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REVIEW

Phase 2 ventricular arrhythmias in acute myocardial infarction: a neglected target for therapeutic antiarrhythmic drug development and for safety pharmacology evaluation

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Ventricular fibrillation (VF), a cause of sudden cardiac death (SCD) in the setting of acute myocardial infarction (MI), remains a major therapeutic challenge. In humans, VF may occur within minutes or hours after the onset of chest pain, so its precise timing in relation to the onset of ischaemia is variable. Moreover, because VF usually occurs unobserved, out of hospital, and is usually lethal in the absence of intervention, its precise timing of onset is actually unknown in most patients. In animal models, the timing of susceptibility to VF is much better characterised. It occurs in two distinct phases. Early VF (defined as phase 1 VF, with possible subphases 1a and 1b in some animal species) occurs during the first 30 min of ischaemia when most myocardial injury is still reversible. Late VF, defined as phase 2 VF, occurs when myocardial necrosis is becoming established (after more than 90 min of ischaemia). Although much is known about the mechanisms and pharmacology of phase 1 VF, little is known about phase 2 VF. By reviewing a range of different types of data we have outlined the likely mechanisms and clinical relevance of phase 2 VF, and have evaluated possible future directions to help evolve a strategy for its suppression by drugs. The possibility that a proarrhythmic effect on phase 2 VF contributes to the adverse cardiac effects of certain cardiac and noncardiac drugs is also discussed in relation to the emerging field of safety pharmacology. It is concluded that suppression of phase 2 as well as phase 1 VF will almost certainly be necessary if drugs of the future are to achieve what drugs of the past and present have failed to achieve: full protection against SCD. Likewise, safety will require avoidance of exacerbation of phase 2 as well as phase 1 VF.
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Abbreviations: CAST, cardiac arrhythmia suppression trial; MI, acute myocardial infarction; SCD, sudden cardiac death; SWORD, survival with oral D-sotalol; VF, ventricular fibrillation; VPB, ventricular premature beats; VT, ventricular tachycardia

SCD, the problems posed by its clinical presentation, and its resistance to drug therapy

Sudden cardiac death (SCD) is a major cause of premature death in the U.K. (Wannamethee *et al*., 1995) and North America (Prystowsky, 2004). Lethal cardiac arrhythmias, especially VF, are accepted as a major cause of SCD (Campbell, 1983; 1987). Importantly, ventricular fibrillation (VF)-related mortality is not declining despite an overall decline in the prevalence of coronary artery disease (Zheng *et al*., 2001).

In the human population, susceptibility to VF appears to be highest during the first few hours after a coronary event, persisting for several hours before waning, although the precise temporal pattern of susceptibility is poorly characterised (Campbell *et al*., 1981; Adgey *et al*., 1982). In animal models of SCD in which local (regional) ischaemia and infarction are elicited by coronary artery ligation, it has been clearly established that VF can occur in two distinct and separate phases, the first associated with the period of reversible injury (the condition where reperfusion of ischaemic tissue leads to cell recovery) and the second associated with the period of infarct evolution (Johnston *et al*., 1983a, b; Curtis, 1998). In humans, equivalent information on the timing of VF is difficult to obtain, but a susceptibility similar to that seen in animal models, occurring during both reversible and irreversible injury, can be inferred from data showing that approximately 50% of patients successfully resuscitated from VF have evidence of infarction while the other 50% do not (De Vreede-Swagemakers *et al*., 1998). Therefore, although difficult to prove beyond doubt, it would appear that separate phases of VF, as defined from animal studies, may occur in humans and that each may contribute to SCD. For reasons that will become apparent, this has important therapeutic implications.

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When VF arises in man, it usually does so without warning and therefore, usually, before the victim has been admitted to hospital, and survival is less than 5% (Prystowsky, 2004). As a consequence, the only feasible means of evaluating possible new therapy in humans is a large controlled clinical trial of prophylactic intervention, with SCD as the primary end point (Pratt *et al*., 1998). This approach has proven problematic, however, and there is no accepted methodology for assigning patients to treatment according to a stratification of their SCD risk (Huikuri *et al*., 2003). For reasons of practicality, in the past, surrogate end points for VF have been used clinically as a guide to therapy, with the likelihood of benefit with a drug estimated on the basis of its ability to suppress spontaneously occurring ventricular premature beats (VPBs) or more severe arrhythmias induced by programmed electrical stimulation (e.g., Bourke *et al*., 1995). However, it is now established that these end points do not accurately predict the effectiveness of drugs in the prevention of SCD (Bourke *et al*., 1995; Goldstein *et al*., 1995); there are no surrogate end points for testing new drugs for VF/SCD.

Moving even further away from VF itself in an attempt to anticipate is occurrence, there are biochemical substances including troponin I, C-reactive protein (CRP) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) that have been used to predict the risk of adverse coronary events in humans (Biasucci, 2004; Oei *et al*., 2005). Whether these may also serve as 'markers' of risk of VF is unknown, and whether they may be applied to selection of therapeutic intervention is even more uncertain. Although recent clinical evidence suggests that reduction of plasma CRP levels with statin therapy can reduce the risk of death (Muhlestein *et al*., 2004; Ridker *et al*., 2005), it is unclear whether this relates to a direct effect on VF. The possibility that these and other substances have direct effects on VF susceptibility is discussed later in this review.

It is not surprising, in view of these considerations, that several large SCD trials of antiarrhythmic drugs in post-MI patients, in which the choice of drugs has been guided in part by surrogate endpoint data, have had outcomes that have been disappointing. For some drugs the outcome has been catastrophic, with survival rate *decreased* by the drug (Pratt *et al*., 1998). This has had a major negative impact on drug development, and few pharmaceutical companies are now prepared to countenance the notion of investing in the development of new antiarrhythmic drugs for VF/SCD.

Of the SCD trials completed, the survival with oral D-sotalol (SWORD) trial, in which post-MI mortality was doubled in the treatment group, remains an enigma since there is no proven explanation for the failure of the drug (Cobbe, 1996; Pratt *et al*., 1998). The cardiac arrhythmia suppression trial (CAST) study, which also revealed a treatment-related increase in death (Goldstein *et al*., 1995), has been subjected to great scrutiny, from which it has been suggested that the test drugs *exacerbated* VF, either during the early phase of ischaemia or *later*, during the infarct evolution phase (Greenberg *et al*., 1995; Hallstrom *et al*., 1995). Importantly, it has been suggested from these and other clinical studies that a drug that suppresses one phase of VF ('early' or 'late') may fail to protect against, or may even facilitate, susceptibility to another phase of VF (Campbell, 1987; Greenberg *et al*., 1995). If the effects of drugs on VF in the human are phase-dependent and the underlying mechanisms of VF correspondingly phase-dependent, this has major relevance to the implementation of any strategy for development of effective new therapeutic interventions.

As the SWORD and CAST trials also show that the circumstances in humans in which antiarrhythmic drugs may be proarrhythmic are poorly characterised, this leads on to an issue separate from the main focus of this article (VF suppression), that of the possible relationship between VF occurring during infarct evolution and the safety of noncardiac drugs. Many non-cardiac drugs can evoke arrhythmias under circumstances that are generally well-recognised, including hypokalaemia and conditions associated with a long QT interval (Malik & Camm, 2001). However, the issue of noncardiac drug-induced proarrhythmia remains an important and controversial area in the emerging science of safety pharmacology, especially with regard to the identification of risk factors that may make an individual more susceptible (Kinter *et al*., 2004; Pugsley, 2004). It is therefore intriguing to note that there have been few, if any, attempts to equate apparent drug-induced proarrhythmia with possible exacerbation of infarct-related VF.

In view of these issues, there is a need to focus on the different phases of VF, to define and distinguish component mechanisms and mediators, and to explore protective strategies, and possible links with drug-induced proarrhythmia, using animal models. Despite the well-documented existence of different phases of VF in animal models of SCD (see Curtis, 1998), we find that there is no definition of the phases that is formally agreed. We offer the following: with one-stage maintained coronary obstruction, the early phase of VF occurring during the first 30 min of ischaemia is defined as phase 1, and the later phase, beginning approximately 90 min after the start of ischaemia, during infarct evolution, is defined as phase 2. For reasons explained below (which are largely to do with its neglect), phase 2 VF is the focus of the present article.

Animal models of ischaemia and infarction and their use in phase 2 VF study

Different models, utilising *in vitro* isolated buffer-perfused hearts (Bricknell & Opie, 1978; Daugherty *et al*., 1986; Hearse *et al*., 1999) and intact anaesthetised (Janse *et al*., 1979; Clark *et al*., 1980; Coker, 1989) and conscious animals (Curtis *et al*., 1984; Schwartz *et al*., 1984), have been employed to investigate the pathophysiological phenomena associated with ischaemia and infarction, and their modulation by drugs. Some of these models are suitable for the study of arrhythmias and others are not. With regard to the main focus of this article few, unfortunately, have been characterised for possible use in the study of phase 2 VF.

Isolated cardiac myocytes have been used for the study of the effects of ischaemia on cellular electrophysiology (Cerbai *et al*., 1991; Entman *et al*., 1992; Wu & Corr, 1994; Vanden Hoek *et al*., 1997). However, ischaemia must be simulated. Investigators seek to achieve this by superfusion with hypoxic solution (Buerke *et al*., 1994; Silverman *et al*., 1997; Nakano *et al*., 1998), by metabolic inhibition of ATP synthesis using inhibitors of oxidative phosphorylation (Williams *et al*., 2001; Rodrigo *et al*., 2002), or by superfusion with a solution mimicking the extracellular space in ischaemic myocardium

(i.e., glucose-free, acidic solution containing high concentrations of lactate and K^+, sometimes with added inhibitors of glycolysis) (Vanden Hoek *et al*., 1997; Wilders *et al*., 1999; Levraut *et al*., 2003). However, none of these approaches truly mimics whole heart ischaemia, and none have been designed to mimic the milieu associated with phase 2 VF. Moreover, isolated cardiac myocytes are not suitable for the study of VF (whether it be phase 1 or 2) or its suppression, *a priori*, since re-entry, the mechanism of VF and other arrhythmia propagation through the myocardial syncytium (Janse, 1991) cannot occur or be modelled in single-cells. Furthermore, the temporal variation of interstitial accumulation of substances leaving the ischaemic cell (whether associated with phase 1 or phase 2 VF) cannot easily be replicated in single-cell superfusion studies, making it difficult to assess the role of these substances in mediating the relevant electrophysiological dysfunction. These considerations indicate that whole hearts (*in vivo* and/or *in vitro*) with regional ischaemia are preferred for modelling VF/SCD, and essential if VF itself is the desired study variable.

Global ischaemia (affecting the whole heart) is modelled only *in vitro* (Bricknell & Opie, 1978; Kleber, 1983; Fukunami & Hearse, 1985). It is not known whether infarct-related phase 2 VF occurs in globally ischaemic hearts as it has not been studied. Globally ischaemic hearts develop asystole (no VF) within a few minutes of cessation of coronary flow, and asystole is sustained for the duration of ischaemia and the experiment, typically 30 min (Ridley *et al*., 1992). It may be the case that globally ischaemic hearts reanimate electrophysiologically after more sustained ischaemia and fibrillate, but this has not been tested in the laboratory (at least, not under normothermic and pharmacologically unadulterated conditions). The global ischaemia models can be modified by partially reducing coronary perfusion so that a residual flow (usually 10% of control flow) is retained. This is termed low-flow ischaemia, and can be achieved most conveniently, *in vitro*, by reducing the delivery pressure of the solution perfusing the heart or by controlling coronary flow using a pump (Culling *et al*., 1984; Cave *et al*., 1997; De Jonge & De Jong, 1999; Gogelein *et al*., 2001). Again, it is not known whether phase 2 VF occurs in these models.

Regional ischaemia, that involving a limited part of the heart, may be studied *in vitro* (Daugherty *et al*., 1986; Coker, 1989; Curtis *et al*., 1993a, b; D'alonzo *et al*., 1994) and *in vivo* (Clark *et al*., 1980; Billman, 1994). Regional ischaemia models have provided the majority of the emerging data on phase 2 arrhythmias and their suppression by drugs.

Characteristics of phase 2 *versus* phase 1 VF in regional ischaemia models *in vivo*

Regional ischaemia is usually achieved by tying a ligature around a left coronary artery, leaving perfusion of the remainder of the arterial bed intact (Harris, 1950; Johns & Olson, 1954; Rushmer *et al*., 1963; Johnston *et al*., 1983a, b; Curtis, 1998). Regional ischaemia is highly arrhythmogenic *in vivo* and *in vitro*, with the majority of control hearts experiencing VF at some point during the experiment in some species (Curtis, 1998). In animal models, there is a well-characterised transition period during which reversible injury changes to irreversible injury, whereupon reperfusion becomes incapable of salvaging the tissue. The process of transition requires a minimum of 20 min of continuous and severe ischaemia to begin, and a minimum of 60 min of continuous ischaemia to be complete (Garcia-Dorado *et al*., 1987). A bell-shaped relationship has been found to exist between the size of the ischaemic zone and susceptibility to phase 1 VF, with maximum susceptibility occurring when ischaemic zone size is between 30 and 50% of total ventricular weight, and this has important implications about arrhythmogenic mechanisms (Curtis & Hearse, 1989a, b; Ridley *et al*., 1992). Unfortunately, the relationship between the size of the infarcting zone and susceptibility to phase 2 VF is unknown.

During the last 50 years, the regional ischaemia models most widely used for preclinical SCD drug research have been adapted from those of Harris (1950), Kenedi & Losonci (1973), Schwartz *et al*. (1984) and Lucchesi (Patterson *et al*., 1982). There are other, older models (Tillmanns *et al*., 1983), but they have not greatly contributed to the study of VF/SCD. Of those cited above, in their chronological order, Harris (1948) initially developed a one-stage complete coronary artery ligation model in the anaesthetised dog and found a high susceptibility to lethal VF during the first 10 min of ischaemia, but opted in subsequent studies to focus on arrhythmogenic mechanisms associated with sustained ischaemia. To achieve this, Harris (1950) developed the 'two-stage ligation model', described in detail by Hashimoto *et al*. (1982). However, the model is not suitable for studies on VF because the first stage of coronary ligation, occurring at time zero, is intended to only partially occlude a coronary artery, thus generating low-flow regional ischaemia with minimal electrophysiological dysfunction and few arrhythmias. Consequently, early ischaemia-induced VF (that which is now referred to as phase 1 VF) is deliberately absent. VF may occur later, after a subsequent, second-stage, complete coronary ligation, but it is unclear whether this is a manifestation of delayed phase 1 VF or is true phase 2 VF (as characterised in other models). Moreover, the late-occurring VF is itself rare in this model (Hashimoto *et al*., 1982), so it appears that the two-stage ligation process itself delays or suppresses VF, making it as hard to study in the model as it would be to classify. As a consequence, the main end points measured in the Harris model have been the less severe arrhythmias, VPBs and ventricular tachycardia (VT). Despite this, the model has been used extensively (Hashimoto *et al*., 1982). The model has, however, been adapted to allow VF to be studied by the use of programmed electrical stimulation of the ventricles (Aidonidis *et al*., 1995) under the supposition that electrically evoked VF may serve as a surrogate end point for coronary ligation-induced spontaneous VF. Unfortunately, this can now be seen as an unwise approach, since spontaneous VPBs and VT and electrically induced VF are now known to have only limited predictive value when used as surrogates for VF in clinical studies designed to select drug therapy on an individual patient basis for prevention of SCD and to predict the effect of a drug on long-term survival (Bourke *et al*., 1995; Goldstein *et al*., 1995), as mentioned earlier.

The Kenedi & Losonci (1973) model involves one-stage coronary artery ligation, and was described first as a method for use in rats. It would perhaps be better known as the Parratt/Szekeres/Walker model, as these were the investigators who independently adapted it, and used it extensively for the study of VF and its suppression (for reviews, see Curtis

et al., 1987; Clements-Jewery & Curtis, 2003). The model was initially developed to downsize to small animals and simplify the first Harris (1948) model to allow convenient study of the arrhythmias occurring during a brief (30 min) period of ischaemia (e.g., Au *et al*., 1979), but was subsequently modified by simply extending the duration of the experiment. This seemingly trivial modification allowed unequivocal identification for the first time of a second phase of VF, occurring during infarct evolution (e.g., Clark *et al*., 1980). Characterisation studies have established that conscious rats surviving the first 30 min of ischaemia (which is associated with phase 1 VF and reversible myocardial injury) always experience a hiatus of normal sinus rhythm, which is then followed by the onset of a second phase of arrhythmias including VF (Figure 1). Phase 2 VF occurs spontaneously (i.e. without the need for electrical stimulation of the ventricle) in this model after about 2 h of sustained ischaemia, coinciding with the establishment of irreversible injury (Curtis *et al*., 1987). The onset of VF occurs much earlier and the prevalence of VF is much greater than that in the Harris two-stage ligation dog model (Harris, 1950), implying that the severity of ischaemia determines the likelihood and latency to the onset of phase 2 VF. The severity of ischaemia is minimised in the Harris dogs because the initial occlusion is partial, and also because most dog hearts (unlike rat hearts, in which phase 2 VF is best characterised) exhibit substantial collateral coronary blood flow (Meesmann, 1982; Curtis, 1998).

Presently, there is insufficient evidence to determine precisely the extent to which the timing and severity of phase 2 VF differ between species. Differences may be anticipated, since there are differences for phase 1 VF, with some species, including rats, exhibiting a single period of susceptibility and others, including the dog, exhibiting subcomponents known as phase 1a and phase 1b (Curtis, 1998). Despite this, there are similarities between species. For example, it is well established that rats have a much reduced susceptibility to arrhythmias once they have survived 24 h of continuous regional ischaemia (Clark *et al*., 1980; Curtis *et al*., 1987), and limited information appears to indicate the same to be the case in dogs (Kaumann & Aramendia, 1968). Although few attempts have been made to undertake characterisation in other species, phase 2 VF does occur where studied, in the anaesthetised pig (Pugsley *et al*., 1995) and possibly also the conscious baboon (Vatner *et al*., 1988b).

The Schwartz/Billman and Lucchesi canine models, which involve one-stage complete obstruction of a coronary artery in conjunction with exercise or with the superimposition of acute ischaemia in hearts with a previous infarction, have been used extensively to examine drug effects on phase 1 VF but, as a consequence of this focus, the duration of ischaemia has always been brief (<30 min) and so it is unknown whether in these models phase 2 VF would also occur were ischaemia to be maintained for longer.

All in all, VF susceptibility following coronary obstruction in animals appears to have condition-dependent subcomponents that appear to be model-dependent and difficult to link with specific clinical counterparts but, nevertheless, two distinct main phases of susceptibility appear to exist. Although the relative importance of phase 2 *versus* phase 1 VF may be unclear and difficult to ascertain in humans (Campbell, 1987), phase 2 VF has been observed *in vivo* following permanent one-stage coronary ligation in all animal species examined to date, as noted above, so it would be no more wise to discount its possible clinical relevance than to discount the relevance of phase 1 VF. Indeed, although the finding does not reflect the majority of data derived from the model (Curtis *et al*., 1987), there is a study using the conscious rat that found the susceptibility to phase 2 VF to be much greater (17/21 animals) than susceptibility to phase 1 VF (4/23 animals) during permanent coronary occlusion (Opitz *et al*., 1995). An example trace of phase 2 VF with accompanying blood pressure recording is shown in Figure 2.

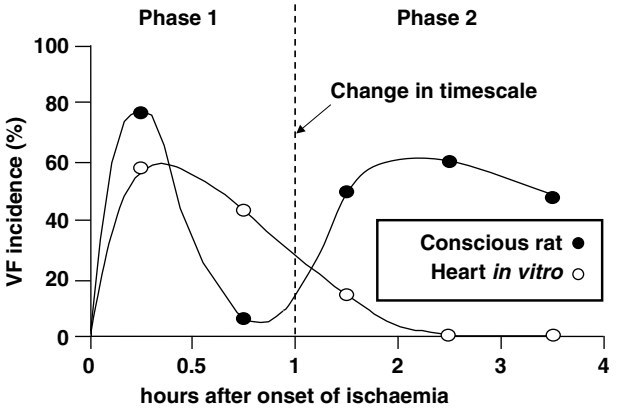

Figure 1 Time course of onset of ventricular fibrillation in conscious rats (●) ($n=18$) and isolated Langendorff perfused rat hearts (○) ($n=12$) subjected to left coronary artery occlusion at the same site. The distinct phases of VF are termed phase 1 (occurring <2 h after coronary occlusion) and phase 2 (occurring >2 h after coronary occlusion). Reentry and the flow of 'injury current' are the likely mechanisms responsible for initiation of phase 1 VF, while reentry and abnormal automaticity are the likely corresponding mechanisms for phase 2 VF. Figure modified and adapted from Curtis (1993).

Biochemical and electrophysiological events underlying phase 2 *versus* phase 1 VF

The distinct phases of VF in animal models, depicted in Figure 1, coincide with distinct biochemical and electrophysiological changes at the cellular and intercellular levels. Although their role in mediating VF is unproven (this remains the case even for phase 1 VF, according to strict criteria set out some years ago; Curtis *et al*., 1993b), these processes are well characterised (Curtis *et al*., 1993a, b; Allessie *et al*., 1995; Cascio *et al*., 1995) and we describe them here only briefly. Specific metabolic dysfunction that occurs during the period of phase 1 VF includes depletion of ATP and accumulation of ADP and lactate as a result of anaerobic glycolysis (Bricknell & Opie, 1978). Electrophysiological changes during the phase 1 period include a depolarisation of the resting membrane potential, and an accumulation of extracellular K^+ which is typically triphasic, with the initial rise and plateau phases occurring during the first 30 min period after coronary obstruction, commonly denoted as 'acute ischaemia', coinciding well with the time when phase 1 arrhythmias occur (Hirche *et al*., 1980). The rise in membrane potential attenuates the amplitude and upstroke velocity of the action potential as a result of Na^+ channel inactivation, and action potential

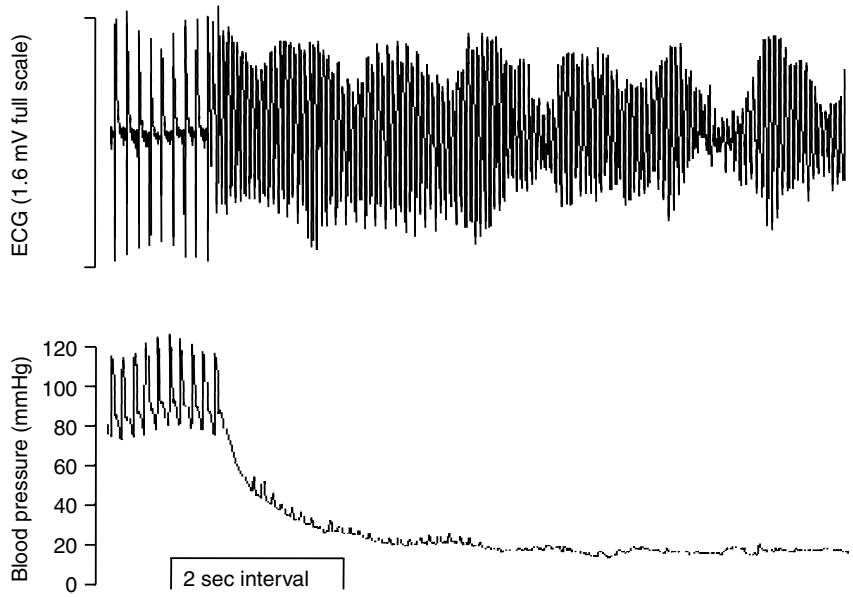

Figure 2 Example of phase 2 VF in a pentobarbitone-anaesthetised rat. Accompanying changes in blood pressure are shown beneath the ECG trace.

duration typically lengthens and then shortens with prolonged ischaemia probably as a result of enhanced outward repolarising K^+ currents (Cascio *et al*., 1995). The phase 1 period is also associated with the intercellular accumulation of many biochemicals, including catecholamines, K^+, and amphiphiles such as lysophosphatidylcholine and platelet-activating factor, and although many have arrhythmogenic properties, no individual substance has yet been identified as being both sufficient and necessary for mediation of phase 1 VF (Curtis, 1993; Curtis *et al*., 1993b; Clements-Jewery & Curtis, 2003; Baker & Curtis, 2004).

Phase 2 VF coincides with the establishment of infarction (Ravingerova *et al*., 1995). In collateral-deficient hearts, the infarction process begins after approximately 15–20 min of sustained ischaemia (Hort & Dacanalis, 1965), and can be complete by approximately 1 h since reperfusion begun after this time will not salvage any myocardial tissue (Garcia-Dorado *et al*., 1987). However, the healing process may take between 24 and 48 h to complete (Bolli & Marban, 1999). This process of evolution of the infarct is accompanied by a number of metabolic, ionic and electrophysiological changes. Metabolically, this is characterised by low glycogen and high lactate intracellular contents, with virtual cessation of anaerobic glycolysis resulting in ATP and creatine phosphate levels below 10% and 2% of normal, respectively (Jennings *et al*., 1990). In addition, the adenine nucleotide pool consists chiefly of AMP and there are high intracellular contents of inosine and hypoxanthine as a result of adenosine deamination (Jennings *et al*., 1990). With respect to ionic changes, the extracellular K^+ accumulation that begins during the phase 1 period of ischaemia proceeds monophasically during infarct evolution. In the *in situ* pig heart, the rise in extracellular K^+ continues for at least the first hour of ischaemia (the limit of the cited study period), without sign of slowdown, and with extracellular K^+ levels eventually exceeding 20 mM (Hill & Gettes, 1980). Intracellular K^+ levels decline correspondingly, and a substantial reduction in intracellular K^+ activity has been recorded in excised Purkinje fibres after 3 h of sustained ischaemia (Hanna *et al*., 1988). Infarction is also characterised by a high cardiac intracellular Na^+ and Ca^{2+} content (Buja *et al*., 1975; Van Echteld *et al*., 1991). It remains uncertain which of these changes are sufficient and necessary for mediation of phase 2 VF.

Purkinje fibres, surviving but dysfunctional, have been proposed to be the main arrhythmogenic foci during the infarct evolution period (Friedman *et al*., 1973; Horowitz *et al*., 1976). After 1 h of ischaemia, Purkinje and surviving muscle fibres exhibit reduced resting potentials, reduced action potential amplitudes and reduced upstroke velocities (Fenoglio *et al*., 1979). The action potential duration, prolonged in muscle fibres, is shortened in Purkinje fibres, facilitating re-entry (Fenoglio *et al*., 1979).

In view of these considerations and the evident differences between reversible ischaemia and the evolving infarct in terms of the biochemical and electrophysiological milieu, it is unsurprising that differences appear to exist in the electrophysiological processes underlying phase 1 and phase 2 VF. Whereas re-entry and flow of 'injury current' (Janse *et al*., 1980) coincide with the appearance of phase 1 VF (Janse, 1991), re-entry and abnormal automaticity (delayed after-depolarisations) are regarded as likely prevalent mechanisms during infarct development (Wit, 1989). On the other hand, there are no major differences in terms of ECG and haemodynamic characteristics in the few seconds preceding the occurrence of phase 2 *versus* phase 1 VF, according to data from our laboratory (Table 1). Table 1 shows that 30% of phase 1 and 30% of phase 2 VF are associated with preceding VPBs, bigeminy or salvos, and 60% of phase 1 VF and 50% of phase 2 VF occurred as a result of 'degeneration' of VT. The only substantial differences between the antecedents of phase 1 and phase 2 VF are the magnitudes of the ECG's R and Q waves, which reflects the evolution of the infarct but does not delineate different underlying arrhythmogenic mechanisms. The future elucidation of underlying mechanisms is relevant, and perhaps essential, to rational drug targeting.

Table 1 ECG and haemodynamic variables immediately prior to the onset of phase 1 or phase 2 VF

Phase of VF	*Blood pressure*		*ECG intervals*			*ECG magnitude*		*Proportion of VF that was preceded by VPBs/salvos/bigeminy (%)*	*Proportion of VF preceded by degenerating VT (%)*	*Occurrence of spontaneous defibrillation (%)*
	Systolic	*Diastolic*	*RR*	*QT*	*PR*	*R wave*	*Q wave*			
Phase 1	100±8	65±7	150±8	48±4	40±1	0.91±0.09	0.18±0.04	30	60	0
Phase 2	110±6	76±4	140±5	54±1	41±1	0.63±0.06*	0.47±0.05*	30	50	10

In each case, data were taken from $n=10$ consecutive fibrillating anaesthetised rats (subjected to 4 h coronary occlusion). The 5 s period preceding the occurrence of VF was used to obtain the values above, taking note of whether VF occurred abruptly or was preceded by ectopy (VPBs, etc) or by a degenerating form of VT. Only one rat in the data set developed both phase 1 and phase 2 VF *Denotes $P<0.05$ *versus* Phase 1 VF, assuming that the data set represents a random sample of the population.

The effect of drugs on phase 2 VF, and limitations of available data

Actions of drugs on phase 1 VF is not a topic that will be dealt with here (it was our intention to refer readers to a review, but there are none suitable that incorporate recent data from preclinical studies – perhaps the time is right for an update). With regard to phase 2 VF, although the data are limited, there are a number of studies that have explored the effects of drugs. These studies have largely been conducted in rats *in vivo*, the findings of which are summarised in Table 2. Although there is some variation in the type of anaesthetic, age of animal and laboratory of study, the database is insufficiently large for detailed subanalysis. However, it is apparent that there is a clear lack of effectiveness of most drugs tested at 'reasonable' dosage (i.e., dosage achieving intended molecular target selectivity). Perhaps the most important consideration of all is that many classes of potentially interesting drugs of relevance to ischaemic heart disease and arrhythmogenesis, such as angiotensin-converting enzyme inhibitors, sodium–proton exchange inhibitors, thrombin antagonists, antiplatelet drugs and platelet-activating factor (PAF) antagonists, have yet to be tested for their effects on phase 2 VF. Likewise, selective I_{Kr} blockers have not been examined for their potential to exacerbate phase 2 VF, a safety pharmacology issue revisited later in this article.

There are several problems with the data summarising drug effects on phase 2 VF, shown in Table 2. Most of the drugs were given as pretreatment and in most studies no check was made to ensure that blood levels of test drugs were adequate during the phase 2 period. Additionally, death due to phase 1 VF reduced group sizes in some studies (e.g., Curtis *et al*., 1984), giving rise to scope for type-2 statistical errors.

The reason why almost all the data on drug actions on phase 2 VF come from studies using rats is unclear, but it probably reflects the perceived favourable bioassay characteristics of rat permanent coronary ligation models that make them so convenient for phase 1 VF studies (Curtis *et al*., 1987), encouraging exploration of phase 2 VF – because it can be done, and easily. However, there are differences between rats that have recovered from preparative surgery and are subjected to coronary ligation while conscious *versus* rats that are prepared acutely and subjected to coronary ligation under anaesthesia which will inform model choice in any future research strategy. In anaesthetised rats, the control incidence of phase 2 VF can be variable and sometimes too low for detecting antiarrhythmic effects of drugs (Table 3), indicating that the favourable bioassay characteristics achieved for the study of phase 1 VF in the model are not always replicated for phase 2 VF. On the other hand, in conscious rats, although the control incidence of phase 2 VF is higher and more reproducible (Table 4), there are ethical and technical issues which make it more of a challenge to justify undertaking studies. Nevertheless, since differences do exist between the acutely prepared anaesthetised and the conscious settings, it can be argued that one or the other is providing potentially misleading data. Unfortunately, in the absence of a clinical template, it is not clear which, and more work is indicated, although the case for more conscious animal work would seem to be compelling.

As a further caveat, there has been a widespread preconception that the rat is an irrelevant species for the study of any

Table 2 The effect of drugs on phase 1 and phase 2 VF in the same animals *in vivo*

Drug	*Species*	*Anaesthesia*	*Dose*	*Reduction of phase 1 VF?*	*Reduction of phase 2 VF?*
Class I drugs					
Lidocaine[1]	Rat	None	$2\,mg\,kg^{-1}$ i.v.	Yes	Yes
Lidocaine[2]	Rat	Pentobarbitone	$10\,mg\,kg^{-1} + 5\,mg\,kg^{-1}\,h^{-1}$ i.v.	Yes	No
Quinidine[3]	Rat	None	$20\,mg\,kg^{-1}$ i.v.	Yes	No
Disopyramide[3]	Rat	None	$10\,mg\,kg^{-1}$ i.v.	No	No
Mexiletine[4]	Rat	None	$20\,mg\,kg^{-1}$ i.v.	Yes	No
Quinacainol[5]	Rat	None	$4\,mg\,kg^{-1}$ i.v.	Yes	No
Class II drugs					
Labetalol[6]	Rat	None	$5\,mg\,kg^{-1}$ i.v.	No	No
Oxprenolol[7]	Rat	Pentobarbitone	$50\,mg\,kg^{-1}$ p.o. b.i.d	Yes	No
Pindolol[1]	Rat	None	$15\,\mu g\,kg^{-1}$ i.v.	Yes	Yes
Propranolol[6]	Rat	None	$0.2\,mg\,kg^{-1} + 0.1\,\mu g\,kg^{-1}\,min^{-1}$ i.v.	No	No
Class III drugs					
Tedisamil[8]	Rat	None	$2\,mg\,kg^{-1}$ i.v.	Yes	No
Class IV drugs					
Nifedipine[9]	Rat	None	0.5, $2\,mg\,kg^{-1}$ i.v.	Yes	No
Nifedipine[9]	Rat	None	$10\,mg\,kg^{-1}$ i.v.	Yes	Yes
Verapamil[10]	Rat	None	$20\,mg\,kg^{-1}$ i.v.	Yes	No
Felodipine[11]	Rat	None	$4\,mg\,kg^{-1}$ i.v.	No	No
Anipamil[12]	Pig	Pentobarbitone	$5\,mg\,kg^{-1} + 0.5\,mg\,kg^{-1}\,min^{-1}$	No	No
Miscellaneous interventions					
Blood K^+ elevation[13]	Rat	None	A range of KCl infusions	Yes	Yes
Nafazatrom[14]	Rat	Ether	$100\,mg\,kg^{-1}$ b.i.d.	No	Yes
Aspirin[15]	Rat	None	$100\,mg\,kg^{-1}$ i.v.	No	No

Data are taken from: (1) (Lepran *et al*. (1983), (2) Clark *et al*. (1980), (3) Johnston *et al*. (1983a, b), (4) Igwemezie *et al*. (1992), (5) Howard *et al*. (1992), (6) Botting *et al*. (1983), (7) Campbell *et al*. (1984), (8) Beatch *et al*. (1991), (9) Curtis & Walker (1988), (10) Curtis *et al*. (1984), (11) Curtis *et al*. (1985a). (12) Pugsley *et al*. (1995), (13) Saint *et al*. (1992), (14) Fiedler, (1983), (15) Johnston *et al*. (1983a, b).

Table 3 Incidences of phase 2 VF in anaesthetised rats obtained by different investigators

Incidence of phase 2 VF (%)	N	*Reference*
46	24	Clark *et al*. (1980)
37	19	Campbell *et al*. (1984)
24	17	Campbell *et al*. (1984)
19	9	Curtis *et al*. (1985a–c)

Mean$\pm$s.d.: $32\pm12\%$.

Note that there are several other publications in which phase 1 and phase 2 VF incidence is recorded combined, meaning that it is not possible to identify the control incidence of phase 2 VF (e.g., Beatch & McNeill, 1988).

Table 4 Incidences of phase 2 VF in conscious rats

Incidence of phase 2 VF	*Year*
90	1981
70	1981
50	1981
70	1983
55	1983
75	1984
60	1984
75	1985
75	1985
100	1986
89	1988
66	1992
81	1995

Mean$\pm$s.d.: $74\pm14\%$. Data are taken from Curtis *et al*. (1987); Opitz *et al*. (1995); Saint *et al*. (1992). For each year, the data represent the group mean incidence from groups of sizes ranging from 4 to 9 (variation due to variation in numbers of deaths occurring from phase 1 VF and cardiac output failure prior to the start of the phase 2 observation period).

form of ventricular arrhythmia whether it be induced by ischaemia (phase 1) or by infarction (phase 2). This is not because there is any evidence that arrhythmogenesis is atypical in the rat, nor because of evidence that the clinical effectiveness of drugs cannot be predicted in rats, but because the rat heart has characteristics that differ markedly from the human. These characteristics, along with those typical of hearts from dogs and humans, are displayed in Table 5. It is certainly true that the rat is unsuitable for evaluating the possible actions (protective or proarrhythmic) of delayed rectifying K^+ channel-blocking drugs, since the relevant channels (HERG/minK) are not functional in rat ventricle (Tande *et al*., 1990). Although there is no *a priori* reason for concern regarding most other putative drug targets and the use of rat hearts, the issue of appropriate species choice for the study of phase 2 VF does remain unresolved (as it does for most therapeutic areas where there are few truly effective drugs – without a clinical template, it is impossible to judge the suitability of any model).

The general paucity of data on the actions of drugs on phase 2 VF may be largely a historical quirk resulting from the fact that researchers (including ourselves) have tended to focus primarily on phase 1 VF and neglect subsequent events. The

Table 5 Typical characteristics of hearts from rats, dogs and humans

Species	*Typical heart weight: body weight ratio* (g kg^{-1})	*Typical body weight* (Kg)	*Typical heart rate* (beats min^{-1})	*APD* (msec)
Rat	3.33[a]	0.3[a]	400 *in vivo*[d] 250–350 *in vitro*[d]	30–60[e]
Canine	~7[b]	23[b]	90–140 *in vivo*[b]	200–240[f,g]
Human	~5[c]	70[c]	70–90	~300[f,g]

Data are taken from: [a]Curtis (1998), [b]Bayon *et al*. (1994), [c]Hanzlick & Rydzewski (1990), [d]Curtis (1998), [e]Curtis *et al*. (1987), [f]Malik & Camm (2001), [f,g]Haverkamp *et al*. (2000).

reasons for this are not necessarily scientifically justified. Thus, because phase 2 VF experiments take much longer to complete, and are therefore less appealing to perform than corresponding phase 1 VF experiments that allow the relatively quick generation of data, there is inevitably a disincentive to make the effort to study phase 2 VF. Additionally, if a heart fibrillates within 20 min of the onset of regional ischaemia (as it does in most animal models; see Curtis, 1998), an investigator may question the need to preserve the heart's rhythm to prolong survival in order to investigate later events. As a result of the influence of these factors, until recently (Clements-Jewery *et al*., 2002a, b), there have been no publications on drug actions on phase 2 VF since 1995 (Pugsley *et al*., 1995) to the best of our knowledge. Perhaps the reasoning outlined below may provide compelling justification to end this hiatus.

Phase 2 VF models and the importance of continued preclinical investigation

There would need to be good reasons to justify further use of available phase 2 VF models (especially rat models) in the light of the considerations outlined. In fact there are several. From a practical viewpoint, study is possible. For example, with rats there are the advantages of their ability to survive phase 1 VF if resuscitation is attempted by the simple technique of 'thump-version' (flicking the chest with a forefinger) with a success rate of up to 100% (Curtis & Walker, 1986; 1988) allowing study of phase 2 VF to proceed. Importantly, phase 2 VF occurs *spontaneously* following coronary ligation in rats (Curtis *et al*., 1987), permitting detection of drug actions without the need for programmed electrical stimulation. Furthermore, available rat drug data do not contradict available clinical data on SCD suppression, despite the intrinsic differences between rat and human hearts. Thus, clinically relevant doses of Class I and IV agents, which have no effect on SCD in humans (Antman *et al*., 1992), are ineffective against phase 2 VF in rats (Clark *et al*., 1980; Curtis *et al*., 1984; Igwemezie *et al*., 1992), while moderate blood K^+ elevation, which is known to suppress SCD in man (Nordrehaug & von der Lippe, 1983), is protective against phase 2 (and phase 1) VF (Curtis *et al*., 1985b, c; Saint *et al*., 1992). This accords encouragingly with the criteria of positive model reinforcement, postulated as an alternative approach to model justification in the absence of an established clinical template (Curtis, 1998).

This also leads on to another important justification for continued preclinical research on phase 2 VF and its suppression. Our premise is that the temporal changes in the biochemical and associated electrophysiological milieu that occur during the transition from acute ischaemia to infarction and were described above (Wit, 1989; Janse, 1991; Curtis *et al*., 1993a, b) have pharmacological importance. It is interesting, therefore, that many of the drugs that are known to prevent phase 1 VF in rats at low dosage are *ineffective* (or are effective, but only at unacceptably high doses) against phase 2 VF in the same species/model (Curtis *et al*., 1984; Curtis & Walker, 1988; Beatch *et al*., 1991; Igwemezie *et al*., 1992). In other words, drugs appear to act differently on phase 2 *versus* phase 1 VF. This is perhaps the most important issue raised in the present article. From a clinical perspective, it means that if a reduction in overall susceptibility to SCD is ever to be achieved by a new antiarrhythmic drug or any indirect form of antiarrhythmic intervention, VF suppression will need to occur during infarct evolution as well as during acute ischaemia. Selective suppression of phase 1 VF will merely delay death for an hour or so. In the past, it was not seen as necessary that drugs be shown to suppress phase 2 as well as phase 1 VF in animals before being tested in humans. The outcome, a series of drugs that have failed to suppress SCD, is therefore unsurprising. Unfortunately, because the distinction between different phases of VF has often been overlooked, a misreading of the implications of the failure of individual models to predict drug effectiveness against SCD has generated a widespread lack of confidence in the value of animal models of VF (Allessie *et al*., 1995). It is true that an individual model of one aspect of the pathophysiological milieu may not be predictive, but, provided a drug is tested in a second appropriate model relevant to the remainder of the milieu, the individual models are likely to have value by together providing a predictive data set. The wise industrial drug development strategist will delay making a decision about whether to progress a putative new drug for prevention of SCD until data from a range of models are available.

The consequence of all these complex considerations is that, even though it may be difficult to ascertain the exact clinical relevance of phase 2 VF, there is sufficient evidence to warrant an interest in its mechanism and control. This has been a neglected area of study.

Future directions

The importance of avoiding reliance in the use of surrogate end points for VF, such as less severe but more easily-evoked arrhythmias (e.g., VPBs) or cellular electrophysiological variables, was explained earlier. However, this rather limits the scope for the study of the mechanisms and control of phase 2 VF. Mechanisms of different diseases are increasingly being explored by exploiting the possibilities afforded by transgenic

mice. However, mouse hearts are notoriously resistant to developing VF in response to coronary ligation (there are no published studies in which VF was successfully elicited by coronary ligation in the mouse, to our knowledge). This means that it is necessary to utilise other species, all of which are unsuitable for routine transgenic manipulation. (A reliable mouse model of phase 2 (and phase 1) VF would be highly desirable.) It also means that study of the ability of selective pharmacological tools (i.e. carefully selected drugs at carefully selected concentrations) to prevent phase 2 VF using models in which phase 2 VF can be reliably evoked remains the best available approach. This approach has already provided a certain amount of information on phase 2 VF mechanisms, particularly concerning the involvement of blood-borne cells and substances, and the sympathetic nervous system. Additional clues have been provided by comparison of outcomes in different, but related models of phase 2 VF.

Comparison of outcomes in different rat models has revealed two possible mechanisms for phase 2 VF, one discussed here and the other discussed below, that might provide a basis for future drug discovery. The data are as follows. In the *in vivo* setting, more than 90% of control collateral-deficient dogs (Meesmann, 1982) or rats (Curtis & Walker, 1988) develop phase 1 VF, provided the ischaemic zone is sufficiently large. In rats (there are no equivalent dog data), 80–100% of defibrillated surviving control animals go on to develop phase 2 VF (Curtis & Walker, 1988). Likewise, in isolated buffer-perfused (blood-free) hearts, more than 80% develop phase 1 VF (Curtis, 1998). However, phase 2 VF is *absent* in buffer-perfused hearts (Curtis, 1998; Ravingerova *et al*., 1995; Clements-Jewery *et al*., 2002a). This is intriguing, all the more because microscopy has shown that in this experimental preparation sustained coronary artery ligation leads to severe structural damage to myocytes and coronary endothelium that is typical of infarcting myocardium (Ravingerova *et al*., 1995). This indicates that there is an absence of phase 2 VF in buffer-perfused hearts, and that this is not due to a lack of severe injury and infarction. Moreover, the marked susceptibility to *phase 1* VF in this preparation (Curtis & Hearse, 1989a, b; Clements-Jewery *et al*., 2002a) means that these hearts are not intrinsically resistant to developing VF. These observations indicate that the critical arrhythmogenic components necessary to cause phase 2 VF *in vivo* are *absent* in isolated buffer-perfused hearts. Although it is possible to offer an alternative explanation and argue that there is something *provided* by *in vitro* buffer perfusion that suppresses phase 2 VF, for example, the provision of tissue oedema (by the lack of colloid), or provision of a supranormal coronary flow in uninvolved tissue (caused by the lack of colloid and by the absence of red blood cells), this would seem to be unlikely in the absence of a rational hypothesis to account for the supposition. Moreover, the existence of a positive protective effect by these or other mechanisms is hard to reconcile with the total lack of equivalent suppression of phase 1 VF, which occurs in abundance in the same hearts (see Figure 1). Thus, an absence of an arrhythmogenic factor would seem to be the better explanation for the lack of phase 2 VF in buffer-perfused hearts. The identity of this arrhythmogenic component is intriguing, not least because it would appear to be unnecessary for the initiation of phase 1 VF.

Of the components that are absent in buffer-perfused hearts, blood is an obvious subject for consideration as a necessary mediator of phase 2 VF. Of all the blood components, there are reasons for considering neutrophils and other white blood cells and platelets as promising candidate mediators. It is well known that infarction generates an acute inflammatory response involving these cells (Frangogiannis *et al*., 2002). Furthermore, interpolation between published studies suggests that there is an apparent temporal concordance between myocardial accumulation of neutrophils from the blood (Sasaki *et al*., 1988; Entman *et al*., 1993) and the onset of phase 2 VF (Clark *et al*., 1980; Beatch *et al*., 1991). Additionally, the anti-inflammatory drug nafazatrom, which impedes neutrophil accumulation by inhibiting 5-lipoxygenase-dependent production of the chemoattractant, leukotriene B_4, appears to afford some protection against phase 2 VF, although it could be argued that the published data are somewhat inconsistent and difficult to interpret (Fiedler, 1983; 1985). Evidence for a role for neutrophils and related cells in phase 2 VF is therefore intriguing but incomplete.

An attempt has been made to determine whether phase 2 VF can occur in neutrophil-containing isolated blood-perfused hearts, but the outcome was ambiguous owing to limitations of the model which included nonspecific activation of neutrophils and their loss from the perfusion circuit (Clements-Jewery *et al*., 2002a). Further work (using *in vivo* approaches) is indicated. It may be premature to contemplate possible mechanisms by which neutrophils may mediate phase 2 VF, but these cells are a source of numerous chemical substances that have the potential to adversely affect cardiac electrophysiology (Jordan *et al*., 1999).

In addition to neutrophils, there are other components of the acute inflammatory response that could play a role in determining susceptibility to phase 2 VF. Importantly, the effects of many of these components on cardiac electrophysiology, especially during the development of infarction, have yet to be characterised. For example, activated platelets are a source of serotonin, which can alter coronary blood flow and increase vascular permeability (Borgdorff *et al*., 1994), complement accumulation within infarcting tissue (Rossen *et al*., 1985; Crawford *et al*., 1988) can trigger mast cell degranulation and histamine release (Frangogiannis *et al*., 1998; 2002), mast cells are also a source of leukotrienes and prostaglandins (Gordon *et al*., 1990), and there are also a number of other chemoattractant substances, such as leukotriene B_4 (Sasaki *et al*., 1988), PAF (Annable *et al*., 1985; Flores *et al*., 1994) and the interleukin IL-8 and IL-6 (Kukielka *et al*., 1995a, b), which are present within the infarcting tissue and are putative mediators of phase 2 VF. It is also possible that inflammatory substances shown to predict risk of adverse coronary events, such as C-reactive protein and Lp-PLA_2 (Biasucci, 2004; Oei *et al*., 2005), may have an arrhythmogenic propensity.

In addition to a role for neutrophils and other inflammatory cells, it is possible that phase 2 VF is initiated by an action of the autonomic nervous system. The absence of sympathetic innervation and of soluble blood components such as catecholamines is a possible explanation for the absence of phase 2 VF in isolated perfused hearts (Ravingerova *et al*., 1995). Moreover, it is possible to evoke VF in dogs with myocardial infarction by activating sympathetic drive to the heart (e.g., Schwartz *et al*., 1984). Unfortunately, although there are many studies that have focused on catecholamines and phase 1 VF (Bolli *et al*., 1984) or have utilised the Harris

2-stage ligation model with its inherent limitations to undertake equivalent studies (Scherlag *et al*., 1989), there are few studies that specifically address the role of locally released catecholamines in the initiation of phase 2 VF. If there is a role for catecholamines, their action is presumably mediated in the uninvolved or border zone region of the infarcting myocardium since, although catecholamine accumulation in the involved region increases during the first hour of ischaemia (Lameris *et al*., 2000), it has been shown that 1 h after coronary ligation β_1-adrenoceptors (the likely molecular target of arrhythmogenic catecholamines) become uncoupled from adenylyl cyclase (Vatner *et al*., 1988a, b). Clinically, β_1 blockers can reduce mortality following myocardial infarction, but the reduction is limited and it is not clear whether this results from an effect on VF (Antman *et al*., 1992). In a recent study (Clements-Jewery *et al*., 2002a), catecholamine replenishment of isolated buffer-perfused rat hearts was found to be unable to restore the susceptibility to phase 2 VF seen *in vivo*. Thus, there may be a role for catecholamines, perhaps in conjunction with other substances, in mediating phase 2 VF, but it does not appear to be a necessary role.

The principal conclusion of these deliberations is that it is evident that further work is required to establish the exact role played by the host of putative (and indeed also the unheralded) intercellular and intracellular mediators of phase 2 VF.

Phase 2 VF and safety pharmacology

The battery of preclinical tests used in drug development to evaluate drug safety is evolving. The US Department of Health and Human Services has instigated an International Conference on Harmonization that has made recommendations on general aspects of safety pharmacology studies for human pharmaceuticals (FDA, 2001), but a guideline on how to deal with possible proarrhythmic effects of drugs, especially noncardiac drugs, remains in the draft stage (FDA, 2002). The reason for this is that it has proven difficult for a consensus to be reached on which core battery of tests should be undertaken in the laboratory in order to have reasonable assurance that a drug will not cause an unacceptable increase in the likelihood of cardiac arrhythmias.

The relevance of this to the present article is that, because individual models of specific types of arrhythmia (such as phase 1 VF) do not by themselves allow accurate prediction of drug effectiveness as *antiarrhythmic* protection against SCD, it is likely that an equivalent scenario exists for *proarrhythmic* effects of cardiac and noncardiac drugs. The logic underpinning a judgement of safety should be no different from that for judging effectiveness. Therefore, just as an antiarrhythmic drug must suppress VF in all settings likely to be encountered in ischaemic heart disease (i.e., ischaemia, infarction and perhaps also reperfusion) in order to be judged effective against SCD, it seems reasonable to propose that a judgement of safety from pro-arrhythmic activity requires demonstration of safety in the settings in which the drug will be used. Thus, it may be necessary to determine a drug's proarrhythmic activity during ischaemia and infarction (and perhaps also reperfusion, a topic beyond the scope of the present article). One may also conceive that yet further models could be deemed necessary to discount the possibility of drug-induced proarrhythmia, including models of heart failure and Na^+ and K^+ channelopathy (reviewed in Clancy & Kass, 2005) – for example, models with mutations in the *SCN5A* and *KCNH2* genes that encode the cardiac Na^+ channel, $Na_v1.5$ and the HERG K^+ ion channel, respectively. However, because the use of surrogate end points (such as I_{Kr} in isolated myocytes) has proven unsuccessful in predicting drug *antiarrhythmic* effectiveness against SCD (e.g., Waldo *et al*., 1996), the value of the use of surrogate end points in drug *proarrhythmic* safety evaluation may also be questioned. Thus, it may be wise to include in the evolving battery of ICH guidelines direct testing for exacerbation of phase 2 VF (as well as phase 1 VF and possibly reperfusion-induced VF) using animal models.

Experimental data on drug-induced proarrhythmia during the phase 2 VF-susceptible period are lacking. Even obvious experiments have yet to be undertaken. For example, it would be of interest to examine whether d-sotalol can exacerbate phase 2 VF as a possible explanation for its hitherto inexplicable exacerbation of SCD in the SWORD trial (Waldo *et al*., 1996).

Conclusion

Phase 2 VF occurs spontaneously in most animals surviving phase 1 VF. However, its mechanism differs from that of phase 1 VF and it is affected by drugs differently from phase 1 VF. Its suppression by antiarrhythmic drugs is likely to be necessary if these drugs are to be effective against SCD. Likewise, cardiac and noncardiac drugs may need to be shown to not exacerbate phase 2 VF in order to be judged safe.

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