Characterisation, utilisation and clinical relevance of isolated perfused heart models of ischaemia-induced ventricular fibrillation

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Abstract

The isolated perfused heart has been used increasingly during the last decade as a model for identifying actions of drugs on ventricular fibrillation (VF) induced by myocardial ischaemia. In addition, it has been used to explore the mechanisms responsible for the initiation and maintenance of VF, the concept of endogenous myocardial protection and the phenomenon of preconditioning. This article is a review of the available data (effects of drugs, sources of variation, comparison with other models and man, etc.) and an attempt to evaluate the possible clinical relevance. For several reasons, it is not possible to make a precise judgement on the absolute value of the model in terms of its ability to accurately predict the effectiveness of drugs in the prevention of sudden cardiac death, the main reason being the lack of a positive control, i.e. a drug with proven effectiveness against sudden cardiac death caused by VF in man. Nevertheless, the means by which one may reliably and reproducibly generate ischaemia-induced VF in different isolated heart preparations, and the factors (such as species, heart rate, perfusion constituents and involved zone size) that determine the incidence of VF are now well documented. Careful selection of species and experimental conditions permits the isolated heart of smaller inexpensive animals to function as a first line model for detecting anti-VF activity of probable relevance to phase 1 arrhythmogenesis (i.e., arrhythmogenesis during the first 30 min of ischaemia). In view of the absence of a clinical template from which to evaluate how well it predicts drug effectiveness in man, this model’s clinical relevance, like that of all other preparations and models, can yet be neither accepted nor dismissed. Recent publication patterns suggest an increasing use of the model. Therefore, recommendations are made to facilitate its effective use.

Keywords: Anti-fibrillatory drugs; Arrhythmia models; Global ischaemia; Ischaemia; Langendorff preparation; Sudden cardiac death; Ventricular fibrillation; Working heart preparation

1. Methods

Data included in this article were assembled from personal archives (covering publications pre-1980) and an on-line literature search using pairs of search terms from the following: in vitro, isolated, fibrillation, arrhythmia and ischaemia. To avoid selection bias, all ‘full’ publications on drug suppression of VF induced by ischaemia in isolated heart preparations have been cited. If there are any omissions, this is by oversight rather than design. The isolated heart has been used extensively by the pharmaceutical industry as a first line screen (both in-house and via external collaboration). As a consequence, there is much data that has been published only in abstract form (and one suspects much other data that has not been, and never will be, published in any form). By contrast, more cumbersome preparations, including most in vivo models (especially those utilising large animals such as dogs, cats and pigs) that generate data more slowly (and expensive-ly), generally give rise to a full publication each time a drug is tested (at the very least, in order to express publicly some justification for undertaking the experiment). In this article, the numerous abstracts on arrhythmias in isolated hearts have been ignored, since the extent of peer review is not possible to determine in every instance. Articles that mention VF en passant have been cited only if they

Time for primary review 37 days.
provide numerical data on the incidence of VF. Articles that present only combined VF plus ventricular tachycardia (VT) incidences or do not give actual VF incidence data have not been included in the survey of drug effects.

2. Historical overview

Ischaemia-induced VF was first described in an isolated heart by Gunn [1]. Fibrillar contraction (electrogram recording was not made) was elicited by an air embolism introduced into the coronary vasculature. The candid reporting in the article acknowledged a tendency for the perfused heart to develop VF spontaneously (the perfusion solution was glucose-free, and coronary flow values were not reported), and there was no attempt to discriminate quantitatively between embolism-induced VF and spontaneous (presumably injured-preparation-induced) VF. One anecdote demonstrates VF apparently induced within 10 s by an air embolus [1]. Having on occasion unfortunately allowed air to pass through the coronary circulation of the isolated rat heart without eliciting so much as a ventricular premature beat (VPB), one is inclined to regard this early anecdotal report as unreliable. It has gone largely unnoticed.

Despite an awareness of ischaemia-induced VF, and the emergence over several decades of a variety of in vivo preparations for its study [2], there was no mention again of ischaemia-induced VF in the isolated heart until the mid-1970s. In 1975, Opie’s group [3] attempted left coronary artery ligation in the isolated working rat heart preparation. Ligation was achieved by adapting a technique used in vivo for small animals, originally described in the 1940s [4]. None of the hearts in Opie’s study developed VF or VT during 60 min regional ischaemia. In this study, coronary ligation lowered coronary flow by only 27%, and Krebs’ solution (K⁺ = 5.9 mequiv./l) was used to perfuse the hearts. Today, we know that the extent of flow reduction indicates the extent of the involved region, and both this and the K⁺ content of the perfusion solution determine the likelihood (incidence) of VF in isolated hearts [5,6], as discussed in more detail later. In the light of this knowledge, Opie’s protocol [3] would not be expected to give rise to severe ischaemia-induced arrhythmias.

Three years after this first study, Bricknell and Opie [7] subjected rat hearts to an 85% reduction in global coronary flow as part of a series of experiments on cardiac biochemistry during ischaemia (and reperfusion). They observed that none of the hearts fibrillated during ischaemia. Today, we know that low flow global ischaemia can be effected in rat hearts to cause VF in 80–90% of controls if coronary flow is reduced by more than 85% [8]. Interestingly, in zero flow preparations, VF incidence is close to zero [9]. Zero flow global ischaemia causes asystole, whereas sinus rhythm may persist if some flow is maintained but, even if asystole is prevented by SA and AV nodal superfusion during zero flow global ventricular ischaemia, the incidence of VF remains close to zero [6]. This could be interpreted to imply that some residual flow in the ventricle is required for the initiation of ischaemia-induced VF, although there are other data that do not support this (see later). Global ischaemia preparations have not achieved widespread use for the study of ischaemia-induced VF and fewer than ten full publications have appeared to date.

Regional ischaemia in isolated hearts today is a more widespread technique for examining ischaemia-induced VF. However, the first full publication, from Hearse’s group, appeared only in 1985 [10]. In this study, an attempt was made to raise baseline VF incidence in the rat Langendorff preparation by perfusing with adrenaline or forskolin, in order to explore the role of cAMP as an endogenous mediator of VF. To achieve this end, a low baseline control incidence of VF was a necessary prerequisite. The control incidence of VF was indeed low (8%), but this would be considered anomalous for the rat Langendorff preparation today (see Table 1). In the light of present knowledge [5], the high perfusate K⁺ concentration used (6.5 mequiv./l) can be expected to have contributed to the low control incidence of VF.

Regional ischaemia in the isolated heart is commonly achieved by sewing a suture under the left anterior descending (or, in rat, left main) coronary artery close to its origin, and compressing the artery against tubing through which the suture is threaded to effect a ligature, in a manner akin to that used in vivo [11]. Since the early 1990s, there has been an increase in the use of the rat Langendorff preparation for exploring ischaemia-induced VF (more than 30 publications to date), whereas the other isolated heart preparations, rat, guinea pig and rabbit global ischaemia, and rabbit and marmoset regional ischaemia, have been used less than fifteen times collectively. Fig. 1 shows the accumulated use of the rat Langendorff regional ischaemia preparation.

In addition there are six–sixteen publications for each of the following: rat working heart with regional ischaemia, rat global ischaemia (working and Langendorff perfusion mode) and fewer than five publications each for guinea pig global ischaemia (working and Langendorff perfusion mode), rabbit regional ischaemia and marmoset regional ischaemia.

One of the more puzzling aspects of the use of isolated hearts for generating ischaemia-induced VF is the late appearance of the model in comparison with others. In particular, coronary ligation-induced VF in large animals, first described in the latter part of the last century, was an established technique by the 1940s, particularly as a result of the work of Harris [12]. There may be several explanations. First, until the 1970s, it had been possible to carry out in vivo experiments in large animals inexpensively and without the need for the kind of complex ethical approval required in most countries today. Therefore, there had been
Table 1
Ischaemia-induced arrhythmias in isolated hearts and their prevention: Class I and II (and related) drugs

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation (type of ischaemia)</th>
<th>Duration of ischaemia (min)</th>
<th>Drug and concentration</th>
<th>Effect on Control VF incidence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Lignocaine, 10 μM</td>
<td>↓VF 100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>20</td>
<td>Propranolol, 1 μM</td>
<td>↓VF 75</td>
<td>[21]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>(−)-Propranolol, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>(+)-Propranolol, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Atenolol, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Betaxolol, 0.25 μM</td>
<td>No effect 100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>ICI18551, 0.5 μM</td>
<td>↓VF 100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Phentolamine, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Phentolamine, 10 μM</td>
<td>↓VF 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Phentolamine, 3 μM</td>
<td>↓VF 100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Prazosin, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Prazosin, 10 μM</td>
<td>↓VF 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Trimazosin, 10 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Guanethidine, 1 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Phenoxybenzamine, 10 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Normetanephrine, 3 μM</td>
<td>No effect 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>6-OHDA i.c.</td>
<td>↓VF 38</td>
<td>[38]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Desipramine, 1 μM</td>
<td>↓VF 64</td>
<td>[28]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Adrenaline, 1 μM</td>
<td>↑VF 8</td>
<td>[10]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Forskolin, 2 μM</td>
<td>No effect 8</td>
<td>[10]</td>
</tr>
</tbody>
</table>

6-OHDA=6-hydroxydopamine sufficient to reduce cardiac noradrenaline content; i.c.=intracardiac; L=Langendorff model; low flow=global reduction in flow to below 5% of control; W=working heart model; VF=ventricular fibrillation; ↓=reduction; ↑=increase; an effect is a significant change in the incidence of VF.

little motivation to develop isolated heart models. Second, hearts from small animals (those most suitable for convenient in vitro perfusion) were perceived to be resistant to the development of ischaemia-induced VF. In a study of ligation of coronary arteries in vivo in rats, hamsters and mice, performed in 1954, it was stated that VF did not occur [13]. Unfortunately, in this study, the ECG was not recorded and the chest was closed immediately after coronary ligation, preventing even ‘visual’ detection of VF. We now know that although data for most small animals is scanty, in male rats at least, coronary ligation elicits VF highly reproducibly in vivo [14]. Additionally, to ligate a large coronary artery in a small animal heart requires an act of faith. The large arteries are not visible to the naked eye in perfused preparations, and ligation requires prior knowledge of where the arteries are located. Moreover, their small size means that coronary arteries can neither be conveniently isolated nor ligated without the inclusion of cardiac muscle within the occluding snare. Despite evidence that ensnaring muscle alone does not cause infarction [13], these anticipated difficulties may have been sufficient to put off investigators from using isolated hearts (from small animals). Today we know that not only can an involved region be produced simply by coronary ligation in a small animal’s isolated heart, but also that the size of the involved zone can be varied precisely and accurately by choosing different sites for placement of the occlusive snare [6,15].

Following the first clear description of coronary ligation-induced VF in small animals (rats) in vivo [16], and the increasing use of isolated hearts for metabolic studies (e.g., [7]), an emerging interest in oxygen-derived free radicals and their role in reperfusion-induced injury stimulated the use of isolated hearts in the examination of reperfusion-induced VF (see Manning and Hearse, [17]). Since reperfusion requires that there is ischaemia beforehand, the isolated heart subsequently became used for the study of ischaemia-induced VF. Once factors influencing the susceptibility to ischaemia-induced VF in isolated hearts had

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Fig. 1. Cumulative publication of full papers on ischaemia-induced VF in the rat Langendorff preparation.

Fig. 1. Cumulative publication of full papers on ischaemia-induced VF in the rat Langendorff preparation.
eventually been characterised (e.g., [5,6,18]), the preparation finally came of age in the 1990s, when, for the first time, Circulation and Circulation Research published work on new approaches to suppression of ischaemia-induced VF that utilised the isolated perfused heart from different species [19–21].

Due to an apparent lack of awareness of the strong influence of certain factors (such as $K^+$) on susceptibility to ischaemia-induced VF in earlier studies, and the relatively small number of studies that have been performed to date, it is difficult to fully evaluate the principal issues (i.e., bioassay characteristics and potential clinical relevance) that determine the suitability of the available isolated heart preparations, with one exception, the rat Langendorff regional ischaemia preparation, which has been used fairly extensively for detecting anti-VF actions of drugs [8,19,21–36], and is well characterised [5,6,18].

The study of VF in globally ischaemic hearts came about from the use of the preparation to study the metabolic effects of ischaemia [7]. Global ischaemia can be achieved simply by clamping the aortic cannula (Langendorff preparation) or the left atrial cannula (working heart preparation). However, one would not instinctively choose a global ischaemia preparation if the study of VF related to ischaemic heart disease were the primary objective (global ischaemia is liable to be encountered in man only during cardiopulmonary bypass, or once VF has begun). On the other hand, any study of ischaemia or reperfusion that requires the use of the guinea pig must use global rather than regional ischaemia, due to the extensive collateral vascularisation of the guinea pig heart, which precludes the production of regional ischaemia by single arterial ligation [37]. The majority of such studies have utilised low flow rather than zero flow global ischaemia (e.g., [38]). One reason for this is that zero flow global ischaemia causes sinus arrest, AV block and asystole and very little VF in the isolated heart [6], whereas low flow ischaemia causes sufficient VF to allow detection of anti-VF drug action in some, although not all, studies (Table 1).

The dual perfusion preparation [39] is a recent modification of the isolated heart model of ischaemia-induced VF. This technique makes use of a dual lumen catheter that allows independent perfusion of left and right coronary beds. Left regional ischaemia can be elicited without the need for coronary artery ligation, and the site of action of anti-VF drugs can be determined by selective perfusion of the left or right coronary beds with a test solution [40].

3. Bioassay characteristics

3.1. Bioassay requirements

Any experimental preparation intended for use in examining the actions of drugs on ischaemia-induced VF must exhibit two key characteristics; a control incidence of VF that is sufficiently high to allow detection of protective drug actions with precision but without the need for more than ten–twelve preparations per group, and a control incidence of VF that varies little between studies, to permit accurate detection of drug action. Bioassay characteristics of isolated heart preparations have been defined or may be deduced from the literature (which is extensive in the case of the rat Langendorff preparation). Tables 1–4 summarise control VF incidences and responses to drugs during ischaemia in the isolated heart. There appears to be a wide range of values. However, this can be explained by consideration of the sources of variation.

3.2. Sources of variation

3.2.1. Perfusion $K^+$ content

One important source of variation is the constituents of the control perfusion solution. The control $K^+$ content has varied between studies from 2.7 [41] to 6.5 mequiv./l [10]. Standard Krebs’ solution contains 5.9 mequiv./l $K^+$. In two studies, it has been demonstrated that variation of $K^+$ content between 2 and 8 mequiv./l can change VF incidence during ischaemia from 100 to 0% [5,29]. This is similar to the relationship between blood $K^+$ content and the prevalence of sudden cardiac death in patients with ischaemic heart disease [42–44].

3.2.2. Involved (ischaemic) zone size

Another important source of variation is the size of the involved zone. VF occurring during regional ischaemia was reported to exhibit a bell-shaped relationship with involved zone size, with maximum susceptibility occurring when approximately 50% of the combined weight of the left and right ventricles was involved [6]. In rabbit and marmoset hearts, the control incidence of ischaemia-induced VF was found to be low, even with a large (40%) involved zone size and clear evidence of ischaemia, as indicated by the observation that more than 50% of hearts developed VF during subsequent reperfusion [20]. The relationship between VF susceptibility and involved zone size in man is not known. Experiments in vivo using rats [14] and dogs [45] have demonstrated a relationship that is qualitatively similar to that for Langendorff perfused rat hearts. The reason why ischaemia-induced VF exhibits a bell-shaped relationship with involved zone size is intriguing and may indicate that ischaemia-induced VF initiates via an interaction between involved and uninvolved regions, perhaps via flow of injury current [6].

3.2.3. Depth of ischaemia

The depth (severity) of ischaemia within the involved zone also determines VF incidence. Experiments performed in vivo on dogs, which show a wide variation in collateral supply to the involved zone, have revealed that residual flow is an important source of variation for
Table 2
Ischaemia-induced arrhythmias in isolated hearts and their prevention: Class III and related drugs

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation (type of ischaemia)</th>
<th>Duration of ischaemia (min)</th>
<th>Drug and concentration</th>
<th>Effect on VF</th>
<th>Control VF incidence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Tedisamil, 0.1–3 μM</td>
<td>No effect</td>
<td>92</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Tedisamil, 3 μM</td>
<td>No effect</td>
<td>92</td>
<td>[24]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Tedisamil, 3 μM</td>
<td>No effect</td>
<td>92</td>
<td>[24]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Tacrine, ≥1 μM</td>
<td>↓VF</td>
<td>70</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>RP58866, 1, 3 and 10 μM</td>
<td>↓VF</td>
<td>92</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>Rabbit L (regional)</td>
<td>30</td>
<td>RP58866, 3 μM</td>
<td>No ↑VF</td>
<td>0</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>Marmoset L (regional)</td>
<td>30</td>
<td>RP58866, 3 μM</td>
<td>No ↑VF</td>
<td>11</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Bretylium, 1 μM</td>
<td>No effect</td>
<td>64</td>
<td>[28]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>UK66,914, 0.3 and 1 μM</td>
<td>No effect</td>
<td>78</td>
<td>[25]</td>
<td></td>
</tr>
<tr>
<td>Guinea pig L (low flow)</td>
<td>30</td>
<td>UK66,914, 3 μM</td>
<td>No effect</td>
<td>100</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>Rabbit L (regional)</td>
<td>30</td>
<td>UK66,914, 0.3 and 1 μM</td>
<td>No ↑VF</td>
<td>15</td>
<td>[25]</td>
<td></td>
</tr>
<tr>
<td>Guinea pig L (low flow)</td>
<td>30</td>
<td>4-Aminopyridine, 30 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Gibenclamide, 1 μM</td>
<td>↓VF</td>
<td>86</td>
<td>[91]</td>
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<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Gibenclamide, 1 μM</td>
<td>↓VF</td>
<td>86</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>Guinea pig L (low flow)</td>
<td>30</td>
<td>Gibenclamide, 3 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>15</td>
<td>Gibenclamide, 10 μM</td>
<td>↓VF</td>
<td>60</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Gibenclamide, 10 μM</td>
<td>No effect</td>
<td>100</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Gibenclamide, 10 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>Guinea pig L (low flow)</td>
<td>30</td>
<td>Glizipide, 30 (not ≥10) μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
<td></td>
</tr>
<tr>
<td>Rat L (low flow)</td>
<td>30</td>
<td>Tolbutamide, 1 mM</td>
<td>↓VF</td>
<td>86</td>
<td>[8]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>15</td>
<td>Cromakalim, 10 μM</td>
<td>↓VF</td>
<td>60</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>Cromakalim, 10 μM</td>
<td>No effect</td>
<td>92</td>
<td>[24]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>15</td>
<td>Pinacidil, 10 μM</td>
<td>↓VF</td>
<td>60</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>RP49356, 10 μM</td>
<td>No effect</td>
<td>100</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>15</td>
<td>K⁺, 6 vs. 2, 8 vs. 4 mM</td>
<td>↓VF</td>
<td>60 (4 mM K⁺)</td>
<td>[29]</td>
<td></td>
</tr>
<tr>
<td>Rat L (regional)</td>
<td>30</td>
<td>K⁺, 4, 6 or 8 vs. 2 mM</td>
<td>↓VF</td>
<td>42 (4 mM K⁺)</td>
<td>[5]</td>
<td></td>
</tr>
</tbody>
</table>

L=Langendorff model; low flow=global reduction in flow to below 5% of control; W=working heart model; VF=ventricular fibrillation; ↓=reduction; ↑=increase; an effect is a significant change in the incidence of VF.

Ischaemia-induced VF [46]. The rat and rabbit heart [37] and also the marmoset [20] exhibit a low level of residual flow, amounting to less than 6% of uninvolved zone flow. This minimises variation in the depth of ischaemia. A within-laboratory assessment of data from conscious rats over a period of several years revealed that the control incidence of VF was both reproducible and high [14], and is an important characteristic of a preparation. Its complexity over a period of several years revealed that the control incidence of VF was both reproducible and high [14], and is an important characteristic of a preparation. Its complexity, species-, model- and condition-dependence and its relationship to the human disease are discussed below.

3.2.4. Heart rate

Heart rate is an important source of variation. The onset of ischaemia-induced VF is delayed by bradycardia [18]. When a study is time-limited (30 min ischaemia is commonly used for the study of phase 1 arrhythmias), VF incidence may become very low if the heart rate is slow [18], precluding detection of anti-VF drug action. In global ischaemia studies, bradycardia is an inevitable consequence of SA and AV nodal ischaemia. Because heart rate is species-dependent, optimum heart rates are also species-dependent. Sinus rate is lower in crystalloid (blood-free) perfused hearts than it is in vivo, even when temperature and other sources of variation are controlled (see later for details on model refinement, Section 4). Characteristic mean rates for isolated rat, guinea pig and rabbit hearts are about 280, 190 and 180 beats/min, respectively [20,38].

3.3. Natural history: Phase 1a and phase 1b VF

The time-course of appearance of ischaemia-induced VF is an important characteristic of a preparation. Its complexity, species-, model- and condition-dependence and its relationship to the human disease are discussed below.

3.3.1. Human template

Estimates of VF natural history in man are not precise as they generally provide only the incidence within the first hour of symptoms. Nor are they accurate, since they do not include patients who die from VF before being seen by a physician (i.e., outside of hospital, the main sub-population that dies from VF). The time-course during the first 30 min of ischaemia is unknown; indeed, the time-course over several hours after the onset of ischaemia is sketchy, due to uncertainty over the exact time of onset of ischaemia, and the variable presence of drugs, even in the low percentage of patients that get to see a physician during the interval between the onset of symptoms and sudden cardiac death [47–49]. There is even variation between the few studies...
Table 3
Ischaemia-induced arrhythmias in isolated hearts and their prevention: Class IV, related drugs and miscellaneous

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation (type of ischaemia)</th>
<th>Duration of ischaemia (min)</th>
<th>Drug and concentration</th>
<th>Effect on VF</th>
<th>Control VF incidence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Verapamil, 3 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>Nifedipine, 0.03 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Caffeine, 1 mM</td>
<td>↓VF</td>
<td>38</td>
<td>[30]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Ryanodine, ≥1 nM</td>
<td>↓VF</td>
<td>38</td>
<td>[30]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>RS56865, 0.1 μM (not ≤0.01 μM)</td>
<td>↓VF</td>
<td>67</td>
<td>[34]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>RS56865, 1 μM</td>
<td>↓VF</td>
<td>100</td>
<td>[60]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>15</td>
<td>(+)-(S)-202-791, 0.3 μM</td>
<td>↑VF</td>
<td>0</td>
<td>[35]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>20</td>
<td>LiCl, 50 mM</td>
<td>↓VF</td>
<td>75</td>
<td>[21]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>NO₃⁺:Cl⁻, ≥50:50</td>
<td>↓VF</td>
<td>58</td>
<td>[6]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>NO₃⁻:Cl⁻, ≥100:00</td>
<td>↓VF</td>
<td>67</td>
<td>[33]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Cl⁻:Cl⁻, ≥100:00</td>
<td>↓VF</td>
<td>67</td>
<td>[33]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Methylsulphate:Cl⁻, ≥100:00</td>
<td>↑VF</td>
<td>67</td>
<td>[33]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Glutamate, 2 mM</td>
<td>No effect</td>
<td>100</td>
<td>[92]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>L (low flow)</td>
<td>30</td>
<td>Preconditioning</td>
<td>↓VF</td>
<td>43</td>
<td>[93]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Preconditioning</td>
<td>↓VF</td>
<td>66 (blood perfused)</td>
<td>[94]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>10</td>
<td>E. coli endotoxin, 2.5 mg/kg</td>
<td>↓VF</td>
<td>53</td>
<td>[95]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>60</td>
<td>ANP, 0.02–2 μM</td>
<td>No effect</td>
<td>58</td>
<td>[96]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>60</td>
<td>Methylene blue, 20 μM</td>
<td>No effect</td>
<td>50</td>
<td>[97]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>60</td>
<td>Zaproprast, 100 μM</td>
<td>No effect</td>
<td>50</td>
<td>[97]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Cinetidine, 1 μM</td>
<td>↓VF</td>
<td>57</td>
<td>[31]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Ranitidine, up to 10 μM</td>
<td>No effect</td>
<td>100</td>
<td>[31]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Allopurinol, 100 μM</td>
<td>↓VF</td>
<td>75</td>
<td>[32]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>Dynorphin, 10 μg/heart</td>
<td>No effect</td>
<td>75</td>
<td>[32]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>ITF-1300, 1–10 mg/l</td>
<td>↓VF</td>
<td>100</td>
<td>[27]</td>
</tr>
<tr>
<td>Rat</td>
<td>W (regional)</td>
<td>30</td>
<td>BN 50739, 1 μM</td>
<td>↓VF</td>
<td>75</td>
<td>[98]</td>
</tr>
<tr>
<td>Rat</td>
<td>W (regional)</td>
<td>30</td>
<td>BN 52021, 60 μM</td>
<td>No effect</td>
<td>78</td>
<td>[99]</td>
</tr>
<tr>
<td>Rat</td>
<td>W (regional)</td>
<td>30</td>
<td>BN 52021, 15 and 30 μM</td>
<td>No effect</td>
<td>78</td>
<td>[99]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>PAF, 1 nM</td>
<td>↑VF</td>
<td>40</td>
<td>[100]</td>
</tr>
</tbody>
</table>

Compound 202-791=isopropyl 4-([1,3-benzoxadiazo-4-yl]-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridine-carboxylate; L=Langendorff model; low flow=global reduction in flow to below 5% of control; W=working heart model; VF=ventricular fibrillation; ↓=reduction; ↑=increase; an effect is a significant change in the incidence of VF.

Table 4
Ischaemia-induced arrhythmias in isolated hearts: Studies with very low control VF incidences

<table>
<thead>
<tr>
<th>Species</th>
<th>Preparation (type of ischaemia)</th>
<th>Duration of ischaemia (min)</th>
<th>Drug and concentration</th>
<th>Control VF incidence (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>15</td>
<td>–</td>
<td>0</td>
<td>[36]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>–</td>
<td>0 (K⁺=2.7 mM)</td>
<td>[41]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>–</td>
<td>0 (flow ↓85%)</td>
<td>[7]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>–</td>
<td>0 (flow ↓85%)</td>
<td>[7]</td>
</tr>
<tr>
<td>Rat</td>
<td>L (regional)</td>
<td>30</td>
<td>–</td>
<td>0 (K⁺=5.9 mM)</td>
<td>[3]</td>
</tr>
<tr>
<td>Rabbit</td>
<td>L (regional)</td>
<td>40</td>
<td>–</td>
<td>0 (K⁺=3 mM)</td>
<td>[40]</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>L (low flow)</td>
<td>30</td>
<td>–</td>
<td>25</td>
<td>[85]</td>
</tr>
</tbody>
</table>

L=Langendorff model; low flow=global reduction in flow to below 5% of control unless stated; VF=ventricular fibrillation; ↓=reduction; ↑=increase; an effect is a significant change in the incidence of VF.

that have reported incidences of in-hospital VF recorded during the first hour after the onset of chest pain, e.g., from 2 [47] to 10% [50].

Because of this lack of precision and accuracy, Fig. 2a indicates that the profile of phase 1 VF is not known in man. It remains to be determined whether phase 1 is mono- or bimodal, and if there is a separation between phase 1 VF (occurring during the first 30 min of ischaemia) and phase 2 VF (occurring later). The relationship in man between phase 1 VF and involved zone size, collateral
Fig. 2. Time-course of phase 1 ischaemia-induced VF in man and a different primate, rat and guinea pig whole heart preparations, in vivo and in vitro. The methods for obtaining the data, the anaesthetics used (in vivo studies) and other methodological considerations are described in the text. The source material was: references [50] and [47] (part a), [53] (part b), [103] (part c), [104] (part d), accumulated unpublished data from 40 consecutive control hearts from my laboratory, hearts perfused with Krebs' modified to contain 3 mequiv/l K⁺ and 1.4 mequiv/l Ca²⁺ (part e), [20] (part f), [9] (part g) and [38] (part h).
flow, blood \( K^+ \) content, heart rate and other determinants of susceptibility to VF, as revealed by animal experimentation, are unknown.

### 3.3.2. Characterisation of phase 1a and phase 1b VF

In some models, phase 1 VF is bimodal. The ‘immediate’ and ‘delayed’ arrhythmias (ectopic beats and VT as well as VF), described by Kaplinsky et al. [51] in anaesthetised dog, were defined as occurring 2–10 and 12–30 min after the start of ischaemia. Likewise, the ‘phase 1a’ and ‘1b’ arrhythmias described by Meesman et al. [46] in the same model were those occurring before and after the twelfth minute of ischaemia. The terms ‘phase 1a’ and ‘1b’ were coined on the basis of the arrhythmia time-course that had been observed in a model. Importantly, the terms were not coined on the basis of any foreknowledge of the possible existence of discrete mechanisms of initiation of VF at different times after the onset of ischaemia. Although there is some evidence that phase 1a arrhythmias are associated with re-entry [51], it is not possible with present techniques to identify the exact electrophysiological mechanism (re-entry versus flow of injury current, etc.) responsible for the initiation of ischaemia-induced VF, whether it be ‘phase 1a’ or ‘1b’, with certainty [52], or to discriminate between them other than by inference. Therefore, it may be safer to desist from assigning the appellation ‘1a’ or ‘1b’ to VF occurring during ischaemia in other models if the time-course is observed to be monomodal. It would be safer to call it ‘monomodal phase 1’ VF. An attempt has been made here to explore whether or not (i) the pattern of VF seen in the anaesthetised dog (from which the 1a and 1b phase concept was derived) is robust (i.e., consistent between different dog studies) and (ii) the pattern is conserved (i.e., consistent between different species and models). Some important recommendations have been made on the basis of this analysis.

### 3.3.3. Species- and model-dependent variation in phase 1 VF distribution and severity

The time-course of phase 1 VF in animal models of different types and species are shown in Figs. 2 and 3 Fig. 4. In evaluating these data, it must be emphasised that species and setting are only two of many possible sources of variation. Most laboratories tend to establish their own techniques, with variations in involved zone size, perfusion constituents (in vitro models), anaesthetic (in vivo models) as well as species, each potentially contributing to differences in outcome. This, together with the fact that the majority of published studies on the action of drugs do not illustrate the precise natural history of phase 1 VF, makes interpretation of data very difficult. Indeed, even when studies do illustrate the time-course of VF onset, the ways the data are presented are inconsistent and potentially misleading. Importantly, the true time-course should take into consideration ‘censoring’ by the occurrence of VF (or death by other causes) over time. The presence of sustained VF in vitro, or death of an animal in vivo, reduce the effective group size. If hearts are defibrillated (or VF spontaneously terminates), the effective group size fluctuates with time.

#### 3.3.3.1. Recalculation of the time-course of phase 1 VF onset

In Figs. 2–4, the time-course of phase 1 VF onset has been recalculated using data derived from original publications, taking into account animal deaths and (in the case of rat experiments) fluctuations in group size associated with transient VF or defibrillation. In order to generate figures that can be contrasted, the group incidence of new episodes of VF was calculated for the sequential time intervals, 0–1, 1–2, 2–5, 5–10, 10–15...25–30 min after the onset of ischaemia for each model. The data should strictly have been represented by histograms, but curves were drawn instead for illustrative purposes. In each instance, the total incidence of VF over a 30-min period is also shown (as a single histogram).

#### 3.3.3.2. Time-course of phase 1 VF onset in conscious primates

Although human data are not available, there are data for the conscious baboon [53]. The incidence of VF was found to be zero during the first 30 min of ischaemia in this model (Fig. 2b). However, it would be hazardous to extrapolate from this observation that phase 1 VF does not occur in man. In fact, the involved zones of the six control baboons studied ranged from only 6 to 25% of the total ventricular weight [53]. In conscious rats or anaesthetised dogs, involved zones of this size would be insufficient to elicit phase 1 VF in accordance with a precise relationship between involved zone size and VF susceptibility [14,45,54]. Clearly, the available in vivo primate data is insufficient. A precise profile of ischaemia-induced VF would be of particular value as it would provide a primate template with which to evaluate other more inexpensive and ethically justifiable models designed for use in screening drugs.

#### 3.3.3.3. Time-course of phase 1 VF onset in conscious rat versus isolated rat heart

At the other end of the species spectrum, the conscious rat was reported to exhibit a high incidence of phase 1 VF early (3–10 min) after the start of ischaemia, with a monomodal time-course (Fig. 2c); there was no evidence of the bimodal distribution reported for the anaesthetised dog [51,55]. In pentobarbitone-anaesthetised rats, the profile was similar, albeit with a slightly delayed peak (at 5–10 min), and a slightly lower total incidence of VF over 30 min (Fig. 2d). In a recent study in which rats were thoracotomised and subjected to coronary ligation under brief ether anaesthesia, then monitored while conscious, a much lower VF incidence of less than 20% was reported, with all phase 1 VF occurring within 10 min of the onset of ischaemia [56]. This may be a sex-related anomaly, since, in contrast with most studies,
Fig. 3. Time-course of phase 1 ischaemia-induced VF in different rabbit and pig whole heart preparations, in vivo and in vitro. The methods for obtaining the data, the anaesthetics used (in vivo studies) and other methodological considerations are described in the text. The source material was: reference [62] (part a), [63] (part b), [64] (part c), [20] (part d), [65] (parts e and f), [66] (part g) and [67] (part h).
Fig. 4. Time-course of phase 1 ischaemia-induced VF in different dog and cat preparations, in vivo. The methods for obtaining the data, the anaesthetics used and other methodological considerations are described in the text. The source material was: reference [69] (part a), [55] (part b), [51] (part c), [67] (part d), [55] (part e), [70] (part f) and [71] (part g).
female animals were used [56], and female rats have been reported to be more resistant to phase 1 VF than males, in vivo [57]. This profile for the rat in vivo may be contrasted with that for the rat Langendorff preparation with regional ischaemia. In the Langendorff preparation, when involved zone size was similar to that produced in vivo, and perfusion K⁺ and other factors that determine VF susceptibility were optimised, the time-course of appearance of ischaemia-induced VF was monomodal (like that in rats in vivo) but peak susceptibility was delayed (compared with that in rats in vivo) to 15–20 min (Fig. 2e). When ischaemia was allowed to persist beyond 30 min in this preparation, the incidence of VF declined over the ensuing 2 h, until no further new episodes appeared [58]. This was quite different from the time-course in conscious rats in which, following the waning of phase 1 VF, there occurred a severe second wave of VF (phase 2 VF) beginning ~90 min after the start of ischaemia [14,56,59].

The timing of the peak of phase 1 VF in the rat Langendorff preparation was found to be dependent on sinus rate; it occurred sooner when the heart rate was increased [18]. When identifiable and established determinants of VF were modulated to maximise susceptibility [5,6,24–26], 90–100% of controls experienced VF (Tables 1–3).

3.3.3.4. Time-course of phase 1 VF onset in primates in vitro, and in global ischaemia preparations. The time-course for other isolated heart models of ischaemia is less well defined. In regionally ischaemic marmoset hearts (perfused with a solution similar to that giving a high incidence of VF in rat hearts), and in low flow globally ischaemic rat and guinea pig hearts, there appears to be little or no ischaemia-induced VF (Fig. 2f–h), although, in the low flow global ischaemic guinea pig heart, there is some variation; Gwilt et al. [60] reported a 100% incidence of VF, a value vastly different from that shown in Fig. 2h. The Gwilt data is not shown in the figure because the time-course of VF onset was not reported; the low incidence of ischaemia-induced VF reported in some low flow global ischaemia studies may be a consequence of the high K⁺ content of the perfusion medium used, 4.6 mequiv./l in one rat study [9] and 5.8 mequiv./l in one guinea pig study [38]. Equivalent values of K⁺ content have been shown to be sufficient to markedly suppress regional ischaemia-induced phase 1 VF in the perfused rat hearts [5], as discussed earlier. The onset of VF in global ischaemia preparations (i.e., in guinea pig hearts), when documented, has been reported to occur later than 15 min after the start of ischaemia (Fig. 2h).

Thus, one may tentatively propose that, in crystalloid perfused hearts from different species, the timing of ischaemia-induced VF appears to be determined principally by the experimental set up per se (i.e., isolated heart perfusion versus the in vivo setting) and possibly by the perfusion constituents used, with the species and type of ischaemia (regional versus global) playing less of a role. On the other hand, the incidence of ischaemia-induced VF is critically dependent on perfusion constituents, species and the type of ischaemia. There are no data available from crystalloid perfused hearts from larger species (i.e., dog) with which to explore the robustness of these observations. Limited information from dog hearts perfused with a non-crystalloid blood/Tyrode mix [61] indicated that VF occurred during 8 min of regional ischaemia, but that the incidence was low (31%).

3.3.3.5. Time-course of phase 1 VF onset in the rabbit in vivo and in vitro. In anaesthetised rabbits and isolated rabbit hearts, no consistent pattern has emerged for the time-course of phase 1 VF (Fig. 3a–d). It is difficult to pinpoint the basis for this variation. In anaesthetised rabbit studies, Coker [62], using diazepam, fentanyl and fluanisone anaesthesia, and Bril et al. [63], using diazepam and pentobarbitone anaesthesia, both reported a monomodal profile of VF, but this was consistent with the original definition of phase 1a VF in only one of the two studies [62]. VF incidence peaked 10–15 min after the start of ischaemia in the other study [63], exactly at the transition point between 1a and 1b VF, as described for the anaesthetised dog [51,55]. In contrast, using pentobarbitone anaesthesia, Barrett et al. [64] found a bimodal pattern of VF, although this did not fit with the anaesthetised dog profile reported by Kaplinsky et al. [51] and Meesman [55], since the cut-off between the first and second peaks occurred 3–5 min after the start of ischaemia (Fig. 3c) rather than at 12 min. These data are important for two reasons. They show that, even when phase 1 VF is bimodal, the location of the peaks varies between species. They also show that there cannot be a universal definition of phase 1a and 1b VF based on the precise timing of VF onset since the timing is species-dependent, even when the time-course is bimodal.

In rabbit hearts perfused with a similar solution, and subjected to regional ischaemia of a similar zone size to that giving a high incidence of VF in regionally ischaemic rat hearts, it was found that the ischaemia-induced VF incidence was zero (Fig. 3d). Thus, whereas the Langendorff regionally ischaemic rat hearts differ from the rat in vivo only in terms of the timing of phase 1 VF, the regionally ischaemic rabbit heart appears to differ from the rabbit in vivo in terms of the overall incidence of phase 1 VF. However, the different anaesthetic regimen used complicates interpretation of these findings. Moreover, it could be argued that the perfusion conditions that optimise susceptibility to phase 1 VF in the regionally ischaemic rat and rabbit heart may differ. Optimum conditions may also differ for the marmoset heart, another preparation that appears to be resistant to phase 1 VF during regional ischaemia in vitro (Fig. 2f). It may be worthwhile to explore this possibility, although more information on basal blood levels of major ions in these species would be a necessary guide to experimentation.
3.3.3.6. Time-course of phase 1 VF onset in the pig, dog and cat. In pig, dog, and cat isolated perfused hearts, there is no clear information on the time-course of ischaemia-induced VF (and no data at all for crystalloid perfused hearts from these species, to my knowledge). In pigs in vivo, the outcome of different studies varies in a manner similar to that described for rabbits, with the exception that, whereas in some rabbit studies VF occurred no later than 12 min after the start of ischaemia, in some pig studies, VF occurred no earlier than 12 min after the start of ischaemia. Fig. 3e–f illustrate data from Bardaji et al. [65] that revealed a profile that Mesman et al. [46,55] would describe as phase 1b VF; the type of anaesthetic used (chloralose in Fig. 3e and thiopentone in Fig. 3f) did not seem to be a major determinant of outcome. In contrast, McDonald et al. [66], using droperidol, ketamine, pentobarbital, fentanyl and pancuronium anaesthesia, reported a bimodal profile of phase 1 VF (Fig. 3g). The transition between the first and second peak of VF occurred some minutes later than the transition in anaesthetised rabbits [63] but sooner than the transition between ‘phase 1a’ and ‘1b’ VF reported by Kaplinsky et al. [51] and Mesman [55] for anaesthetised dogs. By further contrast, Benfey et al. [67], using pentobarbitone anaesthesia, observed a monomodal profile with VF occurring over a wide time interval (5–25 min; Fig. 3h), a pattern different from any yet described for any other preparation or species. The pig heart (like that of rat, rabbit and marmoset [20,37]) is collateral-deficient [68], so within-species variation in study outcomes cannot be attributed to variation in collateral distribution. Indeed, the same consideration applies to the rabbit studies discussed earlier.

One is tempted to suggest, on the basis of data so far reviewed that, if ischaemia elicits phase 1a and 1b VF (as originally defined) in man, then anaesthetised dogs are suitable for the study of phase 1a and 1b VF, anaesthetised pigs are better suited for evaluation of suppression of phase 1b VF, anaesthetised and conscious rats for phase 1a VF, anaesthetised rabbits for phase 1a VF, and isolated rat hearts for phase 1b VF. However, without the necessary clinical template, one cannot evaluate this suggestion. Moreover, one must consider the extent to which the original definition is robust for the dog.

Fig. 4 shows six profiles of ischaemia-induced VF in anaesthetised dog. As mentioned earlier, the original description of phase 1a and 1b VF was made using the anaesthetised preparation, with 12 min of ischaemia as the cut-off between the phases [46,51,55]. However, to illustrate this convincingly requires the use of dogs in which collateral flow is measured and found to be negligible. Total VF incidence was found by Martin and Mesman [69] to be high, with well differentiated 1a and 1b peaks, in collateral-deficient dogs (Fig. 4a; anaesthetic regime not specified), although in an earlier and similar study, Mesman [55] reported a lower total incidence of VF (Fig. 4b, with morphine, chloralose, urethane anaesthesia). In slight contrast, Kaplinsky et al. [51] and Benfey et al. [67], in studies that were not controlled for collateral variation, found a lower overall incidence of phase 1 VF, and less well-defined 1a and 1b VF peaks (pentobarbitone anaesthesia was used in both of these studies; data shown in Fig. 4c–d). In marked contrast, Mesman [55] found that in morphine, chloralose, urethane anaesthetised dogs with functional coronary collaterals, phase 1 VF was monomodal (Fig. 4e), and the peak occurred 10–15 min after the start of ischaemia (the time of transition between phase 1a and 1b VF in Fig. 4a–b). In further contrast, Parratt and Wainwright [70], using thiopentone and chloralose anaesthetised greyhounds (collateral status not described), observed monomodal VF occurring less than 5 min after the start of ischaemia (Fig. 4f), a finding similar to that described by Penkoske et al. [71] for the cat (a species for which available time-course data is limited) anaesthetised with ketamine and chloralose with decamethonium (Fig. 4g). In both of these studies, the overall group incidence of VF was low (Fig. 4).

Conscious dog data is limited. Bolli et al. [54], in an elegant experiment that took into account variation in collateral flow, found that no phase 1a VF occurred at all in collateral-deficient conscious dogs (during 15 min of ischaemia), whereas the incidence in equivalent pentobarbitone-anaesthetised dogs was 25%. Susceptibility to phase 1b VF was not explored. These data differ from rat data (Fig. 2) which indicated that conscious and pentobarbitone-anaesthetised animals had a similar high incidence of VF during the first 15 min of ischaemia.

Thus, the anaesthetised dog, the preparation for which the ‘typical’ profile of phase 1 VF was originally defined, actually exhibits a rather inconsistent pattern of ischaemia-induced VF which, in turn, appears to differ from the profile in the conscious dog. This has important implications, the most obvious of which is that the value of any other model, whether it be in vivo, conscious or anaesthetised, or perfused heart with regional or global ischaemia, cannot be evaluated for its clinical relevance by appraisal of the time-course of ischaemia-induced VF using the anaesthetised dog as the “gold standard”.

3.3.3.7. Nature of, basis for and implications of species-dependent variation. Some of the experiments described above may suggest that species is itself an independent determinant of susceptibility to ischaemia-induced VF. The low incidence of regional ischaemia-induced VF in isolated rabbit and marmoset hearts compared with isolated rat hearts with equivalent sized involved zones [20,25] is one example that supports this notion. These differences are difficult to reconcile. They could be due to a species-dependent relationship between involved zone size and susceptibility to VF (i.e., a qualitative difference based on fundamentally different mechanisms of VF initiation between species). However, this is unlikely since the relationship between VF incidence and involved zone size in the
isolated rat heart [6] is actually qualitatively similar to that found in an entirely different model, the anaesthetised dog preparation with regional ischaemia [45]. The differences could be related to differences in collateral flow, another source of variation discussed earlier. Collateral flow in the rabbit heart is even lower than that in the rat heart [37], yet the isolated rabbit heart seems to be resistant to developing ischaemia-induced VF [20,25]. From this, it may be argued that some degree of collateral flow is necessary for maximising susceptibility to ischaemia-induced VF (as mentioned earlier). Perhaps collateral flow allows a spatial dispersion of dysfunction within the involved zone, facilitating maintenance of re-entry and, if collateral flow is too low, then dysfunction within the ischaemic zone may be too homogeneous to facilitate the maintenance of VF. There are some difficulties with this explanation however, since VF does develop during regional ischaemia in anaesthetised rabbits, as shown in Fig. 3a–c, albeit not with the same high incidence as seen in the anaesthetised rat [14]. This indicates that the low incidence of ischaemia-induced VF in the rabbit Langendorff preparation cannot be attributed simply to low residual flow.

It is possible that the difficulty in explaining the basis for this and other examples of species variation is the result of complex interplay between different variables. Rabbit and marmoset hearts, which are only approximately four–seven and 1.5–2 times the size of the rat’s, have a ventricular APD and QT that are approximately three times the size of the rat’s, yet have an RR interval that is less than twice as long [20]. If ischaemia-induced VF depends on flow of injury current for initiation, and re-entry for maintenance [72,73], these differences might impact greatly on the scope for generation of the ectopic depolarisation that may be necessary for the initiation of VF, and the likelihood of the resultant ‘premature’ wavefront encountering sufficient patches of repolarised tissue to allow the fractionation that may be necessary for the maintenance of VF.

However, the only safe conclusion to draw from Figs. 2–4 is that the natural history profile of ischaemia-induced VF, and the overall susceptibility to ischaemia-induced VF (control group incidence), varies between setting (in vivo versus in vitro), between species and even within species (even when data from a single laboratory is considered, in some cases). It is not possible to fully explain this variation with the data presently available. Many sources of variation (involved zone size, blood K\(^+\) content, collateral flow, etc.) have been identified and one hopes that if every investigator would publish details on these variables for their model, it may become possible to construct a species/setting-independent mathematical model of VF initiation and maintenance.

3.3.4. Re-appraisal of the use of the terms ‘phase 1a’ and ‘1b’ VF

Clearly, the originally described pattern of phase 1a and 1b VF is not robust, nor is it conserved within or between species and models and, so, it needs to be reconsidered. It would be sensible to restrict the use of the terms phase ‘1a’ and ‘1b’ to the VF occurring in anaesthetised dogs with poor coronary collateralisation.

In the short term, the extent of variation between species and models means that labels like 1a and 1b should perhaps be avoided. Their use lends an apparent importance to the twelfth minute of ischaemia that is difficult to justify. It could be the case that the twelfth minute may have relevance only to veterinarians concerned with the health of collateral-deficient dogs undergoing general anaesthesia.

When using a model, one should take care to describe the pattern of phase 1 VF that occurs and state whether it is monomodal or bimodal. If a drug appears to affect selectively the early or late peak of phase 1 VF, then this should of course be reported. However, a much greater understanding of the scale of variation between models and species, and the factors that determine this variation, are necessary before findings can be safely extrapolated from one model to another and to man.

In the longer term, issues will be resolved only when the wider medical community pays more attention to seeking precise and accurate information on the natural history of events acutely following coronary obstruction in man, and the factors that determine the nature of these events. This may require the formation of some kind of taskforce to obtain the information as it pertains to the wider population at risk of a first attack of cardiac ischaemia (as opposed merely to the survivors of acute ischaemia), since the data would otherwise be difficult to obtain.

4. Model refinement

For any given model, regardless of the characteristics of the time-course of ischaemia-induced VF, it is important to control for major sources of variation affecting the incidence of VF. This may be simpler to achieve in the perfused heart model, since there are fewer variables to consider, and most of these can be controlled precisely and accurately. It is possible to carefully select perfusion constituents, modify heart rate, chose and obtain different involved zone sizes and locations, vary study species according to study objective and even vary the mode of perfusion in order to minimise sources of variation, maximise scope for detecting anti-VF action or address specific questions about VF and its modulation, using isolated heart preparations.

4.1. Selection of perfusion K\(^+\) and Ca\(^{2+}\) content

The quantitative relationship between perfusion K\(^+\) content and susceptibility to VF can be manipulated to refine the model. It is possible to select a K\(^+\) concentration
that maximises scope for detection of ischaemia-induced VF. This is 3–4 mequiv./l in the isolated rat heart [5].

There is insufficient information at present to comment in general terms on the ideal Ca\(^{++}\) content of a perfusion solution. In the rat Langendorff preparation, regional ischaemia was found to elicit VF in 100% of control hearts whether perfused with 1.4 or 2.4 mequiv./l Ca\(^{++}\), although the anti-VF effectiveness of a drug with apparent calcium antagonist activity was reduced by the higher Ca\(^{++}\) solution [27]. Thus, Ca\(^{++}\), over the range from physiological levels to twice that, may not influence phase 1 VF susceptibility, although high Ca\(^{++}\) may be capable of surmounting the protective effects of certain drugs.

4.2. Selection of involved zone size

Because susceptibility to ischaemia-induced VF is dependent on the size of the involved region, it is possible to adjust involved zone size in the regionally ischaemic heart to vary the control incidence of VF [6]. This can be achieved easily and accurately in the isolated heart by correct placement of a coronary ligature [6,15,20]. It is possible to maximise the incidence of ischaemia-induced VF by strategic location of the coronary ligature in all species and preparations in which this has been studied, including the dog in vivo [45]. In the perfused rat heart, a 40% involved zone size coupled with a 30-min duration of ischaemia maximises scope for susceptibility to VF, with an incidence close to 100% in control hearts [6,15,20]. In theory, it may be possible to explore proarhythmia by aiming for a 20% involved zone size (giving rise to a low incidence of ischaemia-induced VF [6]) and perfusing with putative proarrhythmic drugs, although this type of study has not yet been attempted.

4.3. Regulation of heart rate

Heart rate is another source of variation that can be manipulated to refine the model. Unwanted bradycardia can occur in perfused hearts for several possible reasons. Cardiac cooling, one possible reason, can be overcome in regional ischaemia studies by ensuring that the perfusion solution is at 37°C when emerging from the aortic cannula at a physiologically relevant flow rate. In low flow global ischaemia preparations, bradycardia results from SA and AV nodal ischaemia, and in zero-flow preparations, this leads to asystole. Bradycardia can be overcome by inserting a small superfusion catheter into the left atrium and delivering warm solution. This technique has been shown to maintain orthograde propagation through the AV node, as well as maintaining the sinus rate, during global ischaemia [6], thereby lending a significant refinement to the preparation. It is possible to surmount bradycardia by cardiac pacing if the bradycardia is not due to cardiac cooling. Pacing allows testing of whether an anti-VF drug acts ‘directly’ on VF, or prevents VF as a secondary consequence of causing bradycardia. It is preferable to pace at a supraventricular site for this purpose since ventricular pacing has been shown to facilitate arrhythmogenesis, presumably by virtue of the creation of a site of ectopic automaticity [74], although supraventricular pacing is ineffective in globally ischaemic hearts, due to the exacerbation of complete AV block [15].

5. Detection of antiarrhythmic activity-pitfalls

5.1. Definition of VF

There are certain difficulties that arise with the measurement of anti-VF activity in isolated hearts that should be avoided. One of these is the problem of arrhythmia diagnosis. Many different definitions of VF are given in the literature, so inter-study comparisons may be unfeasible. This problem is not restricted to isolated heart models and, even in man, discrimination between VF and other ventricular tachyarrhythmias can be hazardous [75]. Careful adherence to the guidelines of the Lambeth Conventions [76], which provides objective definitions for VF and other arrhythmias, can avoid these difficulties.

5.2. Problem of self-terminating VF

Self-terminating VF occurs commonly in perfused heart experiments. This probably reflects the fact that almost all studies have been done using hearts from small animals. Although VF can self-terminate in larger hearts, even in man [75], the small size of rat, guinea pig and other hearts used for perfusion is probably a more important basis for self-termination than the isolated heart perfusion mode of experimentation itself, since self-termination is common also in conscious and anaesthetised rats [14]. The relationship between VF cycle frequency, VF duration and QT interval has been examined in the isolated rat heart [22]. The data provided evidence that the ability of ischaemia-induced VF to self-terminate is related to VF wavelength and refractoriness, which supports other evidence that VF persists because it is a manifestation of multiple wavelet re-entry and thus is liable to self-terminate in smaller hearts [22]. This is not a problem in itself, since VF initiation (i.e. incidence) is likely to be the most important drug target relevant to sudden cardiac death, with VF maintenance being of less interest (a drug that switches VF off would need to restrict VF to no more than a couple of seconds in man for it to be of any interest, in comparison with a drug that prevents VF from occurring in the first place). A problem has arisen, nevertheless, is that some investigators have chosen to present VF data in ways that can render interpretation of their observations difficult, for example, expressing group mean VF duration as a numerical value. This is inappropriate since individual values range from 0 s (i.e., no VF) through finite values (self-
terminating VF) to infinity or an arbitrary value (if VF persists until the end of an observation period) [5]. Thus, if a drug is found to abolish VF in a group of hearts, it would be arcane to suggest that VF duration is reduced to 0 ± 0 s. There is useful information to be had from monitoring VF duration but, in order for it to be amenable to analysis that is both consistent with meaningful enumeration and physiological function, it is necessary first to determine VF incidence, then to determine the proportion of those hearts experiencing VF in which at least one episode was sustained [22]. For the rat, a useful arbitrary definition of sustained VF is an episode lasting continuously for 120 s or more since, in conscious rats, ≈ 120 s of continuous VF will kill the animal, even if sinus rhythm is subsequently restored [76].

5.3. Need for randomisation and blinded analysis

Even though the isolated heart model is ‘simple’ in comparison with, for example, a conscious animal model, there is no justification for disregarding good laboratory practice. Randomisation to treatment and blinded analysis of data are essential. The Lambeth Conventions recommended this in 1988 [76], but few publications actually report the use of these procedures. However, this problem is not just restricted to isolated heart studies. Along with the use of inappropriately high drug doses that are incompatible with therapeutic administration in conscious humans (see Tables 1–4), this may be one explanation for the fact that the number of effective drugs in animal studies vastly outweighs the number of drugs that actually go on to become clinically useful in the prevention of VF. Again, these are not problems restricted to the use of the isolated heart. For example, anaesthetised animals can ‘tolerate’ doses of lignocaine that would evoke convulsions in conscious animals (unpublished observations and [64]).

6. Actions of drugs

The tables (Tables 1–4) describe experiments on VF induced by ischaemia in isolated perfused hearts. The tables are arranged in relation to general drug classes.

6.1. Class I drugs

In Table 1, it can be seen that there is surprisingly little data on the effects of Class I drugs on ischaemia-induced VF in isolated hearts. This situation should surely be rectified quickly. It probably reflects the fact that by the time that the isolated heart was first used for the study of ischaemia-induced VF, Class I drugs had already been studied extensively in other models, reducing the level of interest (and the scope for obtaining the necessary grant support) to test them in the isolated heart.

6.2. Class II and related drugs

Drugs affecting cardiac adrenoceptor activation have been examined more extensively (Table 1). The data do not support the idea that block of cardiac adrenoceptor activation prevents ischaemia-induced VF. This has been explained in detail by Daugherty et al. [28]. Although beta blockers have some beneficial effect on death rate post myocardial infarction in man [77], there is no good evidence that clinical benefit is attributable to suppression of ischaemia-induced VF, and the isolated heart data does not suggest that such a property, if it exists at all, is substantial. Perhaps this is why the impact of beta blockers on sudden cardiac death is limited (if beta blockers were truly effective anti-VF agents, then their present widespread use would be having a much greater impact on sudden cardiac death rates).

6.3. Class III and related drugs

Even more Class III and related drugs have been tested, but surprisingly, the drugs with the most well established clinical beneficial effects, amiodarone and sotalol, have not been tested (Table 2). The experiments that have been published do not give a terribly clear picture, with both KATP activators and inhibitors reportedly both effective and ineffective in different studies. One would not expect IK blockers to affect ischaemia-induced VF in rat hearts because, in this species, the heart does not express functionally important IK currents [78]. Rat heart data may be compared with data from hearts of other species to probe whether IK blockade is an effective mechanism for VF suppression [25]. Presumably, if a drug that blocks several K+ currents suppresses VF and widens QT in the rat heart, then this is a good indication that the drug is not selective for IK, and mediates its effects in the rat by affecting molecular targets other than IK [79]. It would be worthwhile to explore this contingency for amiodarone and sotalol.

6.4. Class IV and related drugs

The effects of Class IV and related drugs are shown in Table 3. Again, it is surprising that calcium antagonists have not been tested for effects against left regional ischaemia-induced VF in perfused hearts. The same considerations noted for Class I drugs probably apply. Table 3 also shows the effects of miscellaneous interventions. The preconditioning phenomenon appears to protect against ischaemia-induced VF in perfused hearts.

Table 4 illustrates studies, in some of which VF was measured only en passant, that reported a low incidence of VF in control hearts. More recent studies generally found much higher incidences of VF (Tables 1–3).

It would seem that further work is necessary to characterise ischaemia-induced VF in perfused hearts in terms...
of responses to drugs of known pharmacological specificity and selectivity.

7. Possible clinical relevance

7.1. Method of establishing clinical relevance

The most difficult issue to address is that of clinical relevance. Isolated heart preparations allow the generation of phase 1 ischaemia-induced VF. This allows detection of anti-VF drug action, and may permit some ancillary analysis (of the mechanism of drug action). The preparations also allow examination of determinants of VF initiation and maintenance. However, in order to establish (or refute) clinical relevance, one requires a hard clinical template with which to refer. This template has two components, a drug—response profile and a natural history profile. If any preparation has a drug—response profile that mirrors the clinical drug—response profile then its natural history profile becomes unimportant.

Unfortunately, the clinical relevance of preparations for detecting anti-VF action relevant to phase 1 cannot be tested by reference to a current clinical template. We have no drugs that are proven to prevent phase 1 VF in man, because, as mentioned earlier, we have no precise or accurate information on the time-course of VF susceptibility following coronary obstruction in man. At present, it is safest to state only that the Class III agents, DL-sotalol and amiodarone, appear to be beneficial against life-threatening arrhythmias when given to patients who have survived acute myocardial ischaemia [80]. Importantly, there is no detail on the effectiveness of drugs in people who have yet to suffer their first cardiac ischaemic event. The latter may be a difficult problem to address, but its importance must not be disregarded. If ischaemia is severe enough to cause cardiac arrest, the survival rate is only about 20% [81] and the most robust clinical data on anti-VF drugs is derived from post-infarct studies restricted to these minority of survivors [80]. What of the lost 80% of patients, most of whom die out of hospital? Most do not contribute to our knowledge about the natural history of VF (see earlier) or drug effectiveness for the prevention of VF, because they die before being seen by a physician. Thus, a clinical template for evaluating the appropriateness of animal models can be anticipated, paradoxically, only when drugs are available that can be used prophylactically to prevent out-of-hospital VF. This makes any attempt to evaluate a model by reference to a clinical template of drug effectiveness unfeasible.

The absence of either a clear-cut drug—response profile or natural history profile has hampered anti-VF drug development, because it prevents elucidation of the clinical relevance of not just isolated hearts but, in fact, all animal models. Without proven legitimacy in terms of clinical relevance, all models, techniques and approaches become suspect.

7.2. Alternative method of exploring clinical relevance

In view of the scale of the clinical problem (of sudden cardiac death in its wider context), one cannot simply give up the search for anti-VF drugs just because one does not have complete confidence in any experimental preparation. Thus, there has evolved an alternative method for determining whether or not a preparation is of possible value. The method has never been formalised, and the following attempt to do so is therefore necessary in order to give context to the conclusion of this article.

7.2.1. Positive reinforcement

Any preparation has a set of characteristics. The closer those characteristics appear to resemble the characteristics of the human disease, the more comfortable one becomes with the preparation. This could be described as positive reinforcement of the preparation. For example, the Lucchesi model [82] and the Schwartz–Stone model [83] are two in vivo models that use a species (dog) with a heart that is closer to man’s in size and anatomy than that of the rat or rabbit, is perfused with blood and has intact innervation (unlike a Krebs’/Tyrode perfused isolated heart), and use a procedure that gives rise to ischaemia in a heart with a healed infarct either at rest [82] or following a period of exercise [83] in the conscious setting. These characteristics are evidently lacking in, for example, an isolated guinea pig heart perfused with Krebs’ solution and subjected to low flow global ischaemia [38,84]. The conscious dog models are therefore positively reinforced whereas the latter is not.

7.2.2. Negative reinforcement

A preparation can additionally be negatively reinforced. For example, any rat heart preparation (in vivo or isolated) is negatively reinforced by the lack of functional expression of IK (r or s) in the ventricles [78]. In fact, this is one of the few examples where a preparation has an unequivocal limitation; clearly, any selective IK blocking agent will not possess antiarrhythmic activity in the rat heart [79], albeit, until it is clear beyond doubt that IK blockade can reduce ischaemia-induced VF in man, this apparent limitation remains theoretical.

The differences between preparations in terms of the natural history of arrhythmogenesis also gives cause for negative reinforcement of a preparation. However, unless one is certain which preparation is ‘correct’, then the existence of a difference can be regarded as no more than a cause for concern. There is certainly much variation in phase 1 VF natural history, as shown in Figs. 2–4 and discussed earlier. In addition, phase 2 VF, the second major peak [14] that appears about 90 min after permanent occlusion and lasts for at least another 3 h, is completely
absent in the rat Langendorff preparation [58]. The safest way of interpreting these data is to regard the presence of differences as negatively reinforcing all models to some extent.

If individual isolated heart studies are considered, it is evident that some data provide positive reinforcement, and some negative. As an example of positive reinforcement, phase 1 VF is clearly reduced by raising the K⁺ concentration in the solution delivered to the uninvolved region of the perfused regionally ischaemic rat heart [5,29]. This effect parallels the inverse relationship between blood K⁺ and sudden cardiac death in man [42–44]. As an example of negative reinforcement, verapamil, a drug that has had no impact on sudden cardiac death [77], can suppress VF in the guinea pig low flow ischaemia preparation [60]. These, and other numerous equivalent examples, are interesting, but none alone are sufficient to condemn any model or approach.

7.2.3. Current practice
It is evident from this that positive and negative reinforcement cannot provide scientifically acceptable proof of the absolute (or even relative) merit of different preparations. A selection of the examples highlighted in this section might lead a naive investigator to conclude that the Lucchesi or Schwartz–Stone dog models should be used to detect new anti-VF drugs, and the isolated heart should never be used. Indeed, until the 1990s, the literature gives the impression that this was an accepted consensus. Yet, these conscious dog models are now used infrequently. The reasons for this are unclear. Cost may be a factor. However, the models have not yet been proven to be the most clinically relevant in absolute terms. For this to be so, they would need to meet the criteria of being uniquely able to allow detection of the beneficial effects of a drug that is also effective in man against sudden cardiac death and uniquely able to predict ineffectiveness and the detrimental effects of proarrhythmic drugs. It is not feasible to attempt to review all data with conscious dog models here, but, suffice to state, these criteria are not met (if they were, then the present crisis in confidence in anti-VF drug development, and the requirement for articles such as this, would not exist). On the other hand, the isolated heart has emerged as a system for detecting anti-VF action of drugs (Tables 1–4). The reason for this is unclear. Again, cost may be a factor. However, the facility for rapid data generation and the versatility of the model means that it can be possible not only to detect anti-VF activity but also to ascertain concentration–response relationships and likely mechanisms of action at the molecular and cellular level. This means that, provided that the drug’s molecular target is expressed in the human heart, then the data itself provides positive reinforcement of the preparation. Concern about absolute clinical relevance seems not to be a major issue, nor can it be as yet. The advantages of isolated hearts are clearly perceived to outweigh the disadvantages. The reasons for this are intriguing, and are discussed further in the following section.

8. Recommendations

8.1. Choosing an isolated heart preparation
If isolated heart preparations are to be used for detecting new anti-VF drugs or exploring the pathophysiology of VF, then the model refinements described earlier may be of value. In a broader context, it may be worthwhile to consider where the preparations may fit into the context of drug discovery.

No new drug today is simply a random screened chemical. Drugs are designed to have specific molecular actions (on molecular targets such as receptors, enzymes and ion channels). In drug development, animal models are needed to provide preclinical information on whether or not there is a reasonable chance that the drug may work to treat a disease. Thus, if it is the case that phase 1 VF contributes to sudden cardiac death in man, then animal models are needed to permit detection of suppression of this form of VF. If a drug is synthesised and shown to affect a specific molecular target, then the choice of preparation can be made rationally on the basis of knowledge of the expression of the molecular target in the heart of a given species, and the utility of the species as a bioassay for detecting anti-VF action.

If the molecular target is expressed in the rat, then the bioassay characteristics of the regional ischaemia Langendorff preparation, and the growing data base for this preparation, make it ideal for first line use. The overwhelming dominance of this preparation among isolated heart options (see Tables 1–4), and the increase in its use as the use of other preparations declines, attest to the increasing perception of its utility. However, popularity does not necessarily equate with appropriateness, and parallels with politics may be made — popular new governments are commonly voted out of office the next time an election takes place.

The guinea pig low flow global ischaemia preparation may be used as an alternative if the molecular target is not functionally expressed in rat heart. This is particularly relevant if the target is IK. However, more work is required to standardise the preparation, since there is some variation in the incidence of phase 1 VF between studies, ranging from 100% [60] to 30% [85].

8.2. False positives and false negatives
The two main limitations of isolated heart preparations are the same as those for any other preparation or model: potential for failure to detect protective actions of potentially clinically useful drugs (false negatives), and the potential for generation of false positives. With regard to
false negatives, until it is possible to show that the molecular or cellular mechanisms responsible for phase 1 VF are categorically different in man from those causing the same VF in an animal preparation, then one cannot legislate for false negatives as a generality.

Specifically, one would expect a false negative if the drug’s molecular target is not expressed in the preparation (this point has already been addressed). The likelihood of false negatives as a generality may be low for two reasons. (i) The occurrence of phase 1 VF, although uncertain in man, is common to most species, regardless of whether it is mono- or bimodal in time-distribution. This implies that, although quantitative differences may exist in terms of mechanisms of arrhythmogenesis between species, there is a qualitative similarity, so the chance that man is uniquely different would be expected to be low. (ii) There are no known examples of interventions that fail to work in any animal preparation yet reduce sudden cardiac death in man. Nevertheless, as a cautionary note, the two publications from which one may derive the time-course of ischaemia-induced VF in primate hearts reported negligible phase 1 VF in vitro [20] and a complete absence in vivo [53]. If one argues that primates are more clinically relevant than any other animals, then it could be argued that ischaemia-induced VF may not occur at all in man. This is probably an unreasonable proposition, however, since involved zone size was very low in the in vivo primate study cited (as discussed earlier), whereas the perfused primate heart study cited [20] may have been flawed in some other way (by the use of non-optimal perfusion constituents, perhaps).

False positives are a much greater likely source of misleading information. The possibility of false positives can be explored by comparing the actions of ‘old’ drugs in isolated heart preparations with their actions in man. The most reliable drug for making this comparison is lignocaine because it has been used clinically for many years and the data, which clearly indicates a lack of benefit against sudden cardiac death in man, are unequivocal [77]. Unfortunately, lignocaine has been studied only once for its effects on ischaemia-induced VF in perfused hearts; it was effective at 10 μM in the guinea pig low flow global ischaemia preparation [60]. The reason for this false positive may be attributable to an inappropriately high drug concentration. It is unfeasible to expect a plasma protein-unbound concentration of 10 μM lignocaine to be maintained throughout the period that a human might be susceptible to phase 1 VF, let alone be maintained during the period when other arrhythmias associated with coronary artery disease (i.e., phase 2 and reperfusion-induced VF) occur.

False positives, therefore, are easily achieved in isolated hearts by virtue of the fact that drug concentrations may be elevated with equanimity to any level, short of that eliciting AV block or asystole. It is important therefore that drugs be tested only over the concentration range that is commensurate with relative selectivity for the intended molecular target. On the basis of experimental and clinical data with lignocaine, it may be argued that although the isolated heart data suggest that the sodium channel may represent a viable molecular target for phase 1 VF suppression, consideration of in vivo (ideally conscious) animal model data is required before the efficacy (safety as well as effectiveness) of sodium channel blockade itself can be predicted from animal studies. Moreover, the fact that lignocaine at 10 μM suppresses ischaemia-induced VF in guinea pig hearts subjected to global ischaemia [60] yet fails to prevent sudden cardiac death in man [77] hardly condemns the isolated heart as an experimental model; the real conclusion from the available isolated heart data is that it would be a good idea to perform some concentration response studies with a few Class I drugs to test their effectiveness against VF induced by regional ischaemia, something that has unaccountably not been done (Table 1).

8.3. Putting the data into context

This leads on to an important recommendation. It would be foolish to take a drug tested only in isolated hearts into a clinical trial. It is necessary as an intermediate step to obtain in vivo (ideally conscious) animal model data. However, one might ask why bother with isolated heart studies if in vivo animal experimentation is a necessary next step? The answer is simple. In vivo (especially conscious) animal models are not viable for first line testing of every new drug. Animal studies in vivo are time-consuming and technically demanding, and can be expensive, whereas isolated heart studies are simple to perform and may quickly provide abundant and extensive information on the potential effectiveness and concentration-dependence of a drug’s action. The bottom line is that, if a drug is effective in an isolated heart preparation that expresses the drug’s specific molecular target, it may be worth carrying out in vivo animal studies, provided that an effect is achieved with a pharmacological (as opposed to a toxicological) drug concentration — as a rule of thumb, no more than 1 μM, and ideally no more than 0.1 μM. Recent development of the Class III drug, tedisamil, typifies this approach. Its actions on phase 1 arrhythmias were examined several years ago in the rat Langendorff regional ischaemia preparation [22]. The data, together with more encouraging data from the conscious rat with regional ischaemia [86], prompted further studies which only recently culminated in the testing of the drug in the Lucchesi dog model [87,88].

For the future, hearts from transgenic animals (particularly rats, for which there is a large non-transgenic database) expressing human molecular targets may represent an ideal solution to the possible species-related limitations of isolated heart preparations.

There is one further consideration that may be of great relevance. What if clinical data is eventually obtained that
reveals that man exhibits separate 1a and 1b phases of VF, and exhibits phase 2 VF and clinically important reperfusion-induced VF, each potentially contributing to sudden cardiac death? If these different manifestations of VF occur via discrete mechanisms in man, then no single model would be capable of predicting the clinical effectiveness of a drug. Also, the notion of a single broad spectrum anti-VF drug would become less feasible, since, in animals, there is some evidence that an intervention that suppresses one type of VF (e.g., ischaemia-induced) may not be effective against another type (e.g., reperfusion-induced) [5]. Therefore, more than one molecular action (i.e., more than one pharmacologically selective drug) may be required to fully protect a patient from susceptibility to VF. A single animal model expressing all phases of VF during a single experiment would also be unfeasible. Therefore, it would be necessary to use a range of animal models, each allowing discrete and specific study of a specific manifestation of VF, and none alone would be capable of predicting a drug’s overall clinical effectiveness.

From this, one must conclude that a cautious and considered approach should be taken. Thus, the isolated heart has a key role to play. A drug effective against phase 1 VF in hearts in vitro would need to be tested against phase 1 and 2 (and reperfusion-induced) VF in vivo before clinical studies are undertaken. It should provide some comfort (i.e., positive reinforcement) and encouragement to the poly-pharmacist to note that no drug at sensible concentrations has yet been found that unequivocally prevents VF in each possible settings in animal models.

To conclude, a constructive recommendation is (i) the careful use of the isolated heart preparation, working within appropriate concentration ranges to achieve target-specificity and selectivity, and paying attention to the particular advantages offered by individual models and species in relation to the particular pharmacological characteristics of the drug under investigation and (ii) consideration of findings in relation to those from other animal models that exhibit bimodal phase 1 VF, phase 2 VF and reperfusion-induced VF. By this means, it might be possible to select for clinical trial drugs that have the potential to halve, rather than double [89,90], the death rate from VF in man.

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References


