Antiarrhythmic Versus Antifibrillatory Actions: Inference from Experimental Studies

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Pathophysiology of the coronary circulation is a major contributor to altering the myocardial substrate, rendering the heart susceptible to the onset of arrhythmias associated with sudden cardiac death. Antiarrhythmic drug therapy for the prevention of sudden cardiac death has been provided primarily on the basis of trial and error and in some instances based on ill-suited preclinical evaluations. The findings of the Cardiac Arrhythmia Suppression Trial (CAST) requires a reexamination of the manner in which antiarrhythmic drugs are developed before entering into clinical testing. The major deficiency in this area of experimental investigation has been the lack of animal models that would permit preclinical studies to identify potentially useful or deleterious therapeutic agents. Further, CAST has emphasized the need to distinguish between pharmacologic interventions that suppresses nonlethal disturbances of cardiac rhythm as opposed to those agents capable of preventing lethal ventricular tachycardia or ventricular fibrillation. Preclinical models for the testing of antifibrillatory agents must consider the fact that the superimposition of transient ischemic events on an underlying pathophysiologic substrate makes the heart susceptible to lethal arrhythmias. Proarrhythmic events, not observed in the normal heart, may become manifest only when the myocardial substrate has been altered. We describe a model of sudden cardiac death that may more closely simulate the clinical state in humans who are at risk. The experimental results show a good correlation with clinical data regarding agents known to reduce the incidence of lethal arrhythmias as well as those showing proarrhythmic actions.

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A major cause of mortality in the United States as well as in other parts of the industrialized world is attributed to sudden cardiac death, a phenomenon most likely to occur in the diseased heart as a result of altered electrophysiology. "Sudden cardiac death" generally denotes death that is nonviolent, unexpected, witnessed, and instantaneous or occurs within a few minutes of an abrupt change in the previous clinical state.[1] Despite a decrease in the number of sudden cardiac deaths over the past 20 years, the number of fatalities in the United States exceeds 300,000 per year. Approximately 60% of the fatal events occur in individuals without any previous diagnosis of heart disease.[2] Clearly, sudden cardiac death is a major health issue of our times, accounting for 50% of all cardiovascular deaths,[3] and the problem is compounded by the recent realization that not only has pharmacologic management done little to improve the situation, it may have contributed to the onset of the fatal events in susceptible individuals.[4] The extent of the problem has made sudden cardiac death one of the most pressing unresolved clinical and public health concerns.

In most instances of sudden cardiac death, the underlying pathophysiology of the coronary artery circulation renders the heart susceptible to the onset and maintenance of a lethal dysrhythmia. That the "triggering" event is of a transient nature is suggested by the fact that patients resuscitated after sudden coronary death are capable of maintaining a stable cardiac rhythm and, in many instances, do not display signs and symptoms suggestive of permanent myocardial injury. It is of fundamental importance, therefore, to understand what possible electrophysiologic factor (or factors) results in the transition from a stable activation sequence of the ventricular myocardium to one with multiple asynchronous circuits culminating in ventricular fibrillation. It is equally important to know what morphologic and electrophysiologic changes characterize the portion of the myocardium that is capable of serving as a *suitable substrate* for the triggering event that proceeds to ventricular fibrillation.

The ischemically injured heart shows marked heterogeneity with respect to regional coronary artery blood flow to areas subserved by stenosed vessels. Further, ion fluxes, availability of substrate, and the superimposition of neural influences are capable of exerting deleterious effects, which render the heart more susceptible to the triggering event leading to the onset of ventricular fibrillation.

Sudden cardiac death may be considered to involve an interaction between structural derangements of the heart, transient functional disturbances, and the specific electrophysiologic events responsible for the fatal arrhythmia.[3] Coronary atherosclerosis and its associated influences on the heart constitutes the major pathologic finding in the vast majority of persons who succumb to sudden cardiac death. Patients who have experienced a major cardiovascular event are at high risk of sudden cardiac death during the first 6–18 months after the index event, suggesting a time dependence of risk and indicating the need for appropriate therapy in the early period.[5]

THE PREFIBRILLATORY STATE

Understanding the factors that characterize the prefibrillatory state as targets for drug intervention: The role of drug intervention in the prevention of sudden death is based on the premise of there being treatable factors that can be identified in those at risk. One factor, recognized for many years, is the finding of chronic ventricular ectopy in patients after myocardial infarction.[6–11] The assumption was made that premature ventricular complexes appearing in the vulnerable phase of myocardial repolarization were responsible for the initiation of malignant arrhythmias in these individuals. Therefore, it seemed entirely appropriate that an agent capable of suppressing premature ventricular complexes would be effective in preventing sudden death. Indeed, early studies with lidocaine, an effective agent in suppressing ectopy,[12] suggested that its use might be associated with a decreased incidence of ventricular fibrillation.[13] After this, however, there appeared a number of reports using several agents that failed to substantiate this claim and, when reviewed in early meta-analysis, appeared to suggest an adverse trend.[14,15] Nevertheless, the analyses were not without criticism for dissimilarities in study designs and populations, so it was not until the advent of the Cardiac Arrhythmia Suppression Trial (CAST) that the prescribing public was given a clear picture of the issue. CAST was designed specifically to address the question of whether premature ventricular complex suppression was an appropriate surrogate for mortality in the postmyocardial infarction population. Encainide, flecainide, and moricizine were identified in the Cardiac Arrhythmia Pilot Study (CAPS)[16] as "premature ventricular complex killers" and were considered to be the agents of choice for CAST. Despite (ironical) concern over the ethics of a placebo arm, it was included in the major multicenter study, which employed an elegant dose titration in a double-blind manner. Preliminary (CAST I) and final (CAST II) results from this study provided the definitive answer that the use of all 3 agents was associated with significant *increases* in arrhythmic deaths.[17,18] Further, the adverse trends were apparent in all identified subgroups and, in view of the diverging curves, were thought to be ongoing throughout the trial.

An alternative clinical model for the testing of antiarrhythmic drugs is the unmasking of malignant reentrant arrhythmias by electrophysiologic testing. An appropriately timed stimulus, delivered through an in-dwelling cardiac catheter, can induce ventricular tachyarrhythmias in patients at risk of life-threatening disturbances in cardiac rhythm.[19] Further, the same arrhythmias may be prevented by the use of individualized drug therapy.[20,21] The induction of sustained ventricular arrhythmia is considered an objective endpoint by which to evaluate candidates for antiarrhythmic drug therapy. The rate at which inducible ventricular arrhythmias are suppressed during serial antiarrhythmic drug testing in survivors of sudden cardiac death is in the range of 20–50%.[20,22,23] However, even when ventricular fibrillation is induced by programmed electrical stimulation in the electrophysiology laboratory, one cannot be sure that the substrate of the arrhythmia thus generated is identical to that pertaining at the time of sudden death, or that pharmacologic prevention of stimulus-induced ventricular fibrillation reflects protection against sudden death. The question remains, therefore, whether it is the fact that a patient's arrhythmia can be suppressed that is important, or if it is due to the associated drug therapy; in other words, is suppressibility per se associated with a good prognosis? To answer this would require a placebo-controlled trial in which patients with suppressible arrhythmias were compared with patients whose arrhythmias could not be prevented, but before embarking on this we must first be assured that electrophysiologic testing is an appropriate surrogate for mortality. The only study to date that addresses this question is the National

Institutes of Health-sponsored Electrophysiology Study Versus Electrocardiographic Monitoring (ESVEM) trial.[24] ESVEM uses mortality in determining the relative usefulness of electrophysiologic testing and Holter monitoring in assessing several antiarrhythmic agents, including quinidine, procainamide, mexiletine, propafenone, sotalol, and pirmenol (a class IA agent no longer being developed). An imipramine group was removed shortly after the trial was initiated. Until this trial reports in 1993, electrophysiologic testing cannot be recommended for routine use.

To summarize the clinical situation, therefore, whereas certain drugs (e.g., lidocaine, bretylium, amiodarone, and most recently, sotalol) do have the indication for prevention of recurrent ventricular fibrillation, no pharmacologic intervention has yet been shown to decrease the incidence of sudden cardiac death. Further, there appears to be no relevant surrogate endpoint for the evaluation of potential new therapies. The results of CAST have demonstrated that clinical testing of antiarrhythmic drugs cannot rely on a drug's ability to reduce the frequency and/or complexity of ventricular premature depolarizations. Even the application of more elaborate procedures, such as electrophysiologic testing for the evaluation of a drug's antiarrhythmic efficacy, may be misleading with respect to its efficacy in preventing the development of ventricular fibrillation. As long as the endpoint remains anything other than sudden cardiac death, any clinical trial or clinical use of a drug in patients determined to be at high risk will be carried out mostly on an empirical basis. An essential component in approaching the development of effective therapeutic interventions for the prevention of sudden cardiac death must include appropriate animal models for early preclinical testing, based on an understanding of the pathophysiologic milieu pertaining at the time of sudden death. At present, the approach to identifying therapeutic interventions for prevention of lethal arrhythmias is predicated on a drug's ability to alter various characteristics of the membrane action potential of cardiac tissues, alterations in ion channel function, or examination of the drug's ability to prevent or terminate one or more experimentally induced dysrhythmias. Unfortunately, few of the experimental approaches have a close relation to several important questions: First, can the agent under study prevent ventricular fibrillation? Second, can it serve as a candidate drug for a highly focused clinical trial in well-characterized subsets of patients who possess identifiable specific etiologic features placing them at risk of succumbing to sudden cardiac death? Lastly, does the therapeutic agent of interest confer an antifibrillatory action under conditions in which the altered substrate (damaged myocardium) is subjected to transient ischemic events? The latter is of primary importance, since a drug capable of preventing or suppressing spontaneous or electrophysiologically induced reentrant rhythms in the nonischemic myocardium may be proarrhythmic or profibrillatory under conditions in which transient ischemic episodes are superimposed on a vulnerable substrate.

THE MYOCARDIAL SUBSTRATE

The essential substrate for reentrant rhythms and ventricular fibrillation: Postmortem studies indicate that in the majority of cases, ventricular fibrillation is a primary event and not related to acute myocardial infarction.[25,26] Sudden cardiac death is known to occur most commonly in patients with previous myocardial ischemic injury secondary to advanced coronary artery atherosclerosis.[27–29] Further, the finding in many cases of intracoronary thrombus without acute infarction suggests that ischemia, per se, may be acting as the trigger for the genesis of ventricular fibrillation in a vulnerable, electrically unstable ventricular myocardium. The electrophysiologic properties of ischemic myocardium, such as increased excitability, shortening of the ventricular refractory period, slowing of conduction velocity, and increased inhomogeneity in recovery may provide the milieu for the emergence of reentrant rhythms in a heart critically deranged by previous infarction. The concept of ischemia in a region remote from the infarct-related artery acting as the trigger for fatal ventricular arrhythmias was addressed by Schuster and Bulkley.[30] In a study of 2 groups of patients with early postinfarction angina, they found that patients with remote ischemia constituted a group of hemodynamically stable patients who faced an unexpectedly high mortality compared with those patients whose angina arose from the peri-infarcted region. Schwartz and coworkers[31,32] reproduced this phenomenon experimentally when they demonstrated a high incidence of ventricular fibrillation in a chronic canine model of myocardial infarction in which additional ischemia was initiated using a hydraulic coronary artery occluder. Also using a canine model, Kabell and coworkers[33] demonstrated a diminution in infarct collateral blood flow with distant ischemia. Since this was preceded by delayed epicardial activity within the

area of preexisting infarction, it suggested that ischemia might be influencing the substrate of an infarcted area of myocardium to render it more suitable for the emergence of lethal arrhythmias.

Coronary vasospasm has been considered as a triggering mechanism for sudden death, especially since patients with atypical angina have demonstrated serious ventricular arrhythmias during episodes of spasm.[34] Although the majority of survivors of cardiac arrest give no previous or subsequent history of atypical angina, in one study sudden death was observed in 17% of 114 patients with coronary vasospasm followed for a mean of 24 months.[35]

The concept that spasm may be an important contributor to sudden cardiac death is supported by data showing the relation between the disease-free wall of the coronary artery and the severity of obstruction.[36,37] The mean disease-free wall arc length measured 17–23% of the total vessel circumference in eccentric coronary lesions that obstructed 50–90% of the cross-sectional area of the lumen. The ratio persisted regardless of the location of the lesion within the vessel. The normal arc may be capable of responding to vasospastic stimuli, as opposed to atheromatous material occupying the bulk of the arc, which appears firm and unlikely to change configuration in response to humoral or neurogenic stimuli. The potential exists for the dynamic alterations in coronary luminal dimensions most likely to occur along the disease-free circumference of the coronary artery. Thus, a clinically silent atherosclerotic lesion could be converted to a clinically symptomatic and possibly lethal lesion by additional spasm in the plaque-free segment of the vessel. Postmortem examination of human coronary artery segments has led to the suggestion that the atherosclerotic process leads to a decrease in density or sensitivity of the β adrenoceptors in the smooth muscle of the coronary arteries.[38] The conclusion was that the intrinsic properties of human coronary smooth muscle may be one of the mechanisms of coronary spasm. There are no unequivocal markers at the time of necropsy to demonstrate that coronary artery spasm preceded the fatal arrhythmia. However, a distinctive finding in smooth muscle of coronary arteries suggestive of antemortem spasm has been reported in which "hypercontraction" of smooth muscle cells may give rise to dense eosinophilic bands, similar to those seen in reperfused cardiac muscle.[39] Coronary artery contraction bands were found in 75% of the cases examined and were more common in vessels with $<50\%$ cross-sectional area obstruction than vessels with $>50\%$ obstruction.

Another mechanism to be considered in the genesis of lethal arrhythmias is the role of the autonomic nervous system. Alterations in autonomic tone are well recognized in acute myocardial ischemia and may be inherently arrhythmogenic by nature of the increase in myocardial oxygen consumption and alterations in refractoriness. Inhomogenous adrenergic stimulation has been shown to precipitate arrhythmias in a number of animal models,[40] whereas others have demonstrated a possible role of the sympathetic nervous system when acute ischemia is produced in the setting of previous myocardial infarction.[31,32,41] Specifically, sympathetic hyperactivity favors the onset of life-threatening cardiac arrhythmias, whereas vagal activation exerts a protective and antifibrillatory effect.[41] Direct neural recording of vagal activity to the heart confirmed that vigorous reflex vagal activation during acute myocardial ischemia is associated with protection from ventricular fibrillation.[42] Other factors contributing to the precipitating trigger in sudden death include those biochemical alterations (such as hypokalemia and hypomagnesemia) that are known to precipitate fatal arrhythmias in individuals at risk. Thus, a variety of factors may predispose the individual at risk to the development of lethal ventricular arrhythmias (Figure 1). Pathophysiologically, sudden cardiac death involves an interaction between structural abnormalities of the heart, transient functional disturbances, and the specific electrophysiologic events responsible for fatal arrhythmias.

ISCHEMIA MUST BE DIFFERENTIATED FROM INFARCTION

There is a period of healing after myocardial infarction in which the necrotic mass of tissue is converted to a dense, fibrous scar. The healed phase of myocardial infarction is characterized by a chronic alteration in myocardial structure that, in itself, is electrically stable. However, the structural abnormality is capable of influencing electrophysiologic parameters when other events are superimposed on the heart. In contrast, ischemia is a transient event due to an absolute or relative reduction in regional myocardial blood flow. The influence of ischemia on a structurally normal heart has a more favorable outcome compared with an ischemic event superimposed on a heart previously subjected to myocardial infarction. There is compelling evidence to indicate that regional myocardial ischemia superimposed on the previ-

ously damaged heart, in contrast to a normal heart, is more likely to precipitate malignant and potentially lethal ventricular arrhythmia.[43–45] Superimposition of an acute nonocclusive thrombus, an imbalance between oxygen supply and demand, metabolic or electrolyte changes, or neurophysiologic influences may establish the conditions necessary to sustain a reentrant rhythm leading to a lethal arrhythmia. Enhanced coronary artery vasomotor activity abruptly decreasing myocardial blood flow in a region remote from a region of previous myocardial infarction may precipitate symptoms of angina, disturbances in rhythm, and sudden cardiac death.[35,46] Platelet aggregation at sites of coronary vessel damage and the release of vasoactive mediators have been implicated as major contributors to the initiation of lethal cardiac arrhythmias.[47–50]

TABLE I Methods for Preclinical Evaluation of Antiarrhythmic Agents

Chemically induced arrhythmias
Aconitine
Hydrocarbon–catecholamine
Barium chloride
Digitalis glycosides
Potassium channel openers
Electrically induced arrhythmias
Ventricular fibrillation/defibrillation threshold
Repetitive ventricular response
Programmed electrical stimulation
Neurally induced arrhythmias
Application of stimuli to the lateral ventricle of the brain
Electrical stimulation of the autonomic nervous system
Emotional- or exercise-induced stress
Ischemia induced arrhythmias
Acute interruption of regional coronary artery blood flow (Harris 1 or 2 stage)
Acute interruption of regional coronary artery blood flow followed by reperfusion
Acute regional ischemia superimposed on a previously infarcted myocardium

PRECLINICAL ASSESSMENT

Antifibrillatory drug efficacy, using relevant experimental models: It is not our intent to discuss the pros and cons concerning the multitude of experimental models used for the study of antiarrhythmic drugs. Table I presents a listing of some of the more commonly used experimental models. The subject has been reviewed in detail in previous publications.[51–54] Whole animal models for the evaluation of antiarrhythmic activity have relied on arrhythmias induced by cardiotoxic agents, electrical stimuli, or arrhythmias associated with coronary artery occlusion, with or without reperfusion.[53] Other approaches include arrhythmias induced by catecholamines[55] or electrical stimuli[56,57] in the subacute phase of myocardial infarction. Although each of these techniques is capable of generating reliable and reproducible arrhythmias, they fail to provide an opportunity to examine the electrophysiologic environment at the time of ventricular fibrillation, or to study pharmacologic interventions aimed at preventing sudden cardiac death. The preclinical development of antiarrhythmic agents should emphasize the importance of designing animal models to address ventricular fibrillation, since it may represent one of the primary rhythm disturbances associated with sudden cardiac death, particularly in the heart altered by the presence of coronary artery disease. In light of the CAST results,[17] animal models that evaluate a drug's capacity to reduce the number of innocent

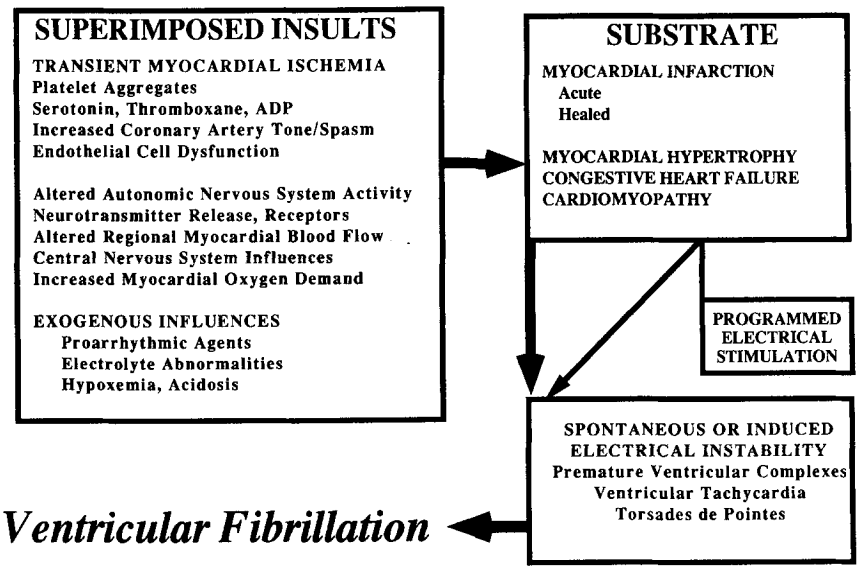

FIGURE 1. Diagram illustrating the potential "insults" capable of contributing to the emergence of fatal ventricular arrhythmias in a heart critically deranged from previous myocardial infarction. The use of programmed electrical stimulation in the postinfarction period is capable of unmasking the electrical instability, which is ultimately responsible for the terminal arrhythmia. (ADP = adenosine diphosphate)

TABLE II Characteristics of the Chronic Canine Model of Sudden Death

Features	Inducible	Noninducible
Anterior infarct size (% left ventricular mass)	24.7 ± 1.7	5.3 ± 1.1*
Time to ischemia (min)	196 ± 39	225 ± 30
Sudden VF (< 1 hour)	11/15	2/15
Delayed VF (< 24 hours)	3/15	0/15
Thrombus mass (mg)	7.2 ± 1.81	11.2 ± 2.3
Posterolateral infarct mass	19.0 ± 1.0 (n = 3)	16.7 ± 2.3 (n = 13)

*p < 0.001.
VF: Ventricular fibrillation.
Summarized from Wilber et al.[58]

ventricular premature depolarizations may be of limited value in new drug development. The ideal drug may be one that is effective against sustained ventricular tachycardia/fibrillation that occurs "spontaneously" in the presence of previous myocardial injury. There may be a clear distinction between antiarrhythmic efficacy and antifibrillatory potential. It may not follow that the latter is simply an extension of the former.

VENTRICULAR TACHYCARDIA AND SUDDEN CARDIAC DEATH

An experimental model: Our group has made extensive use of a conscious canine model that is susceptible to the initiation of stimulus-induced ventricular arrhythmias in the subacute phase of anterior myocardial infarction.[44,53] Of particular interest in this model was the finding that an additional ischemic insult (initiated by a 150 μA anodal current to the left circumflex coronary artery) served as a reliable model for the spontaneous onset of ventricular fibrillation. The study by Patterson et al[44] also demonstrated that previous myocardial damage was a prerequisite for the observed high mortality, since dogs without anterior infarctions exhibited a low risk of ventricular fibrillation. A subsequent study[58] evaluated the model further by looking at the relation between inducible ventricular tachycardia and the subsequent development of ventricular fibrillation. Results suggested that inducible arrhythmias (either sustained or nonsustained) were predictive of spontaneous ventricular fibrillation during posterolateral ischemia. The mass of previously injured myocardium was a critical determinant of both, since animals with inducible arrhythmias (24-hour mortality, 93%) had larger infarct sizes (24.7 ± 1.7% of left ventricular mass) than the animals in which arrhythmias could not be induced at baseline testing (24-hour mortality, 15%; infarct size, 5.3 ± 1.1% of left ventricular mass; Table II). Use of this canine model enabled evaluation of antiarrhythmic activity against arrhythmias thought to share the same reentrant basis as ischemic arrhythmias in humans.[56,59] In addition, the model permits discrimination between antiarrhythmic activity as determined with programmed electrical stimulation versus antifibrillatory activity in the postinfarcted heart subjected to an ischemic event in a region remote from the infarct-related artery.

DETERMINING ANTIFIBRILLATORY ACTIVITY

Experimental procedure: Mongrel dogs of either sex are anesthetized by the intravenous administration of sodium pentobarbital, intubated, and ventilated with room air. Using an aseptic technique, the left jugular vein is isolated and cannulated for subsequent drug administration. A left thoracotomy is performed, and the heart exposed and suspended in a pericardial cradle. The left anterior descending (LAD) coronary artery is dissected free at the tip of the left atrial appendage and the left circumflex (LCX) coronary artery isolated approximately 1 cm from its origin. Anterior wall infarction is achieved by a 2-hour occlusion of the LAD coronary artery followed by reperfusion in the presence of a critical stenosis. An epicardial bipolar electrode is sutured to the left atrial appendage for subsequent atrial pacing. A bipolar plunge electrode is sutured onto the surface of the heart in the region of the right ventricular outflow tract (RVOT) for the subsequent introduction of extrastimuli during programmed electrical stimulation. In addition, 2 bipolar plunge electrodes are sutured to the left ventricular wall; 1 in the distribution of the LAD coronary artery distal to the site of occlusion (infarct zone) and the second in the distribution of the LCX coronary artery (normal zone). Finally, a 30-gauge electrode is inserted into the lumen of the LCX coronary artery and secured by suturing to the heart wall. Figure 2 is a schematic representation of the instrumented canine heart as used in the model of sudden cardiac death.

Programmed electrical stimulation and electrophysiologic testing are performed in the conscious, unsedated animal, 3–5 days after surgical preparation. After determination of the RVOT excitation threshold and refractory period, programmed stimulation continues with the introduction of double (S2, S3) and triple (S2, S3, S4) extrastimuli (4 msec duration at twice RVOT excitation threshold) during sinus rhythm. Previous studies indicated that these stimulation methods will not induce arrhythmias in sham-operated animals.[44]

Electrophysiologic parameters from normal and infarcted myocardium are determined from the construction of strength–interval curves using data obtained from the normal zone and infarct zone electrodes, respectively. Dogs with sustained or nonsustained ventricular tachycardia are allocated randomly to drug or vehicle groups, and electrophysiologic testing and programmed stimulation are repeated in full after drug equilibration.

On completion of the post-treatment stimulation protocol, a direct anodal current of 150 μA is applied to the intimal surface of the LCX coronary artery using a 9 V nickel-cadmium battery and variable resistor. Application of an anodal current to the intimal surface of the vessel results in injury and exposure of the underlying collagen matrix. Platelet aggregates form on the denuded surface of the coronary artery, accompanied by cyclic variations in blood flow and a high incidence of acute ventricular fibrillation within 1 hour from the onset of ischemia as determined by depression or elevation in the ST segment of the electrocardiogram.

Lead II of the electrocardiogram is recorded at preset intervals (30 sec every 15 min) by a programmable cardiocassette recorder. After 24 hours of continuous application of the anodal current or the development of ventricular fibrillation, the heart is excised and any thrombus in the LCX coronary artery is removed and weighed. The heart is sectioned transversely and incubated in a 0.4% solution of triphenyltetrazolium chloride for 15 min. Anterior and posterolateral areas of infarction are identified by their inability to reduce triphenyltetrazolium chloride enzymatically to a brick-red formazan precipitate. Infarct masses in the myocardial regions are quantified by computer-assisted planimetry and expressed as a percentage of total left ventricular mass. Playback of the cardiocassette provides information regarding the time of onset of ischemia (as assessed by the appearance of ventricular ectopy and/or ST-segment changes), the time from ischemia to death, and the percent change in heart rate before death. When last tabulated, a total of 201 inducible, vehicle-treated dogs had been studied in our laboratory; of these, 188 (94%) had died within 24 hours of posterolateral ischemia in the sudden death protocol.[60]

The results of various pharmacologic interventions in the conscious canine model of sudden death are summarized in Table III. The data in Table III illustrate the dichotomy of action of many antiarrhythmic agents when tested both against the arrhythmias of programmed electrical stimulation and in their effects against ischemic ventricular fibrillation. Based on these observations, it could be concluded that there is little, if any, value in predicting antifibrillatory efficacy from a drug's effect on stimulus-induced ventricular tachycardia. It can be seen that clinically relevant plasma concentrations of the class IA agent quinidine were capable of preventing the induction of stimulus-induced arrhythmias, but were ineffective in preventing ventricular fibrillation[61]; conversely, if we ignore for the present the confounding issues with sotalol, β-adrenergic receptor blockade appears to be offering some degree of protection in the sudden death model without influencing stimulus-induced ventricular tachycardia,[54,62–64] a phenomenon shared by the specific bradycardic agent alinidine.[65] Studies with 2 calcium channel antago-

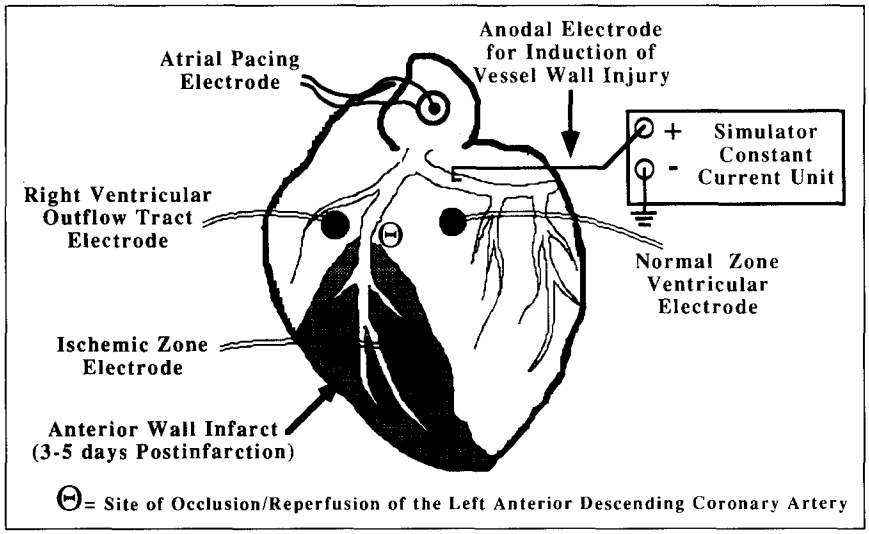

FIGURE 2. The conscious canine model of sudden death (surgical preparation). Anterior myocardial infarction is produced by a 2-hour occlusion of the left anterior descending coronary artery, with subsequent reperfusion in the presence of a critical stenosis. An atrial bipolar epicardial electrode is illustrated, as well as bipolar plunge electrodes in normal myocardium (normal zone), infarcted tissue (infarct zone), and the right ventricular outflow tract. The latter is then used for the introduction of extrastimuli during programmed electrical stimulation, 3–5 days after surgery. A silver wire electrode is illustrated within the lumen of the left circumflex coronary artery. Final introduction of a 150 μA anodal current results in acute posterolateral ischemia and a high incidence of ventricular fibrillation in the sudden death protocol.

TABLE III Drug Efficacy in the Conscious Canine Model of Myocardial Infarction and Sudden Death

Agent	Ventricular Tachycardia Suppression (Programmed Stimulation)	24-Hour Survival (%)
Vehicle	–	6*
Class I		
Quinidine	+	9
Flecainide	–	14
Class II		
Nadolol	–	56–63
Dilevalol	–	20–60
Sotalol	+	63
Celiprolol	–	30
Class III		
D-Sotalol	+	65
Bretylium	+	60
Amiodarone	–	40–80
Clofilium	+	30
CK 3579†	–	70
Sematilide	+	60
E-4031	–	60
UK 68,798	–	42
Class IV		
Diltiazem	–	10
Bepridil	+	30–40
Other		
Alinidine	–	60
Meobentine	–	0
Bethanidine	–	0
Prazosin	–	50
CGS 12970‡	–	30
R 68070§	–	30

*Cumulative.
†Class III with β_1 adrenoceptor antagonist properties.
‡Thromboxane synthetase (TS) inhibitor.
§Combined TS inhibitor and receptor antagonist.
Adapted from: Lynch and Lucchesi.[54]

nists show bepridil to be antiarrhythmic without affecting mortality,[66] whereas diltiazem did not demonstrate any beneficial effect.[67] Studies with other agents not covered by the Vaughan Williams classification offer little additional insight into the antiarrhythmic or antifibrillatory relation.[68–72] Even in the group with the greatest overall effect in the sudden death model, only half of the class III agents tested demonstrated a correlation with antiarrhythmic activity. Thus, it would seem reasonable to conclude that there is little prognostic value from suppression of ventricular tachycardia in this model, other than the ancillary electrophysiologic data obtained at the time of testing, which frequently provide an insight into potential antifibrillatory mechanisms.

CONSCIOUS CANINE MODEL OF SUDDEN CARDIAC DEATH

Clinical significance: A striking observation from Table III is the apparent lack of activity of the class I agents in preventing sudden death in the model. In light of what we have learned from CAST, the study with flecainide was particularly interesting in that 3 of 7 animals, noninducible at baseline and therefore at low risk from posterolateral ischemia, failed to survive the sudden death protocol.[73] Thus potential profibrillatory activity with flecainide had been suggested on the basis of preclinical studies several years before CAST. The class IC agents are characterized by their ability to slow conduction velocity with only minimal effects on the duration of the refractory period of the ventricular myocardium. Flecainide in particular is of interest in that it increases the ventricular effective refractory period and, to a lesser extent, the action potential duration. On the other hand, in Purkinje fibers, action potential duration is decreased as flecainide concentration increases.[74] In contrast to the actions of other antiarrhythmic agents that are sodium channel inhibitors, flecainide, like encainide, exerts a differential effect on repolarization in ventricular muscle and Purkinje fibers, an effect that is likely to aggravate heterogeneity of excitability and refractoriness on the heart and may worsen ventricular tachyarrhythmias under certain experimental or clinical situations.[74] Depending on the length of the reentrant circuit, slowing of conduction velocity without a coincident lengthening of the refractory period may result in multiple reentrant circuits.[75] Quinidine, as well as procainamide, 2 class IA antiarrhythmic agents, produce a prolongation of refractoriness and a rate-dependent depression of conduction velocity. The precise role of these electrophysiologic effects in mediating an antiarrhythmic action is not clear. Studies with procainamide[76] indicate that lesser slowing of conduction velocity and greater prolongation of refractoriness tend to abolish reentry within the reentrant circuit. Drugs that prolong refractoriness appear more likely to be effective against tachycardia caused by reentry than are drugs that produce a slowing of conduction velocity as their major electrophysiologic effect.[77]

The canine model of sudden cardiac death successfully identified the proarrhythmic action of flecainide. The antiarrhythmic and antifibrillatory effects of flecainide acetate during the early postinfarction period were evaluated in the conscious canine model of sudden cardiac death. Ventricular tachycardia remained inducible early after infarction in 8 of 9 dogs receiving an intravenous loading dose of flecainide (2.0 mg/kg body weight) and 7 of 8 dogs receiving saline vehicle. In both the drug and vehicle groups, there was no significant change in the ventricular refractory period or in the cycle length of the induced ventricular tachycardia. With a maintenance intravenous infusion of flecainide,

1.0 mg/kg/hr for 4 hours, the subsequent development of acute posterolateral ischemia resulted in ventricular fibrillation and sudden death in 7 of 8 flecainide-treated and 8 of 8 vehicle-treated dogs. Seven additional postinfarction dogs with noninducible tachycardia during pretreatment programmed stimulation, and thereby considered to be at low risk for the development of ischemic ventricular fibrillation,[58] were given flecainide in an intravenous loading and maintenance dosing regimen. The subsequent occurrence of posterolateral ischemia resulted in the development of ventricular fibrillation in 3 of these 7 dogs. The findings suggest that flecainide acetate may not possess pharmacologic properties useful in managing ventricular tachycardia or in preventing ischemic ventricular fibrillation in the presence of recent myocardial damage.[73,78]

The only pharmacologic intervention shown to have a beneficial effect on sudden death is β-adrenergic receptor antagonism, where a number of studies in the postmyocardial infarction period have confirmed significant protection.[79–83] In this context there also appears to be a good correlation with the conscious canine model of sudden death, since protection has been demonstrated with a number of agents.[54,63,64,84] The antiarrhythmic and antifibrillatory potential of β-adrenoceptor antagonism, however, remains unclear; in particular, there is uncertainty over whether the drugs act by a direct antifibrillatory effect or via a primary antiischemic influence. This point is pursued in the following section.

Although no individual study with a class III agent has yet demonstrated significant antifibrillatory activity, suggestions of a beneficial trend are apparent in a recent meta-analysis.[85] The authors conducted an overview of randomized controlled trials of classes I and III antiarrhythmic agents and updated earlier overviews on classes II and IV antiarrhythmic drugs. A total of 137 trials involving 96,000 patients made up the study population. It was concluded that mortality was increased significantly with class I antiarrhythmic agents, reduced with classes II and III, and not significantly altered with class IV drugs. The data suggest that amiodarone and β-adrenoceptor blocking agents are the only drugs likely to *reduce mortality* while other agents may be ineffective or may actually increase the likelihood of a fatal arrhythmia. With the exception of the ESVEM trial mentioned earlier,[24] most of the major ongoing studies are with amiodarone, with European and Canadian postmyocardial infarction trials, and 2 placebo-controlled trials in heart failure: the Veterans Affairs Congestive Heart Failure trial of antiarrhythmic therapy and the group study of heart failure survival in Argentina (GESICA).

Pharmacologic protection: direct or indirect?

Except where ancillary electrophysiologic properties are part of a particular agent's pharmacologic profile, the β-adrenergic receptor antagonists, as a group, appear to be devoid of a direct effect on the heart. Despite this, several studies have reported significant antiarrhythmic effects with these drugs, both in clinical[86] and in experimental[87,88] studies. Our laboratory has examined several β-adrenoceptor blocking agents for potential antiarrhythmic and antifibrillatory activity in the canine model of sudden cardiac death. Nadolol, a noncardioselective agent, was studied in the sudden death protocol after pretreatment with 1 ($n = 9$) and 8 ($n = 13$) mg/kg. Respective survival figures were 56% and 63% ($p < 0.01$ vs placebo).[84] D-Nadolol, an optical isomer devoid of β-adrenoceptor blocking properties, was ineffective. An interesting feature in this study was the observation that the majority of nadolol-treated dogs that died, did so, not from ventricular fibrillation, but as the result of complete heart block, severe bradycardia, and/or pump failure. This phenomenon was also observed in subsequent studies with dilevalol, the *R,R*-enantiomer of labetalol, where 75% of deaths were accompanied by severe bradyarrhythmias.[54,63] The administration of methylscopolamine to postinfarction animals pretreated with dilevalol, however, significantly reduced mortality (40% vs 100% vehicle-treated; $p < 0.05$), suggesting that dilevalol, like nadolol, was capable of preventing ischemic ventricular fibrillation in this model, but that death was due to the unopposed effects of parasympathetic influences plus the inability of the sinoatrial node to manifest a positive chronotropic action due to the presence of β-adrenoceptor inhibition.

In a series of experiments with celiprolol, a class II drug with intrinsic stimulant properties, it was of significance that the drug was without effect in preventing sudden cardiac death.[64] In particular, ventricular fibrillation was responsible for each of the 7 deaths in the drug-treated group. Although the model is not designed specifically to address the question of intrinsic cardiostimulant phenomena, it was noted that resting heart rate did not change after celiprolol administration, and it is possible that this feature of the drug attenuated any protection during acute posterolateral ischemia. It has been demonstrated, for example, that the propensity of sympathetic stimulation to in-

duce arrhythmias in the late myocardial infarction period may relate primarily to heart rate.[89] Previous studies have shown antagonism of the antiarrhythmic protection afforded by propranolol by overdrive atrial pacing.[90] These studies also reported that β-adrenoceptor antagonists demonstrated an almost linear relation between the reduction in resting heart rate and mortality and noted that drugs with intrinsic sympathomimetic activity produced small reductions in heart rate and lesser effects on mortality.[62] Although it is unclear whether celiprolol's stimulant properties are due entirely to partial agonism,[91] intrinsic sympathomimetic activity is cited as a possible reason why the drug failed to exert a beneficial influence on ventricular arrhythmias in a group of patients with acute myocardial infarction.[92]

In an attempt to clarify the role of heart rate in the genesis of sudden death, we evaluated the antifibrillatory effects of alinidine, the N-allyl derivative of clonidine. Alinidine is one of a number of agents in which the main pharmacologic action appears to be a reduction in heart rate from a direct action on the sinus node.[93,94] Although capable of attenuating the chronotropic response to isoproterenol, these drugs do not operate by antagonism of β-adrenoceptors.[93,95] Similarly, there is no evidence that the specific bradycardic action involves α-adrenergic or muscarinic receptors, or calcium channels.[93,95,96] However, studies in isolated tissues have shown a nonvoltage-dependent decrease in the slope of the slow diastolic depolarization, indicating that the drugs' effects may be mediated by restriction of current through anion-selective channels.[96] In the canine model of sudden cardiac death, alinidine (1 mg/kg) produced a significant ($p < 0.01$) decrease in resting heart rate and prevented ventricular fibrillation in 6 of 10 animals studied ($p < 0.05$ vs concurrent placebo group). In a third group of dogs in which constant atrial pacing maintained heart rates at predrug values throughout the sudden death protocol, mortality was 100% despite pretreatment with alinidine.[65] No changes were observed on parameters of ventricular refractoriness or conduction velocity.

Bradycardic agents, such as the β-adrenoceptor antagonists, are capable of increasing perfusion pressure distal to a coronary artery stenosis,[97] an effect that, for the bradycardic agents at least, appears to be attenuated by atrial pacing to control (predrug) heart rate values.[98] Thus, during posterolateral ischemia, drugs with a negative chronotropic action may contribute to an enhanced collateral flow in the ischemic bed secondary to slowing of heart rate, prolongation of diastole, and presumed reduction in myocardial oxygen consumption.

An additional property of the β-adrenoceptor antagonists is their ability to attenuate the potentially deleterious influence of enhanced adrenergic stimulation. In this respect, it is interesting to consider results in the sudden death model with the α_1-adrenoceptor antagonist prazosin. Despite an inability to alter electrocardiographic intervals, ventricular refractoriness, or the induction of ventricular tachycardia by programmed stimulation, pretreatment with 500 μg/kg of prazosin resulted in a 50% survival rate in the sudden death protocol ($p < 0.05$ vs placebo).[70] This may be of particular significance in view of the recent suggestion that α-adrenergic responsiveness may be enhanced under conditions of myocardial ischemia[99] and that this is correlated with an increase in α-adrenoceptor concentration.[100] Although the relative contributions of α- and β-adrenergic influences in the genesis of ventricular fibrillation remain unclear, it has been suggested that α-mediated prolongation of action potential duration in ischemic areas may combine with β-mediated shortening of action potential duration in nonischemic areas to increase disparity in refractory periods and produce the arrhythmogenic milieu suitable for the emergence of fatal reentrant pathways.[101] Antagonism of either adrenergic pathway (by the respective adrenergic antagonist) could therefore be seen as an indirect reduction in the electrophysiologic derangements leading to ventricular fibrillation and explain the protection afforded by both the β-adrenoceptor antagonists and prazosin in the animal model of sudden cardiac death.

In identifying a common direct electrophysiologic characteristic for antifibrillatory efficacy in the experimental model of sudden cardiac death, it becomes apparent that the greatest overall protection has been seen with agents that have as part of their pharmacologic profile prolongation of the action potential duration (class III activity). Studies with bretylium,[102] amiodarone,[103] sotalol,[104,105] and a number of experimental agents[106–108] have demonstrated significant protection in placebo-controlled studies. The effects of clofilium, an alternative class III drug, were less clear[109] and may relate to a failure to provide an appropriate dosing regimen.

Bretylium was introduced into clinical cardiology in the early 1980s and is currently one of the few drugs marketed as an antifibrillatory agent. Its electrophysiologic properties include direct effects

on cardiac action potential duration and indirect effects mediated via its actions on the autonomic nervous system. Early studies with the drug demonstrated suppression of stimulus-induced ventricular tachycardia[110,111] and elevation in ventricular fibrillation thresholds.[112] In the sudden cardiac death model, bretylium (10 mg/kg intravenously every 6 hours) resulted in significant prolongation of ventricular refractoriness and the survival of 6 of the 10 animals studied ($p < 0.05$ vs placebo). The exact antifibrillatory mechanism of the drug, however, remains obscure; although bretylium has been shown to exert similar electrophysiologic effects in the denervated heart,[113] the significance of its autonomic effects on the development of ventricular fibrillation is unknown. Further, studies with bethanidine[68] and meobentine[69] failed to prevent ventricular fibrillation and sudden death in the same model, despite similar structural and electrophysiologic characteristics.

Amiodarone originally was introduced as an antianginal agent, but subsequently was found to have electrophysiologic features attributable to each of the 4 classes of antiarrhythmic action.[114-116] In addition, the drug reduces the inotropic and chronotropic responses of other agents and has vasodilatory effects on the coronary and systemic vasculature.[117] Its outstanding property, however, is prolongation of the cardiac action potential, prompting its identification as a potential antifibrillatory agent. Despite the observation that alterations in action potential duration and ventricular refractoriness are apparent only with chronic dosing, studies in our laboratory have demonstrated significant antifibrillatory protection after both long- and short-term oral therapy. Although no differences were observed in plasma or myocardial concentrations of amiodarone between the 2 dosing regimens, the greater survival in those animals treated for 10 days (80% vs 60% treated acutely) suggests that long-term therapy may have additional, as yet unidentified actions contributing to greater efficacy. It is known that the electrophysiologic effects of amiodarone resemble closely those of hypothyroidism[118,119]; that this is not due to the iodine moiety of the drug has been shown in experiments where the administration of iodine has had no effect on cardiac action potentials.[120] However, the concomitant administration of amiodarone and thyroid hormone has prevented the repolarization changes seen with amiodarone alone, and thyroidectomy can protect postinfarction animals from ischemic ventricular fibrillation in the sudden death protocol.[121]

The effects of sotalol and its dextrorotatory enantiomer, D-sotalol, have been of particular importance in correlating the antifibrillatory potential of pharmacologic agents with their known electrophysiologic characteristics. Racemic sotalol is a noncardioselective β-adrenoceptor antagonist that produces a dose-dependent prolongation of action potential duration without associated class I (membrane-stabilizing) properties; D-sotalol, however, while retaining the same cardiac electrophysiologic profile, does not share to the same extent the parent compound's β-adrenoceptor blocking properties. The use of D-sotalol allows an assessment of the relative antifibrillatory action of the drug's direct electrophysiologic effects, divorced from the confounding influence of β-adrenoceptor antagonism. Initial studies with racemic sotalol demonstrated a 65% survival in animals treated with the drug and entered into the sudden death protocol.[104] The protective effect was associated with significant prolongation of the QT interval (an electrocardiographic parameter of action potential duration) and bridging diastolic electrical activity of the lead II electrocardiogram, a phenomenon invariably followed by ventricular fibrillation in vehicle-treated animals. In a subsequent study with the D isomer, Lynch and coworkers[105] demonstrated similar electrophysiologic and antifibrillatory effects, but without the attenuation of the ischemia-induced increase in heart rate seen with the parent compound. This suggested that the observed antifibrillatory effect of D-sotalol was not related to antagonism of the β-adrenoceptor, but stemmed directly from prolongation of action potential duration and the increase in the ventricular refractory period.

More recent studies from this laboratory have reinforced the positive trend seen with agents sharing the ability to prolong ventricular refractoriness. The experimental agents CK-3579 and sematilide,[106] E-4031,[107] and UK-68,798[108] have produced protection in placebo-controlled studies in the canine model of sudden cardiac death.

CLASS III ANTIARRHYTHMIC AGENTS

One activity, but uncertain mechanisms: The Vaughan Williams classification was the first serious effort to classify antiarrhythmic agents based on what was known regarding common electrophysiologic characteristics of the available drugs in the early 1970s.[122] It is widely recognized, however, that the classification is not without major inadequacies, not least of which being that the system is essentially a hybrid: Classes I and IV represent

agents that impair ion channels; class II agents inhibit receptors; and class III agents change an electrophysiologic variable (the action potential duration).[123] Although actual mechanisms contributing to the class III effect were not known 20 years ago, the common feature now appears to be interruption of normal potassium efflux by antagonism of one or more of the potassium channels.[124,125] With the increased understanding of the role of various potassium channels in health and disease has come an explosion of publications on the subject and an ever-increasing number of newly discovered channels in various organ systems.[126]

POTASSIUM CURRENTS THAT PREDOMINATE DURING ALTERED METABOLIC STATES

Abnormalities of membrane function arise in response to myocardial ischemia or hypoxia and favor the development of slow conduction and unidirectional block. Both conditions are essential for establishing a reentrant pathway capable of supporting ventricular tachyarrhythmias. Among the electrophysiologic changes observed in the ischemic or hypoxic tissue is the abrupt increase in extracellular potassium concentration accompanied by intracellular acidosis and a decrease in tissue adenosine triphosphate concentration. Myocardial hypoxia is associated with K^+ efflux from cardiac myocytes and a shortening of the action potential duration.[127,128] A mechanism involving opening of K^+ selective channels during ischemia has been proposed to account for ischemia-induced myocyte K^+ loss. In addition, conditions of metabolic inhibition, as with ischemia or hypoxia, lead to the liberation of free fatty acids and a gain in intracellular sodium and calcium ions. The decrease in tissue adenosine triphosphate content, the increase in tissue free fatty acids, and the gain in intracellular sodium and calcium ions, each activate separate potassium channels. The single-channel conductance of the K^+ channels activated under pathophysiologic conditions is greater than that occurring when K^+ channels are operative under normal conditions. Conditions of altered myocardial metabolism resulting from hypoxia and/or ischemia would favor outward rectification, so that the outward current predominates over the inward current. Significant outward current would flow during depolarization, resulting in a decrease in the action potential duration. The accumulation of K^+ in the extracellular space will depolarize the myocardial cell membrane. The net result of the local ionic events is to decrease conduction velocity and shorten the effective refractory period, which in the presence of a suitable myocardial substrate has the potential to result in a lethal arrhythmia. The 3 K^+ channels that predominate under pathophysiologic conditions may act synergistically to favor outward rectification and provide the conditions needed for reentry. The most widely studied of the 3 pathophysiologic channels in the heart is the adenosine triphosphate-dependent potassium channel (K_{ATP}).

With the discovery of a K_{ATP} channel regulating insulin release in the pancreatic islet β cells,[129] and its subsequent determination in the heart,[130] came the realization of a cardiac channel active only in pathologic (hypoxic or ischemic) circumstances, where it could potentially play a crucial role in the genesis of fatal reentrant arrhythmias. Further, the functional or active K_{ATP} channels would become manifest only in myocardial cells in which intracellular adenosine triphosphate was decreased. The concept was supported by the finding that glyburide, a sulfonylurea that (like all members of its class) exerted an antidiabetic effect by promoting the closure of pancreatic K_{ATP} channels, could also reverse the electrophysiologic consequences of ischemia in isolated myocardial cells.[131] At about the same time, independent research had demonstrated that glyburide was effective in preventing the development of ventricular fibrillation in isolated heart preparations under conditions of low intracellular adenosine triphosphate, whether the result of ischemia[132,133] or hypoxia.[134] When hearts are made hypoxic or ischemic in the presence of glyburide, the potassium loss during the early phase is partially blocked by the glyburide.[135–137] Based on these observations, it appears that part of the potassium loss during hypoxia or ischemia can be attributed to activation of the K_{ATP} channel, although other mechanisms may be involved, such as the Na^+-activated and fatty acid sensitive K^+ channels. Thus, arachidonic acid and its metabolites, as well as other unsaturated fatty acids, can modulate K^+ channel activity. Understanding the manner in which pharmacologic interventions modulate the several potassium channels in the ventricular myocardium is complicated by the fact that there seems to be an interaction among the activity of potassium-channel modulators and tissue metabolites. For example, the effectiveness of glyburide to block the K_{ATP} channel depends on the cytosolic concentration of adenosine diphosphate,[138] whereas the ability of pinacidil to act as an opener depends on the cytosolic content of adenosine triphosphate.[139,140] There is no doubt that the tissue content of both adenosine triphos-

phate and adenosine diphosphate will be altered during intervals of hypoxia and ischemia as well as on reperfusion. Thus, alterations in tissue metabolites will influence the final outcome of any experimental protocol and may account for incongruous results among laboratories, because the content of tissue metabolites may vary according to the particular experimental protocol employed.

Our laboratory has confirmed the antifibrillatory effect glyburide in the rabbit isolated heart made hypoxic in the presence of pinacidil[141] (Figure 3). Pinacidil promotes intracellular potassium efflux and significantly reduces action potential duration via an agonist effect on K_{ATP} channels.[139,142] In the normoxic heart under atrial pacing, pinacidil is without discernible effects on cardiac rhythm, despite the fact that a significant decrease in ventricular effective refractory period occurs, presumably due to opening of the K_{ATP} channel. However, in the presence of pinacidil, but less likely in its absence, ventricular fibrillation occurs in >80% of hearts made hypoxic for 12 minutes or occurs shortly after the heart is reoxygenated.[141] The induction of ventricular fibrillation in the presence of pinacidil is dependent on a decrease in myocardial cell adenosine triphosphate content (Figures 4 and 5), thereby suggesting that the myocardial K_{ATP} channel shows increased responsiveness to the agonist effects of pinacidil when it is disinhibited as a result of decreased cellular adenosine triphosphate. Glyburide, known for its ability to inhibit the K_{ATP} channel, prevents the pinacidil-induced decrease in the effective refractory period and significantly reduces the incidence of ventricular fibrillation in the hypoxic/reoxygenated perfused heart.[141] The profibrillatory action of pinacidil is unmasked by myocardial hypoxia or ischemia, either of which will decrease myocardial cell adenosine triphosphate content. It is anticipated that a lowering of myocardial adenosine triphosphate will favor opening of the K_{ATP} channel, especially in the presence of the agonist pinacidil. The 2 events, lowering of cellular adenosine triphosphate and further opening of the K_{ATP} channel by the agonist pinacidil, would favor the rapid outward movement of potassium and a marked decrease in the ventricular refractory period.

It is proposed that a special binding site is located on the intracellular side of the membrane by which adenosine triphosphate closes the K_{ATP} channel.[143] Opening of the K_{ATP} channel occurs when intracellular adenosine triphosphate content is reduced. Under conditions of reduced intracellular adenosine triphosphate, pinacidil is more likely to facilitate an opening of the K_{ATP} channel, thereby enhancing an effect similar to that of a reduced intracellular adenosine triphosphate concentration. It has been suggested that pinacidil opens the K_{ATP} channel by antagonizing adenosine triphosphate binding or that pinacidil binds to a different site and modulates the affinity of the receptor to adenosine triphosphate by a pseudocompetitive action.[144] Consistent with this explanation is the observation that only channels closed by low concentrations of adenosine triphosphate could be opened by potassium channel agonists.[139,145] Therefore, the "open" probability of the K_{ATP}

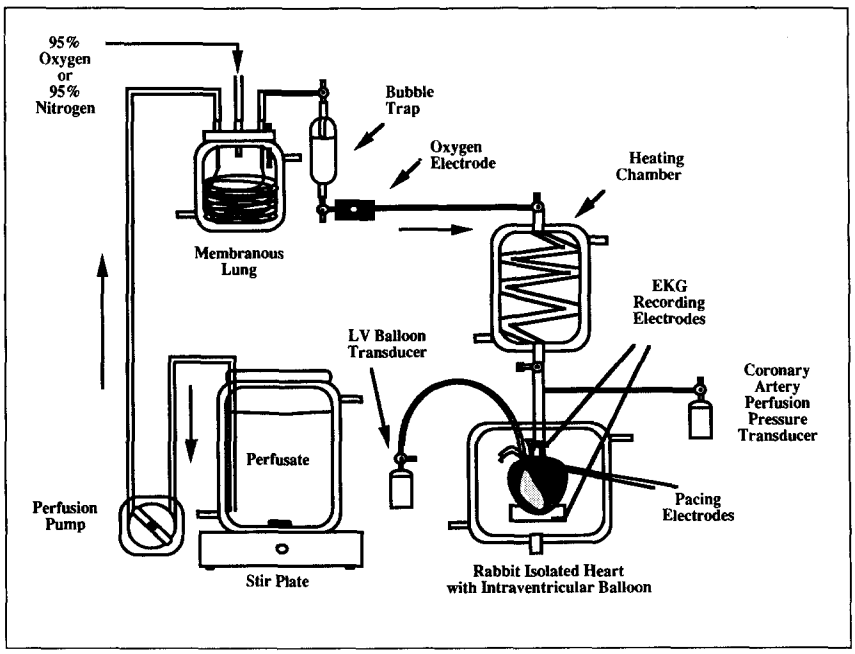

FIGURE 3. Schematic representation of the experimental model for perfusion of the rabbit isolated heart under conditions of varying oxygen tension. The use of a "membrane lung" allows for the rapid change in the oxygen tension of the perfusion medium. After a period of equilibration under normoxic perfusion, the hearts are subjected to 12 minutes of hypoxia and then reoxygenated for 40 minutes. The test drugs are added to the perfusion medium before the induction of hypoxia. Heart rate is maintained constant by atrial pacing and coronary perfusion is not altered throughout the study protocol. (EKG = electrocardiogram; LV = left ventricular)

channel can be significant in the absence of adenosine triphosphate and can be influenced further by the interaction of cofactors formed during ischemia or hypoxia, as discussed above.

The possibility must be entertained that during ischemia there may be a finite probability for the channel to open. Half-maximal sensitivity of the channel increases 4-fold by the addition of adenosine diphosphate and guanosine diphosphate in concentrations known to exist during metabolic inhibition or ischemia.[146,147] A significant open probability of the K_{ATP} channel may be expected under appropriate conditions, even with millimolar concentrations of adenosine triphosphate.[147,148] The K_{ATP} channel-dependent action potential shortening is likely to occur if adenosine triphosphate concentration falls below normal levels (approximately 5 m*M*), as may happen regionally or globally during myocardial ischemia.[149] The known relation between cellular adenosine triphosphate concentration and the functioning of the K_{ATP} channel would suggest that selective channel agonists should be more effective in the ischemic heart than in normal myocardium. Our observations in the intact postinfarcted canine heart and in the hypoxic perfused heart would support the conclusion that K_{ATP} channel openers, while of no deleterious consequence during normal oxygenation, become profibrillatory under conditions of metabolic inhibition leading to a decrease in intracellular adenosine triphosphate concentration.[133,141,150,151]

Confusion arises over whether it is more advantageous to restore an abnormally shortened action potential to a normal action potential by application of a K_{ATP} channel closer (e.g., glyburide) or to shorten further the action potential in cardiac cells by administration of a K_{ATP} channel opener (e.g., pinacidil). Studies designed to examine myocardial recovery of contractile function suggest the latter alternative as the more desirable course of action to prevent the deleterious effects of ischemia and preserve the viability of cardiac cells and recovery of contractile function.[152–156] Future studies must address the issue of whether it is more important to preserve contractile function at the risk of jeopardizing electrophysiologic properties of the heart subjected to metabolic inhibition. Will inhibitors of the K_{ATP} channel, other than glyburide, have the same undesirable effects on recovery of function? To date, class III antiarrhythmic agents known to inhibit the delayed rectifier current have not been

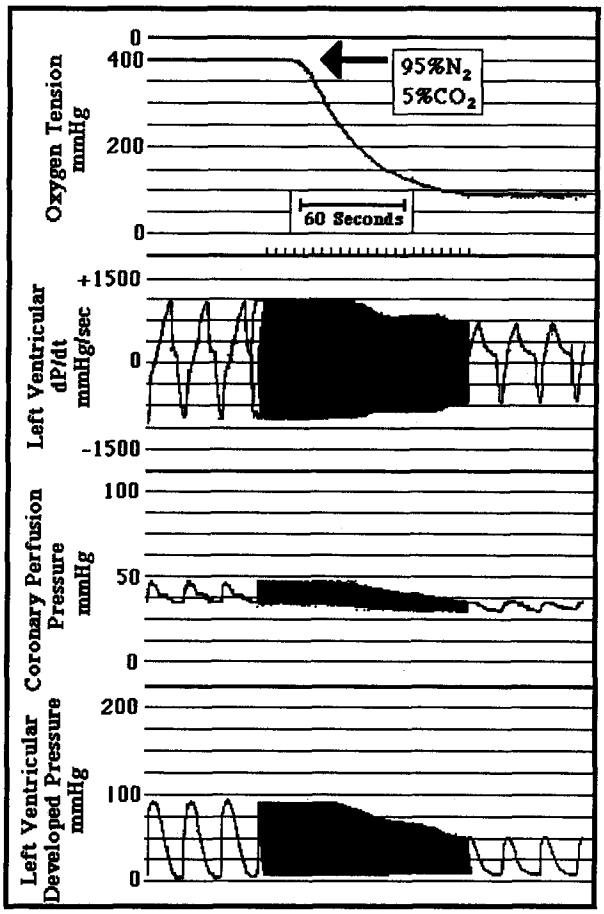

FIGURE 4. Representative recording from the rabbit isolated heart during normoxic and hypoxic perfusion. The tracings from above downward represent: oxygen tension of the perfusion medium, left ventricular ±*dP/dt*, coronary perfusion pressure, and left ventricular developed pressure. The functional effects of changing the gas mixture in the artificial lung become evident within 1 minute as manifested by a decrease in ±*dP/dt*, coronary artery perfusion pressure, and left ventricular developed pressure. Myocardial tissue content of adenosine triphosphate is reduced approximately 50% during the 12-minute exposure to hypoxic perfusion.

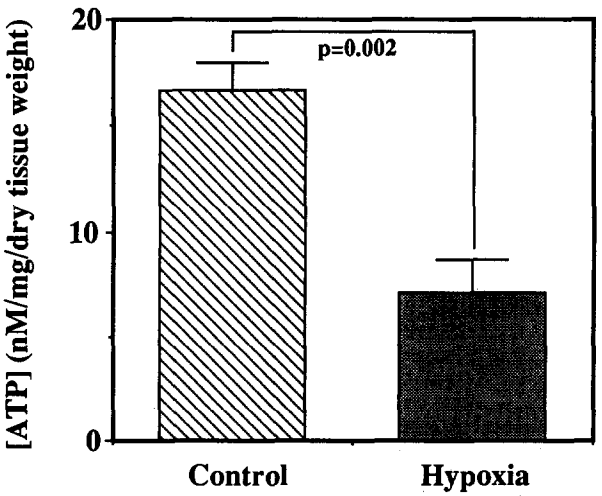

FIGURE 5. Myocardial tissue adenosine triphosphate (ATP) content was determined in a control group of hearts perfused under normoxic conditions and in a second group of hearts subjected to 12 minutes of hypoxic perfusion. The reduction in tissue adenosine triphosphate content was significant when compared with the controls.

shown to influence ischemic myocardial injury[157] but in contrast may exhibit a positive inotropic action as assessed by the measurement of dP/dt.[158] The class III antiarrhythmic agents differ significantly from glyburide with respect to modulating the effects of ischemia on myocardial tissue, since the latter has been reported to have a negative influence on functional recovery and tissue viability after ischemic myocardial injury.[152,159]

Pharmacologic interventions directed at blocking the K_{ATP} channel, thereby preventing a decrease in the ventricular refractory period, may provide a useful approach to the prevention of ventricular fibrillation, without necessarily possessing antifibrillatory activity as manifested by the reduction or prevention in premature ventricular depolarizations. Based on these observations, it is suggested that inhibition of the K_{ATP} channel, by preventing potassium efflux, will antagonize reductions in the action potential duration and will prevent the shortening of refractoriness in ischemic and adenosine triphosphate-depleted myocardial cells. By so doing, disparity in refractory periods can be avoided and with it the risk of emergent ventricular reentrant arrhythmias.

Using a cohort of small-infarct, noninducible dogs similar to those described above in the flecainide study, we evaluated the profibrillatory action of pinacidil in the sudden cardiac death model. Compared with a 24-hour mortality in the placebo group of 20% (incidence of ischemic ventricular fibrillation, 6.7%), mortality in the pinacidil group was 87% (ischemic fibrillation, 60%), a difference statistically significant at the $p < 0.01$ level. Changes in arterial blood pressure did not reach statistical significance, indicating that the profibrillatory effect could not be explained on the basis of hypotension.[150] These studies provide further support for the pivotal role of the K_{ATP} channel in the genesis of fatal cardiac arrhythmias. In the search for the K_{ATP} channel antagonist to be developed as the first potential antifibrillatory agent, the hypoglycemic properties of the sulfonylureas make their evaluation particularly difficult in the intact animal. However, a number of unrelated compounds claim to have K_{ATP}-blocking activity as part of their pharmacologic profile; one in particular, 5-hydroxydecanoic acid, purports to be a "pure" K_{ATP} channel antagonist and appears to attenuate ischemic ventricular fibrillation in the rat heart.[160] The preliminary results with 5-hydroxydecanoic acid require further evaluation in appropriate in vivo models.

Most of our understanding of the channel blocking actions of class III antiarrhythmic agents is derived from voltage clamp studies. Such studies provide a clear demonstration of an hypoxia-induced increase in time-independent K^+ current as being the important factor in shortening of the ventricular action potential. Although the derived information is promising, there remains a void in our knowledge concerning the relation of the observed electrophysiologic and ionic changes to the onset of malignant disturbances in cardiac rhythm under conditions that approximate the clinical situation of sudden cardiac death. The use of the canine model of sudden cardiac death has proved valuable in bridging the gap between the electrophysiologic studies and events as they occur in the intact heart under pathophysiologic conditions. The isolated perfused rabbit heart subjected to hypoxia and tested with the K_{ATP} channel opener, pinacidil, represents a valuable addition to the study of class III antifibrillatory agents. In this model, the induction of ventricular fibrillation is dependent on the hypoxia-induced decrease in tissue adenosine triphosphate content together with the influence of the K_{ATP} channel opener pinacidil to facilitate the opening of the K_{ATP} channel. The ability of glyburide and a number of class III antiarrhythmic agents (E-4031, 5-hydroxydecanoate) to protect against the development of ventricular fibrillation suggests a role for the K_{ATP} channel in the development of ventricular fibrillation (Figure 6). The antifibrillatory action of these agents may be attributed, in part, to the suppression of K^+ release from hypoxic or ischemic myocardium, perhaps through inhibition of the adenosine triphosphate-regulated K^+ channel. We believe that it is the tissue adenosine triphosphate content and not the direct effects of hypoxia or reoxygenation that influences the action of pinacidil on cardiac rhythm. The K^+ channel putatively responsible for ischemia or hypoxia-induced K^+ loss is the adenosine triphosphate-dependent K^+ channel present in cardiac cells.[130] Under normoxic conditions, the adenosine triphosphate-dependent K^+ channel is blocked by high intracellular adenosine triphosphate concentrations. Depletion of cellular adenosine triphosphate (as can occur during hypoxia, ischemia, or in the presence of a metabolic inhibitor) disinhibits the blockade of the channel and allows for K^+ efflux to occur. A role for the adenosine triphosphate-dependent K^+ channel in hypoxia/ischemia-induced K^+ loss has been shown by specific inhibition with glyburide or tolbutamide.[133,135,161] Glyburide inhibits both ischemia-induced myocardial K^+ loss and ischemia-

induced ventricular fibrillation. The latter observation provides evidence that the K^+ loss resulted from opening of the adenosine triphosphate-dependent K^+ channel and that the K^+ efflux was related to the onset of ventricular fibrillation.[135] The results of our studies may be interpreted as suggesting that pharmacologic opening of the adenosine triphosphate-dependent K^+ channel during hypoxia (decrease in myocardial adenosine triphosphate) or on reoxygenation (sustained decrease in myocardial adenosine triphosphate content) makes the myocardium more susceptible to the development of ventricular fibrillation. The suggestion that under certain conditions K^+ channel agonists may facilitate the development of ventricular fibrillation concurs with in vivo data showing the profibrillatory action of pinacidil under conditions of regional myocardial ischemia in the presence of a previous myocardial infarction.[150]

In addition to demonstrating antifibrillatory activity for specific interventions, results obtained in the isolated heart have supported the observations in the intact animal in which profibrillatory events have been uncovered, as was the case with flecainide.[73,162] Class I antiarrhythmic agents (quinidine, aprindine, lidocaine, and flecainide) were selected for study in the isolated heart made hypoxic and treated with pinacidil. Only quinidine prevented the pinacidil-induced ventricular fibrillation. Flecainide in the presence of hypoxia, but in the absence of pinacidil, invariably was associated with the onset of ventricular fibrillation that could be prevented by pretreatment with glyburide.[162] The observations suggest that ventricular fibrillation can be provoked by the potassium channel agonist pinacidil or by flecainide, under conditions that reduce intracellular adenosine triphosphate concentration. Glyburide, a selective antagonist of the K_{ATP} channel, prevented the profibrillatory actions of both pinacidil and flecainide.

CONCLUSION

The lack of effective and safe drugs for the prevention of lethal arrhythmias and sudden cardiac death has served to stimulate renewed interest in the area of drug development and the introduction of several new candidate agents that share a common ability to prolong ventricular refractoriness. Equally important is the recognition that most antiarrhythmic agents have been evaluated with in vitro or in vivo models that have little relevance to the clinical situation of sudden cardiac death. As the recent CAST report[17,18] has emphasized, the final analysis of a drug's ultimate utility will depend on appropriate clinical testing in patients who are at risk of developing sudden and unexpected life-threatening arrhythmias, or ventricular fibrillation, or both.

We can no longer afford to employ the more expedient and less dependable approach to evaluating new agents for their ability to reduce the frequency and/or complexity of ventricular depolarizations or their ability to modify the patient's response to programmed electrical stimulation. Despite the formidable task involved in the clinical assessment of an effective therapy for the prevention of ventricular fibrillation, the challenge could be made more readily attainable by preclinical assessment of a candidate drug, based on studies conducted in relevant animal models using mean-

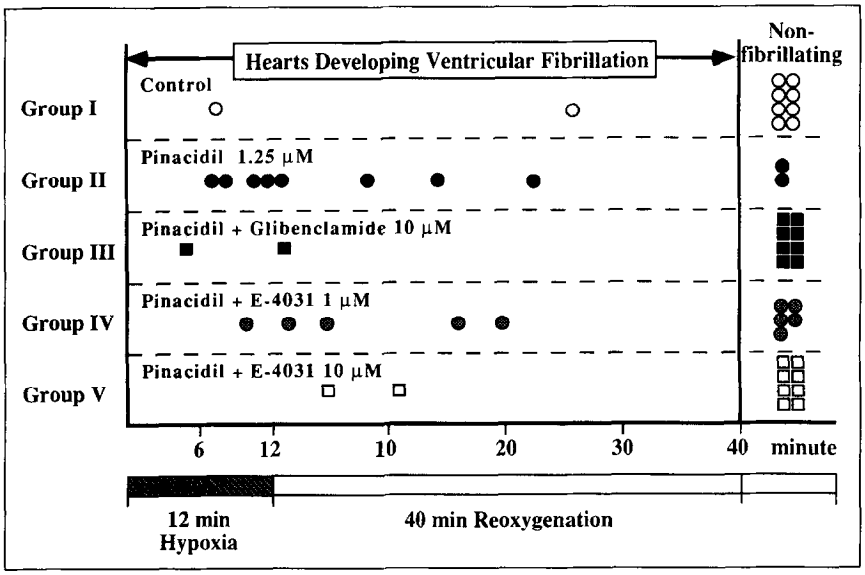

FIGURE 6. Summary of results in the isolated heart model of hypoxia- or pinacidil-induced ventricular fibrillation. Rabbit isolated hearts were subjected to 12 minutes of hypoxia in the absence (control) or presence of pinacidil (1.25 μM) followed by 40 minutes of reoxygenation. Ventricular fibrillation developed in 20% of the control rabbits compared with 80% of the pinacidil-treated hearts. The K_{ATP} channel blocking agent, glyburide (glibenclamide), reduced the incidence of ventricular fibrillation, as did E-4031. The isolated heart model may permit one to identify antifibrillatory agents capable of inhibiting the K_{ATP} channel that becomes functional in response to a decrease in myocardial adenosine triphosphate content associated with the 12 minutes of hypoxic perfusion.

ingful electrophysiologic endpoints that occur spontaneously. To this end, we have employed an animal model of sudden cardiac death in which ventricular fibrillation develops within 1 hour from the onset of an ischemic event in a myocardial substrate that has been identified, through the use of programmed electrical stimulation, to be capable of supporting an arrhythmic mechanism. The conscious, postinfarcted, canine model has been employed by us to confirm antifibrillatory activity in a number of approved antiarrhythmic agents, proposed antifibrillatory potential in several agents at exploratory stages of development, and warn of profibrillatory dangers in others. Although a number of animal models of sudden death exist, it seems desirable to use the model with previous myocardial infarction (healed scar) and new induction of ischemia, which may most closely emulate the situation seen in the CAST.[163] The animal model, together with studies conducted at the cellular, biochemical, and molecular levels, will serve as the conduits for understanding the physiopathologic events leading to lethal arrhythmias and for the development of pharmacologic interventions aimed at preventing sudden cardiac death.[164]

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