indicates variable values 1 min after bolus injection. p values obtained from comparison of pre-drug and 1 min measurements using a students t-test at a significance level of α= 0.05.

3.1.4 Toxic actions of sematilide and almokalant

Both sematilide and almokalant caused Torsade des Pointes (TdP) in large primates. This arrhythmia occurred at doses of 1.2 mg/kg for sematilide and 0.003 mg/kg for almokalant and appeared similar in morphology for both drugs (Figure 5). We did not detect a short-long-short series of ECG complexes or action potentials prior to the onset of the Torsades des Pointes, a characteristic pattern associated with Torsades in rabbits (Carlsson et al., 1993).

3.2 Effects of Ibutilide and E4031 in small primates

3.2.1 Cardiovascular effects of ibutilide and E4031

In monkeys ibutilide and E4031 (0.00001-0.1 mg/kg) had slight vasodepressor and bradycardic actions at doses > 0.003 mg/kg. Ibutilide reduced both systolic and diastolic blood pressure while heart rate remained unaffected (Figure 6a). These actions were more pronounced
for E4031 than for ibutilide (Figure 7a). Over the dose range tested, ibutilide had no effect on the PR or QRS interval of the ECG (Figure 6b). E4031 prolonged the PR interval but only at the highest dose tested, and like ibutilide had no effect on the QRS interval (Figure 7b). Ibutilide and E4031 prolonged the Q-Tc interval of the ECG in a dose-dependent manner. The approximate ED50 for Q-Tc interval widening in this species was approximately 8 µg/kg and 5 µg/kg for ibutilide (Figure 8a) and E4031 (Figure 8b), respectively.

3.2.2 Toxic actions of ibutilide and E4031

Neither ibutilide nor E4031 caused Torsades des Pointes in the small primates at any of the doses tested. However, both compounds did produce coupled extrasystoles (bigeminy) at doses above 0.030 mg/kg. The ectopic beats were ventricular in origin, having a QRS complex with reversed polarity to the normal QRS complex (Figure 9).

3.3 Low and high dose effects of ibutilide and E4031 in rats and guinea pigs at sinus and low heart rates.

3.3.1 Cardiovascular actions of Ibutilide and E4031 in guinea pigs

Figure 10a demonstrates the stability of haemodynamic and ECG variables in guinea pigs over the duration of the experiments. Figure 10b illustrates the stability of ERP measurements, at both low and sinus heart rates, in guinea pigs over the duration of experimentation. In guinea pigs, ibutilide (Figure 11a) and E4031 (Figure 12a) failed to reduce blood pressure over the entire dose range tested (0.001-1.0 mg/kg). However, both compounds reduced heart rate at doses up to 1.0 mg/kg. E4031 was more effective in reducing haemodynamic variables than ibutilide. Ibutilide (Figure 11b) did not increase the P-R or QRS interval at any dose whereas E4031 (Figure 12b) increased the P-R interval only at the highest doses tested. Ibutilide and E4031
prolonged the Q-Tc interval of the guinea pig ECG with approximate ED50 values of 30 μg/kg and 25 μg/kg, respectively (Figure 13). Both ibutilide and E4031 increased the Q-Tc to a similar maximum producing an increase of 65% and 80% from pre-drug values respectively. The Q-Tc interval was not selectively prolonged at slower heart rates by either of the compounds (Figure 14a). At 0.01 mg/kg (approximate ED25% for Q-Tc widening) both compounds increased the effective refractory period to a similar extent at high and low heart rates (Figure 14b). Threshold current for induction of extrasystole (I_t) was increased by both ibutilide and E4031 while maximum following frequency (MFF) was reduced (Table III). VF_t was not appreciably measured in most of these preparations due to the fact that attempting to measure this variable often resulted in precipitation of VT and/or escape rhythms from another part of the ventricular conducting system. Electrical stimulation variables were affected in a similar manner at low heart rates (Table III).

**Table III**  The effects of ibutilide and E4031 on electrical stimulation variables in guinea pigs at low and sinus heart rates.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Heart Rate</th>
<th>Ibutilide</th>
<th>E4031</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I_t (µA)</td>
<td>MFF (Hz)</td>
</tr>
<tr>
<td>Pre-Drug</td>
<td>Low</td>
<td>102±13 86±16 12±2</td>
<td>95±12 74±16 11±3</td>
</tr>
<tr>
<td></td>
<td>1mg/kg</td>
<td>110±18 228±43* 8.5±1*</td>
<td>92±8 211±22* 7.5±2*</td>
</tr>
<tr>
<td></td>
<td>Sinus</td>
<td>219±16 66±10 15.5±3</td>
<td>209±19 85±13 16.5±2</td>
</tr>
<tr>
<td></td>
<td>1mg/kg</td>
<td>182±11* 178±25* 10.0±1*</td>
<td>133±19* 182±36* 11.0±3*</td>
</tr>
</tbody>
</table>

Table III shows the effects of ibutilide and E4031 on threshold current, measured in micro-amps (µA), for induction of extrasystole (I_t) and maximum following frequency (MFF), measured in Hertz (Hz), in guinea pigs at sinus and low heart rates. Data are expressed as mean ± SEM (n=5). * Indicates p<0.05 for comparison with Pre-Drug.
3.3.2 Cardiovascular actions of Ibutilide and E4031 in rats

Saline and time controls for haemodynamic and ECG variables were also stable over the time period required for dose response testing in this species (Figure 15). In rats, both ibutilide (Figure 16a) and E4031 (Figure 17a) reduced blood pressure and heart rate but only at doses > 1.0 mg/kg. Both compounds prolonged the P-R and Q-T intervals at doses > 1.0 mg/kg but failed to increase the QRS interval even at the highest dose tested (0.01-100 mg/kg) (Figures 16b and 17b). Similarly ERP was increased by both compounds, but only at doses > 1.0 mg/kg (Figure 18a and 18b). Electrical stimulation studies also showed a dose dependent increase in VF_t and decrease in MFF. I_t was increased significantly while T_t remained unchanged over the dose range tested (Table IV). This profile was the same for the haemodynamic, ECG and stimulation variables in rats with low heart rates. (Data not shown).

Table IV Effects of ibutilide and E4031 on electrical stimulation variables in rats at sinus heart rates.

<table>
<thead>
<tr>
<th>Variable</th>
<th>(units)</th>
<th>Ibutilide Pre-drug</th>
<th>30 mg/kg</th>
<th>100 mg/kg</th>
<th>E4031 Pre-drug</th>
<th>100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I_t</td>
<td>(μA)</td>
<td>59±14</td>
<td>169±21*</td>
<td>38±11</td>
<td>186±13*</td>
<td></td>
</tr>
<tr>
<td>T_t</td>
<td>(msec)</td>
<td>.28±.06</td>
<td>.33±.06</td>
<td>.34±.04</td>
<td>.32±.03</td>
<td></td>
</tr>
<tr>
<td>VF_t</td>
<td>(μV)</td>
<td>265±59</td>
<td>738±127*</td>
<td>213±40</td>
<td>667±168*</td>
<td></td>
</tr>
<tr>
<td>MFF</td>
<td>(Hz)</td>
<td>17±1</td>
<td>11±1*</td>
<td>16±0.3</td>
<td>10.5±1*</td>
<td></td>
</tr>
</tbody>
</table>

Table IV shows the effects of ibutilide and E4031 on electrical stimulation variables in rats at sinus heart rates (Pre-drug heart rates were: Ibutilide, 323±9 bpm; E4031, 347±17). Data are expressed as mean ± SEM (n=5). * Indicates p<0.05 for comparison with Pre-drug.

3.4 Effects of Ibutilide and E4031 on field stimulated guinea pig vas deferens.
In the field stimulated guinea pig vas deferens preparation both ibutilide (Figure 19a) and E4031 (Figure 19b) produced a biphasic concentration-response curve such that there was enhancement of contractions induced by electrical stimulation at low doses and inhibition of electrically induced contraction at high doses. Minoxidil antagonized the effects of ibutilide and E4031 at low doses and enhanced the inhibitory effects of both drugs at high doses. Prostaglandin E1 also enhanced the inhibitory effects of higher doses of both ibutilide and E4031 (Figure 20).
FIGURES
Diagram 1

SEMATILIDE

\[
\text{CH}_3 - \text{SO}_2 - \text{NH} - \text{C} - \text{NH} - (\text{CH}_2)_2 - \text{N} \left(\text{CH}_2\text{CH}_3\right)
\]

IBUTILIDE

\[
\text{CH}_3 - \text{SO}_2 - \text{NH} - \text{CH} - (\text{CH}_2)_3 - \text{N} \left(\text{CH}_2\text{CH}_3\right)
\]

E-4031

\[
\text{CH}_3 - \text{SO}_2 - \text{NH} - \text{C} - \text{N} \left(\text{CH}_2\text{OH}\right)
\]

SOTALOL

\[
\text{CH}_3 - \text{SO}_2 - \text{NH} - \text{CHCH}_2\text{NHCH(CH}_3\text{)}_2
\]

ALMOKALANT

\[
\text{NC} - \text{C} - \text{OHCH(OH)CH}_2\text{NHCH(CH}_3\text{)(CH}_2\text{)}_3\text{SO(CH}_2\text{)}_2\text{CH}_3
\]
Figure 1 shows a) the effects of sematilide on mean arterial blood pressure (BP, (+)) measured in mmHg and heart rate (HR, (●)) as beats per minute in halothane anaesthetized primates, and b) effects of sematilide on PR (■), QRS (●) and Q-Tc (▲) intervals of the ECG measured in msec. C indicates pre-drug values. Data are expressed as mean ± SEM (n=6). The variable Q-Tc was derived from Q-aT according to Hayes et al. (1994).
Cardiovascular Effects of Sematilide in Primates

+ BP  • HR

Blood Pressure (mmHg)

Heart Rate (bpm)

Dose (mg/kg)

- PR  • QRS  ▲ Q-Tc

PR and QRS Interval (msec)

Q-Tc Interval (msec)

Dose (mg/kg)
Figure 2 shows a) the effects of sematilide on right ventricular effective refractory period at 600 (+) and 400 (▲) msec cycle lengths, and b) effects of sematilide on right ventricular monophasic action potential duration, at 75 % repolarization, at 340 (+), 400 (●), 500 (▲) and 600 (◆) msec cycle lengths in halothane anaesthetized primates. Data are expressed as mean ± SEM (n=6). SEM values have been omitted for the sake of clarity. SEM varied between 3 and 5 % for drug related changes of variables from pre-drug values. Group comparisons were made using repeated measures ANOVA at α=0.05 and Duncan's test for multiple comparisons.
Figure 2a top, 2b bottom

Effects of Sematilide on Ventricular Effective Refractory Period

Effects of Sematilide on Ventricular Monophasic Action Potential Duration
Figure 3 shows the dose dependent effects of sematilide on right ventricular accommodation (+) and restitution (●). Accommodation and restitution are given in units of msec/log unit cycle length as derived from linear regression of APD75 on log cycle length. C indicates pre-drug values.
Effects of Sematilide on Ventricular Accommodation and Restitution

+ Accommodation

● Restitution

Figure 3

Accommodation - Restitution (ms/log unit)

Dose (mg/kg)
Figure 4 shows the negative frequency-dependent actions of a single 0.003 mg/kg dose of almokalant (●) in halothane anaesthetized primates. APD75 is action potential duration at 75 % repolarization while pacing cycle length is measured in milliseconds (msec). Data are expressed as mean ± SD (n=4). Pre-drug values of APD75 at various cycle lengths are indicated by (+).
Negative Frequency Dependent Actions of Almokalant (3μg/kg) in Primates

Pacing Cycle Length (msec)

APD75 (msec)
Figure 5 is an illustration of Torsades des Pointes resulting from the single dose administration of 0.003 mg/kg almokalant. Channel A indicates blood pressure (mmHg), while channel B is the ECG recorded in lead II configuration and channel C is the ECG configuration recorded from a V3 unipolar electrode. Channel D is right ventricular monophasic action potential measured in mV/10 from an Ag/AgCl₂ electrode placed in the right ventricle and channel E is heart rate in beats per minute as calculated from an internal rate meter.
Figure 6 shows a) the effects of ibutilide on systolic blood pressure (Sys BP, (+)), diastolic blood pressure (Diast BP (▲)) and heart rate (HR (●)) in pentobarbitone anaesthetized primates, and b) the effects of ibutilide on PR (■) and QRS (●) intervals of the primate ECG. Data are expressed as average values for n=3 animals. C indicates pre-drug values.
Cardiovascular Effects of Ibutilide in Primates

- + Sys BP
- △ Diast BP
- ● HR

Blood Pressure (mmHg)

Heart Rate (bpm)

- ■ PR
- ○ QRS

PR & QRS Intervals (msec)

Dose (mg/kg)
Figure 7 shows a) the effects of E4031 on systolic blood pressure (Sys BP, (+)), diastolic blood pressure (Diast BP (▲)) and heart rate (HR (●)) in pentobarbitone anaesthetized primates, and b) the effects of E4031 on PR (■) and QRS (●) intervals of the primate ECG. Data are expressed as average values for n=3 animals. C indicates pre-drug values.
Cardiovascular Effects of E4031 in Primates

Blood Pressure (mmHg)

Heart Rate (bpm)

Dose (mg/kg)

Figure 7a top, 7b bottom
Figure 8 shows a) the effects of ibutilide on the Q-Tc (♦) interval of pentobarbitone anaesthetized primates, and b) the effects of E4031 on the Q-Tc (●) interval of pentobarbitone anaesthetized primates. Data are expressed as average values for n=3 animals. Approximate ED50 values for Q-Tc widening in this species are 8 µg/kg and 5 µg/kg for ibutilide and E4031 respectively. Maximum Q-Tc was defined as the greatest increase in Q-Tc without toxic effects. The variable Q-Tc was derived from Q-aT according to Hayes et al. (1994). Pre-drug Q-Tc values were 340 msec and 320 msec for ibutilide and E4031 respectively. Ibutilide and E4031 increased the Q-Tc interval to 510 msec and 694 msec respectively at the highest dose tested.
Figure 8a top, 8b bottom

Effects of Ibutilide and E4031 on Q-Tc
Interval Duration in Primates

- Ibutilide

- E4031
Figure 9 is an illustration of the coupled extra systoles which appeared with both ibutilide and E4031 at doses above 0.030 mg/kg.
Figure 10 shows data for saline and time controls for a) blood pressure (+), heart rate (●), and the ECG measures of PR (▲), QRS (▼) and Q-Tc(■) intervals in anaesthetized guinea pigs, and b) effective refractory period (ERP) at low (L) and sinus (S) heart rates in anaesthetized guinea pigs. Data are expressed as mean ± SEM (n=10, pooled from vagal stimulation and electrical stimulation studies).
Saline and Time Controls: Guinea Pigs
Haemodynamic and ECG Variables

*BP*  *HR*  *PR*  *QRS*  *Q-T*

Saline & Time Controls for ERP at Low (L) & Sinus (S) Rates: Guinea Pigs
*ERP (S)*  *ERP (L)*
Figure 11 shows a) the effects of ibutilide on mean arterial blood pressure (BP (+)) and heart rate (HR (●)), and b) the effects of ibutilide on the PR (■) and QRS (●) intervals of the guinea pig ECG. Data are expressed as mean ± SEM (n=5). C indicates pre-drug values.
Cardiovascular Effects of Ibutilide in Guinea Pigs

Blood Pressure (mmHg)

Heart Rate (bpm)

Dose (m/kg)

Figure 11a top, 11b bottom
Figure 12 shows a) the effects of E4031 on mean arterial blood pressure (BP (+)) and heart rate (HR (●)), and b) the effects of E4031 on the PR (■) and QRS (●) intervals of the guinea pig ECG. Data are expressed as mean ± SEM (n=5). C indicates pre-drug values.
Cardiovascular Effects of E4031 in Guinea Pigs

**Blood Pressure (mmHg)**

- + BP
- ○ HR

**Heart Rate (bpm)**

**PR & QRS Intervals (msec)**

- ■ PR
- ○ QRS

Dose (mg/kg)
Figure 13 shows a) the effects of ibutilide (◆) on the Q-Tc interval of the guinea pig ECG, and b) the effects of E4031 (○) on the Q-Tc interval of the guinea pig ECG. Data are expressed as mean ± SEM (n=5). Approximate ED50 values for Q-Tc widening in this species are 30 μg/kg and 45 μg/kg for ibutilide and E4031 respectively. Maximum Q-Tc was defined as the greatest increase in Q-Tc without toxic effects. The variable Q-Tc was derived from Q-aT according to Hayes et al. (1994). Pre-drug Q-Tc values were 114±10.2 msec and 109±13.3 msec for ibutilide and E4031 respectively. Ibutilide and E4031 increased the Q-Tc interval to 188 msec and 197 msec respectively at the highest dose tested.
Effects of Ibutilide and E4031 on Q-Tc Interval Duration in Guinea Pigs

- Ibutilide

- E4031
Figure 14 shows a) the effects of heart rate on the Q-Tc prolonging actions of ibutilide at low (●) and sinus heart rate (Δ) and E4031 at low (●) and sinus (+) heart rates, while figure b shows the effects of heart rate on the ERP prolonging actions of ibutilide (solid bars) and E4031 (hatched bars) at low and sinus heart rates in guinea pigs. Data are expressed as mean ± SEM (n=5). Pre-drug ERP values at sinus rates were 114±3.1 msec and 100±7.3 msec for ibutilide and E4031 respectively. Pre-drug ERP values at low heart rates were 177±14 msec and 169±8 msec for ibutilide and E4031 respectively.
Effects of Ibutilide & E4031 on Q-Tc in Guinea Pigs at Sinus (S) & Low (L) Rates

Effects of 10 µg/kg Ibutilide & E4031 on ERP in Guinea Pigs at Sinus & Low Rates
Figure 15 Illustrates the stability of haemodynamic and ECG variables in rats at sinus rates. Data are expressed as mean ± SEM (n=10) for animals pooled from electrical stimulation and vagal stimulation studies. Saline and time controls for electrical stimulation variables at low and sinus heart rates were also stable with respect to time (data not shown).
Figure 15

Saline and Time Controls: Rats
Haemodynamic and ECG Variables

PR, QRS and Q-T intervals (msec)

BP (mmHg) and HR (bpm)

Time (min)
Figure 16 shows the effects of ibutilide on a) the mean arterial blood pressure (BP (+)) and heart rate (HR (●)), and b) PR (■), QRS (●) and Q-T (Δ) intervals of the ECG in pentobarbitone anaesthetized rats. The variable Q-T was not corrected for rate based on Hayes et al. (1994). Data are expressed as mean ± SEM (n=5). C indicates pre-drug values.
Cardiovascular Effects of Ibutilide in Rats at Sinus Rate

Dose (mg/kg)

Blood Pressure (mmHg)

Heart Rate (bpm)

Dose (mg/kg)
Figure 17 shows the effects of E4031 on a) the mean arterial blood pressure (BP (+)) and heart rate (HR (●)), and b) PR (□), QRS (●) and Q-T (Δ) intervals of the ECG in pentobarbitone anaesthetized rats. The variable Q-T was not corrected for rate based on Hayes et al. (1994). Data are expressed as mean ± SEM (n=5). C indicates pre-drug values.
Figure 17a top, 17b bottom

Cardiovascular Effects of E4031 in Rats

+ BP  ● HR

Blood Pressure (mmHg)

150
100
50
0

Heart Rate (bpm)

400
300
200
100
0

Dose (mg/kg)

0
.1
1
10
100

0
20
40
60
80
100
120

PR, QRS & QT Intervals (msec)

C

0
.1
1
10
100

PR
QRS
QT
Figure 18 shows the effects of a) ibutilide (●) and b) E4031 (○) on the effective refractory period in rats at sinus rates. ERP was increased in a similar manner in rats subjected to slowing of heart rate through vagal stimulation (data not shown). Data are expressed as mean ± SEM (n=5). C indicates pre-drug values.
Effects Of Ibutilide & E4031 on ERP In Rats at Sinus Rate

- Ibutilide
- E4031
Figure 19 shows the effects of a) ibutilide and b) E4031 on the field stimulated vas deferens in the absence (●) and presence of 2 (■) and 20 (Δ) μM minoxidil. Vas deferentia were stimulated at 50V and 20Hz for a duration of 10 seconds at a pulse width of 1 msec. Data are expressed as mean ± SEM (n=5) for % change in pre-drug peak contraction. Pre-drug baseline tension was .97 ± .13g and peak contraction was 8.60 ± .34g.
Effects of Ibutilide & Minoxidil (2 & 20µM) in Guinea Pig Vas Deferens

Effects of E4031 & Minoxidil (2 & 20 µM) in Guinea Pig Vas Deferens
Figure 20 shows the effects of 75 μM ibutilide (IB, open bars) and 75 μM E4031 (E4, hatched bars) in the absence and presence of 0.3 μM and 30 μM prostaglandin E1 (PGE1). Vas deferentia were stimulated at 50V and 20Hz for a duration of 10 seconds at a pulse width of 1 msec. Data are expressed as mean ± SEM (n=5) for % change in pre-drug peak contraction. Pre-drug baseline tension was 1.32 ± .17g and peak contraction was 8.96 ± .73g.
Effects of PGE1 on Ibutilide and E4031 Inhibited Contraction of Vas Deferens
4 DISCUSSION

4.1 Expected actions of test compounds in primates

4.1.1 Expected actions of delayed rectifier blockers

Sematilide, almokalant and E4031 are potent blockers of the rapidly activating component of the delayed rectifier current ($I_{Kr}$) (Sawanobori et al., 1994; Ohler and Ravens, 1994; Ohler, 1994). As mentioned previously $I_{Kr}$ has been demonstrated in the S.A. and A.V. nodes of a number of species and blockade of this current would be expected to reduce the heart rate. However, in practice, prolongation of the action potential may take place without producing an increase in R-R interval thus leaving heart rate unaffected (Taggart et al., 1990). The blockade of $I_{Kr}$ would also be expected to increase action potential duration and effective refractory period more than would be expected from a reduction in heart rate. Since the Q-Tc interval of the ECG is a good estimate of ventricular action potential duration in primates (Hayes et al., 1994) one would also expect to see an increase in Q-Tc in response to $I_{Kr}$ blockers. On the other hand ECG variables which are dependent on calcium or sodium currents (PR and QRS) would not be expected to change in the presence of $I_{Kr}$ block (Wallace et al., 1991). Due to the relatively uniform distribution of $I_{Kr}$ in most species one would not expect to see a dispersion of repolarization in the presence of $I_{Kr}$ block. Changes in T-wave morphology may be related to dispersion of repolarization whereas an increase in T-wave amplitude may not (Carmeliet, 1993b). Therefore one may or may not see an increase in T-wave amplitude in the presence of $I_{Kr}$ block. Excessive prolongation of the action potential could be expected to produce triggered activity as a result of the recycling of time-dependent Ca$^{2+}$ currents (O'rourke et al., 1994). In the presence of bradycardia this might result in Torsades des Pointes or other ventricular arrhythmias (Weissenburger et al., 1993).
4.1.2 Expected actions of ibutilide

As opposed to the previously mentioned $I_K$ blockers, ibutilide is purported to be an activator of an inward sodium current during the plateau phase of the action potential (Lee et al., 1993). Although this is a distinctly different mechanism from $I_K$ block, the overall effects on cardiovascular variables would be expected to be the same based on the fact that rapid sodium and slow calcium currents would not be affected. One could expect to see a prolongation of the action potential similar to that observed with $I_K$ blockers. This is because action potential duration may be altered by modulation of both inward and outward currents (Hondeghem, 1994).

However if ibutilide's actions are due to sodium channel activation then one might expect to see some differences in action between ibutilide and E4031. For example, this compound does not appear to have negative frequency dependence of action with respect to the prolongation of the ventricular Q-Tc interval and refractoriness (Cimini et al., 1992). Furthermore, ibutilide has been reported to activate outward potassium currents at high doses in vitro (Lee et al., 1993). Such an action would be expected to reduce proarrhythmic potential at high doses by limiting the extent of Q-Tc prolongation and thereby avoid precipitating triggered activity in the presence of bradycardia. In vivo and in vitro studies conducted with ibutilide show that it has the ability to antagonize the action potential prolonging actions of other class III agents (Lee et al., 1993). Ibutilide is reported to be associated with a decreased incidence of tacharrhythmias compared to other class III agents in a rabbit model of proarrrhythmia (Buchanan et al., 1993b) and has antifibrillatory actions in isolated rabbit hearts (Friedrichs et al., 1993). These beneficial effects take place even in the presence of a dose dependent increase of the Q-Tc interval of the ECG (Buchanan et al., 1992).

4.2 Observed effects of test compounds in primates
4.2.1 Cardiovascular actions of delayed rectifier blockers

In studies with sematilide and almokalant in baboons Q-Tc widening correlated positively with the prolongation of the right ventricular monophasic action potential duration and ventricular effective refractory period but a negative correlation was found with cycle length. PR and QRS intervals were not significantly altered over the dose range tested. The negative rate dependence of the electrophysiological effects, along with the inability of both of these compounds to alter haemodynamic and other ECG variables, indicates a selective class III action, findings which agree with other studies both \textit{in vitro} and \textit{in vivo} (Krafte and Volberg, 1994; Carlsson et al., 1991). The observed lack of effect of both of these compounds on the PR and QRS intervals of the ECG suggest that neither compound affects atrial or ventricular conduction in a significant manner. This is an important distinction between class III (potassium channel blocking) and Ic (sodium channel blocking) antiarrhythmic drug action. Excessive slowing of conduction, commonly associated with class Ic compounds, may be proarrhythmic as a result of drug induced conversion of bi-directional block to unidirectional block, thus reestablishing reentry (Scholtz, 1994). The observed correlation between plasma concentration of sematilide and increases in the Q-Tc interval of the ECG is of experimental interest in that we may speculate that the ECG changes may be directly related to certain amounts of drug, therefore allowing us to calculate an ED50 for plasma concentration. This relationship may also be of clinical importance if a certain dose is associated with a required antiarrhythmic action. Designing dosages of drugs which have profiles similar to sematilide is easier than attempting the same task using drugs, such as amiodarone, which do not demonstrate such a straight forward relationship (Woosley et al., 1990; Follath, 1992)

The dose dependent changes in ventricular restitution seen with sematilide suggest that at longer cycle lengths the heterogeneity of the duration of action potentials, elicited by introduction of extra stimuli during periods of steady state pacing, increases. Similarly accommodation of action potential duration during stepwise steady state pacing to progressively shorter cycle lengths
suggests that sematilide increases, dose dependently, the heterogeneity of recovery of action potential duration during increases in heart rate. These results may or may not have implications concerning the proarrhythmic potential of this compound (Cui et al., 1994). The proarrhythmic actions of quinidine in infarcted canine ventricular cells have been ascribed to similar changes in restitution and accommodation of the action potential duration (Karagueuzian et al., 1990). In contrast to the actions of quinidine, propranolol reduces the variability in restitution of action potential duration in infarcted canine ventricular muscle (Varro et al., 1990).

The appearance of the ventricular arrhythmia, Torsades des Pointes, was expected for both sematilide and almokalant. Torsades des Pointes has been demonstrated with almokalant and sematilide in rabbits (Carlsson et al., 1990). This type of arrhythmia is commonly observed in clinical and experimental situations with high doses of other class III drugs (Carlsson et al., 1990; Lazzara, 1993; Cui et al., 1994). Torsades des Pointes has a characteristic morphology which is manifest as periodic reversal of the QRS polarity in the ECG and in some cases is preceded by a somewhat conserved sequence of electrical events (Weissenburger, 1993). Our inability to demonstrate a short-long-short triad of electrical activity prior to the onset of Torsades des Pointes in this preparation does not in any way confirm or deny the necessity of this activity in precipitating Torsades des Pointes.

The mechanisms involved in the generation of Torsades des Pointes are not yet clearly understood. However current models suggest that triggered activity, in the presence of bradycardia and Q-Tc prolongation, and/or re-entry due to dispersion of ventricular repolarization are the most probable causes of this type of arrhythmia (Higham and Campbell, 1994; Higham et al., 1995). It is interesting to note that although both sematilide and almokalant had similar cardiovascular, electrophysiological and toxic effects in our preparations, their chemical structures are quite distinct. This suggests that common actions not related to the structure of a particular class of compounds may be important in the production of drug induced Torsades.

The actions of E4031 in small monkeys were those expected of an I\textsubscript{k} blocker. An increase in PR interval was not expected for this compound. Others have shown that E4031 does not
prolong the PR interval in dogs at doses which also do not affect the QRS interval (Spinelli et al., 1992; Inoue et al., 1994). The lack of effect of this compound on the QRS interval in our studies agrees with numerous other in vivo studies and, like sematilide and almokalant, suggests that this compound does not depress ventricular conduction. A surprising result of these studies was that E4031 was more potent than sematilide in prolonging the Q-Tc interval despite the fact that the baseline heart rate was much higher in the smaller primates. E4031 has been demonstrated to be more potent than sematilide in a variety of preparations but one would have expected the differences in heart rate to counteract this potency difference, at least in part. This would suggest that E4031 may be acting in a manner inconsistent with the negative frequency-dependence observed with in vitro preparations (Hiraoka et al., 1994). Pacing experiments in dogs have shown a reduction in the Q-Tc interval and prolongation of the ventricular effective refractory period at high rates of stimulation (Hiraoka et al., 1994). However, the differences in QTc and refractoriness between E4031 treated and control animals were still significantly greater for drug treated animals at pacing rates up to 3.3 Hz (200 beats per min). E4031 has been shown to produce early after depolarizations, as a result of excessive prolongation of the action potential duration, both in vitro and in vivo (Courtney et al., 1992; Buchanan et al., 1993). Therefore the appearance of coupled extrasystoles at higher doses was not unexpected.

4.2.2 Cardiovascular actions of ibutilide

The actions of ibutilide in small monkeys were similar to those of E4031. A clear distinction between the mechanism of action of ibutilide and that of Ifc blockers was not possible based on results obtained from this species. One would expect similar effects despite any mechanistic differences based on the idea that action potential duration can be prolonged by enhancement of inward currents and blockade of outward currents (Hondeghem, 1994). Responses indicative of the activation of outward K+ currents were not seen at higher doses as the Q-Tc interval was widened in a monotonic dose-dependent fashion and bigeminy was observed in
all monkeys at the highest doses tested. The similarity of ibutilide's actions on ECG and haemodynamic variables, compared with those of E4031, were expected whereas the proarrhythmic potential was not. ED50 estimates for prolongation of the primate Q-Tc interval for both ibutilide and E4031 compare favorably with similar estimates in other species (Chi et al., 1991; Baskin et al., 1991). Neither ibutilide or E4031 caused serious vasodepressor actions even at doses associated with arrhythmias. Limited depression of cardiovascular function is often observed with most selective class III agents (Colatsky and Follmer, 1989). This cardiovascular profile would be expected to be of benefit in the treatment of arrhythmias in patients with compromised myocardial function.

4.3  **Expected actions of ibutilide and E4031 in guinea pigs and rats.**

4.3.1  **Cardiovascular action of ibutilide and E4031 in guinea pigs**

In guinea pigs one would expect to see, with both ibutilide and E4031, cardiovascular actions similar to those observed in the primates. Both compounds would be expected to produce negligible cardiovascular depression without affecting the PR and QRS intervals of the ECG. On the other hand one would expect to see, despite any differences in mechanism of action, a prolongation of the Q-Tc interval of the ECG. If the cardiovascular actions of these compounds is strictly due to block of I_k (or activation of I_{Na} in the case of ibutilide) then the potency of their actions in this species should be similar to that in primates. However due to the higher intrinsic heart rate in this species one would expect that ibutilide may have more potent actions on the Q-Tc interval of the ECG at sinus rate compared to E4031 since it possesses negative rate dependent actions *in vivo* (Hiraoka et al., 1994). Furthermore, at slow heart rates, E4031 should produce a similar degree of Q-Tc prolongation and increase in refractoriness (ERP) as compared to ibutilide. Similarly one might expect that ibutilide would increase ERP more than E4031 in guinea pigs at sinus heart rates.
4.3.2 Cardiovascular action of ibutilide and E4031 in rats

If the cardiovascular actions of these compounds is truly due to different mechanisms of action then these differences should be demonstrable in rats, a species lacking $I_k$. Ibutilide would be expected to affect haemodynamic, ECG and stimulation variables at comparable doses to those in guinea pigs and primates. These effects would be expected in species with and without $I_k$ based on the ubiquity of sodium currents in action potential generation (Driscoll, 1981; Dangman et al., 1988; Le-Guennec and Noble, 1994). Furthermore, these effects would not be expected to be enhanced at low heart rates in this species since ibutilide is reported to lack negative frequency dependence (DiMarco et al., 1994). On the other hand, E4031 would be expected to lack effects in rats. Similarly, due to a lack of $I_k$ in this species, E4031 would not be expected to have differential cardiovascular actions at low and high heart rates.

4.4 Observed effects of ibutilide and E4031 in guinea pigs and rats.

4.4.1 Cardiovascular action of ibutilide and E4031 in guinea pigs

Both ibutilide and E4031 showed cardiovascular actions in guinea pigs which closely resembled those seen in primates. The potency of the two compounds at prolonging the Q-Tc interval in this species was less than that observed for the drugs in primates; however, the maximal increases in Q-Tc interval were close to those seen in primates. The observed lack of negative rate-dependence for E4031 in the guinea pig was not expected and does not agree with previous studies in dogs (Buchanan et al., 1993). However, recent experiments using electrophysiological studies in single cells as well as in vivo have failed to demonstrate a negative frequency dependence of action for this compound (Ohler et al., 1994). The lack of negative frequency dependence observed in guinea pigs would be expected for ibutilide, especially if it is a
sodium channel activator as suggested by Lee et al. (1993), and agrees with other in vitro and in vivo studies (Hester et al., 1991; Cimini and Gibson, 1990; Buchanan et al., 1993). The observation that the maximal increases in the Q-Tc interval were similar for both compounds at low and sinus heart rates also indicates a lack of selective action at low rates for both of these compounds. It was interesting to note that, although both ibutilide and E4031 are structurally similar to sematilide, they apparently lack the negative frequency dependent action seen with sematilide.

4.4.2 Cardiovascular actions of ibutilide and E4031 in rats

The limited effect of E4031 in rats was expected based since it is well known that this species lacks $I_k$ as a repolarizing current (Josephson et al., 1984). At high doses the increases in the PR and QRS intervals was not expected for E4031, an $I_k$ blocker, but rather for drugs, such as local anaesthetics, which block sodium currents (Makielski et al., 1989; Scholtz, 1994). The actions of ibutilide in rats were similar to those for E4031. One might expect ibutilide to increase the Q-T interval and ERP in this species, at very low doses, provided its class III actions are dependent upon plateau sodium current activation and not $I_k$ blockade. However this was not observed. Such results might suggest that the class III action of ibutilide may not be due to activation of an inward sodium current. However, the short duration of the plateau phase of the action potential in rats might preclude this compound from having class III actions, at low doses, in this species if its onset kinetics are slow (Driscoll, 1981).

Both ibutilide and E4031 give clear indications of non-selective ion channel blockade at very high doses. The increases in VF$_t$, ERP and MFF caused by both compounds may be the result of sodium channel blockade. Slowing the rate of phase 0 depolarization by blockade of sodium channels may prolong action potential duration by limiting the voltage and time dependent activation of outward currents, thus causing an increase (or decrease in the case of MFF) in variables which normally indicate the blockade of potassium currents in this species (Howard and
Walker, 1990). On the other hand, block of the transient outward current in this species would also affect the same stimulation variables in a similar manner (Beatch et al., 1991).

Based upon the results from our studies in rats we cannot say whether or not one or both of the above mentioned actions is responsible for our observations. However, both ibutilide and E4031 have been reported to act without effects on the transient outward current in vitro (Lee et al., 1993; Hiraoka et al., 1994; DiMarco et al., 1994). Therefore one might suggest that the actions of both compounds at high doses is the result of sodium channel blockade.

4.5 Expected and observed actions of ibutilide and E4031 in vitro.

4.5.1 Minoxidil sensitive modulation of field stimulated guinea pig vas deferens

Resting potential in the guinea pig vas deferens ultimately depends upon potassium currents (Sjostrand, 1973). Blockade of potassium currents in this preparation would be expected to produce an increase in contraction due to depolarization of the muscle membrane and enhanced activation of membrane calcium currents during stimulation (McGrath, 1978). Block of sodium currents would be expected to decrease contractions by blocking the transmission from excitatory nerve terminals (Sneddon et al., 1992). Ibutilide and E4031 had similar actions in guinea pig vas deferens consistent with a profile of potassium channel blockade at low doses and sodium channel block at high doses.

Antagonism of the low dose effects of both compounds in this preparation by the non-selective potassium channel opener, minoxidil, supports the idea of potassium channel blockade by these compounds at low doses. Minoxidil also augmented the inhibitory actions of high doses of ibutilide and E4031. Such results suggest that the actions of minoxidil are additive but separate from those of ibutilide and E4031. Further reduction in electrically stimulated contractions of ibutilide and E4031 inhibited preparations in the presence of prostaglandin E₁, suggest that prostaglandin E₁ is acting synergistically with both compounds. Prostaglandin E₁ is known to
inhibit the release of neurotransmitter in the stimulated vas deferens by actions on nerve terminal receptors, thus reducing the amount of transmitter released during depolarization (Savage et al., 1993; Christian and Poyser, 1994). If the actions of ibutilide and E4031 involve blockade of sodium channels, then one would expect to see additive actions in the presence of prostaglandin E$_1$. 
CONCLUSIONS

All of the drugs tested had potent class III actions in species which express delayed rectifier potassium currents. The reverse use-dependent and toxic actions in primates were apparent with, but not limited to, drugs with structural similarity to sotalol. Ibutilide and E4031 had similar actions in all species *in vivo* and in an *in vitro* preparation. The similarity of their actions in a species which lacks $I_k$ was not expected based on proposed differences in their mechanisms of action. The similarity of the actions of ibutilide and E4031 over a broad range of heart rates was also not expected based on observations from previous studies. Further studies would be necessary to demonstrate differential mechanisms of action for ibutilide and E4031 *in vivo*. 
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