Update review

Voltage-gated calcium-channels and antiarrhythmic drug action

Andrew A. Grace*.*, A. John Camm

*Departments of Medicine and Biochemistry, University of Cambridge and the Department of Cardiology, Papworth Hospital, Cambridge CB3 8RE, UK

bDepartment of Cardiological Sciences, St. George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Keywords: Antiarrhythmic agents; Atrial function; Ca-channel; Impulse formation; Membrane potential; Ventricular arrhythmias; Ventricular function

1. Introduction

Calcium-channels, blockers have an established role in the management of cardiac arrhythmias [1–5]. They were identified empirically with the idea of achieving selective inhibition of voltage-gated calcium-channels and vasodilatation [6,7], but early laboratory studies of the hemo-
dynamic and vascular effects of verapamil happened also to demonstrate efficacy against cardiac arrhythmias [1,8]. The importance of the landmark paper by Vaughan Williams and Singh [9] is that it proposed a novel discrete mechanism for the drug control of arrhythmias that did not rely on the three mechanisms viz. local anaesthetic, anti-
sympathetic or delay in repolarization already described [10]. There were two major outcomes of this publication. First, the expanded and refined classification of antiar-
 rhythmic drug actions heralded a period of development in antiarrhythmic drug strategies and encouraged critical thinking about the mechanisms of drug action. This contributed in turn to a more complete evaluation of antiarrhythmic drug therapy in general [11,12]. Second, intravenous verapamil entered clinical practice leading to a complete change in the treatment of acute paroxysmal supraventricular tachycardia [13–15]. The deployment of calcium-channel blockers in cardiac arrhythmias moved, of course, in parallel with increased use of this class of drug in coronary artery disease and hypertension [16,17]. This growth was unencumbered until more recently when it has been clouded by controversy [17–21].

This commentary will examine the cellular and molecular mechanisms of action and current applications of calcium-channel blockers in cardiac arrhythmias. We contend that there have been major advances in understanding the molecular mechanisms of action of these agents and this has substantially contributed to a greatly expanded knowledge of cardiac (and indeed non-cardiac) ion channel structure-function relationships [22]. This explosion of knowledge however has not yet resulted in any useful new drugs [23]. Despite this lack of progress in terms of completed drug design calcium-channel blockade remains an important adjunctive mechanism in the antiarrhythmic repertoire. As a specific pharmacological action it remains a desirable feature of new antiarrhythmic drugs [24,25].

2. Calcium-channel blockers: homing in on drug targets

Calcium-channels are membrane-spanning proteins that allow the controlled delivery of calcium into the cell [22,26–28]. They are crucial to normal cardiac function and are responsible for the generation of the heart beat, the action potential plateau, the control of impulse propagation through the atrio-ventricular junction node and contraction of atrial and ventricular muscle [29]. The channels activate after membrane depolarization leading to (slow) calcium influx producing a modest increase in cytosolic calcium, this triggering the opening of ryanodine-sensitive intracellular calcium-channels with excitation–contraction coupling [27]. Two types of cardiac cell membrane calcium-channels have been identified. The T-type calcium-channel is present in most excitable tissue and in the heart is localised relatively selectively to pacemaker cells [30,31]. These channels are not currently thought to be important in the generation of cardiac arrhythmias [31]. In addition, clinical modulation of the T-type channel is not currently a treatment option (see below). In contrast the L-type voltage-dependent calcium-channel has been characterised in great detail and provides the principal planned target of most calcium-channel blockers [2,32]. From a structural standpoint, L-type channels are large glycoprotein complexes consisting of α1, β and α2/β subunits. At the core
are the $\alpha_1$ subunits which serve as calcium conductance pores whilst the auxiliary subunits ($\beta, \alpha_2/\delta$) modulate function [22,26].

Calcium-channel blockers are responsible for concentration- and dose-dependent inhibition of L-type calcium-channels [16,32]. The action is highly tissue-specific and more-or-less confined to the heart and vascular wall. The development of the most commonly used agents arose from empiric observation rather than systematic drug design [7,33]. The convenient short-hand grouping tends to conceal their chemical diversity but this is revealed in their individual pharmacology and patterns of therapeutic efficacy [32].

Verapamil, the prototype calcium-channel blocker, was developed from its parent, papaverine, as a coronary vasodilator [7,33]. It remains, after almost four decades, the calcium-channel blocker of usual choice in the management of cardiac arrhythmias. L-type calcium-channel blockers are categorised into three distinct groups based on structure: phenylalkylamines (e.g. verapamil, gallopamil), the benzoazepines (e.g. diltiazem), and the dihydropyridines (e.g. nifedipine, amlodipine, isradipine, nicardipine) [32]. Phenylalkylamines and benzoazepines stereo-selectively interact with high-affinity binding domains on the $\alpha_1$-subunit of the channel complex and produce a block with electrophysiologically useful actions [32,34]. Dihydropyridines are essentially devoid of electrophysiological action in vivo but at the bench have been the more important reagents in the analysis of ion channel function [22,35].

Calcium-channel block may also result with agents having other dominant electrophysiological effects [3]. For example, azimilide, aimed principally at potassium channels blocks L-type calcium-channels at clinically relevant concentrations [36] whilst dofetilide and E4031 have little effect [3]. The overall electrophysiological importance of these observations is unknown but may well be implied from the observations of future clinical trials. Support for the notion that calcium-channel blockade may be a useful adjunctive drug action comes from studies of amiodarone [24,25,37,38]. Amiodarone binds to and then blocks calcium-channels predominantly during the inactive or resting state [39] associated with the suppression of calcium-dependant action potentials in vitro [37,40]. The clinical correlate is the well documented amiodarone-induced slowing of atrio-ventricular conduction the characteristics of which are most consistent with underlying calcium current inhibition [37,40]. Other drugs that inhibit calcium-channels include propafenone, [41,42], flecaïnide [3], and terfenadine [43]. Terfenadine is interesting in that it inhibits the L-type calcium-channels with an $IC_{50}$ of 0.14 mmol l$^{-1}$, similar to that for the inhibition of $I_K$, and also within the range of clinically relevant concentrations (0.01–0.1 mmol l$^{-1}$) [43].

There is a large literature and considerable debate regarding the interaction of calcium-channels and beta-adrenergic receptors [29,44–46]. Specifically, beta-adrenergic receptor activation regulates calcium-channels by cAMP-dependent protein kinase-A phosphorylation of the L-type calcium-channel $\alpha_1$-subunit thereby reducing calcium influx [29]. Accordingly, beta-blockers modulate calcium fluxes indirectly but have little apparent direct effect on calcium-channels [3].

3. Calcium-channel blockers: model experimental systems

The ouabain-model of arrhythmia in the paper of Singh and Vaughan-Williams [9] is principally the result of delayed afterdepolarizations and triggered activity [47]. The antiarrhythmic action of calcium-channel blockers have also been studied using ischemia and reperfusion [48] or electrically induced ventricular fibrillation [49]. The clinical relevance of the ouabain and ischemia-reperfusion models to most clinically relevant arrhythmias is questionable and underlines the few available relevant tractable animal models [50]. Interestingly there is a model-independent cross-species similarity in that macro-re-entrant ventricular arrhythmias are almost invariably insensitive to L-type calcium-channel blockers [51] correlating well with clinical experience [2]. Indeed verapamil, diltiazem and mibebradil tend to increase both the rate and duration of ventricular tachycardia in animal models [52].

Clinical observations have contributed disproportionately to an understanding of the integrated action of calcium-channel blockers which in turn has also highlighted potential arrhythmia mechanisms [53,54]. The most striking examples of the imputation of mechanism are with atrio-ventricular re-entrant arrhythmias and normal heart ventricular tachycardia [53]. These examples were used as prominent illustrations of the vulnerable parameter concept in the Sicilian Gambit [53,55]. Indeed triggered activity was suggested as a mechanism in idiopathic left ventricular tachycardia (ILVT) based on verapamil sensitivity [54,56]. Of course, caution is required in interpretations based simply on drug sensitivity and re-entry rather than triggered activity is now the perceived mechanism of ILVT [54,56].

3.1. Voltage-gated calcium-channels and cardiac arrhythmias

Calcium-channel blockers exert little or no effect on atrial, ventricular, His-Purkinje or bypass tract conduction or refractoriness [2,5,16] and have no action on macro-re-entrant ventricular arrhythmias in either animal models [51] or in patients [2]. The principal aims of the drug treatment of arrhythmias are the reduction of both symptoms and risk. Calcium-channel blockers may occasionally reduce risk in the occasional rare case of polymorphic ventricular tachycardia due to coronary artery spasm [57].
but otherwise calcium-channel blockers are more commonly associated with enhanced risk in a range of patient groups (Ref. [58] and see below).

3.2. Calcium-channel blockers: modulation of conduction across the atrio-ventricular junction

The most striking clinically useful action of calcium-channel blockers are on the atrio-ventricular node [4,13,24]. The first successes were predicted by the knowledge that the slow calcium current is critical to atrio-ventricular node function [59]. In the first invasive electrophysiological studies prolongation of atrio-ventricular junctional conduction by verapamil was beautifully demonstrated and associated with lengthening of both anterograde and retrograde atrio-ventricular node refractoriness [60]. The macro-electrophysiological responses of the atrio-ventricular node have now been examined in detail [2,5]. One key observation is that calcium-channel recovery from inactivation is time-dependent even after repolarization is complete [24]. This accounts for decremental responses to premature stimulation and is exaggerated with both verapamil and diltiazem [2]. Interestingly, also, effects of both verapamil and diltiazem on atrio-ventricular nodal refractoriness are dependent on underlying rate (Fig. 1) [59,61]. This links in to the in vitro data demonstrating state-dependency of heterologously expressed calcium-channels [22].

In general diltiazem and verapamil have similar antarrhythmic efficacy when directed at the atrio-ventricular node although more data are available on verapamil [4,24]. It should be borne in mind, especially with dihydropyridines, that reflex sympathetic activation may counteract any direct electrophysiological effects producing sudden increases in heart rate [2].

3.3. Calcium-channel blockers: afterdepolarizations and triggered activity

Occasional arrhythmias occurring in the structurally normal heart are due to triggered activity [47]. The clinical relevance of triggered activity is not totally clear but early and late afterdepolarizations are associated [47,62]. Early afterdepolarizations are triggered by an increased influx of calcium during prolonged repolarization leading to calcium overload and occur especially with potassium channel dysfunction as in congenital and acquired long-QT syndromes [47]. Diltiazem suppresses cesium-induced early afterdepolarizations in vivo supporting a role for calcium-channel blockers [47,63]. There are few reports of calcium-channel blockers being used to suppress early afterdepolarizations: 

3.4. Calcium-channel blockers: heart failure and hypertrophy

Widespread molecular and cellular changes affecting calcium handling characterise the hypertrophic and failing heart [68–70]. These changes are complex and controversial [68]. The prediction of the nature of drug–receptor interactions under these conditions is further complicated by the fact that with the onset of heart failure drug-binding sites may have modified characteristics [68].

Altered calcium homeostasis is thought relevant to the generation of ventricular arrhythmias in heart failure although how is not clear [71]. What is known is that in animal models arrhythmias are not suppressed by calcium-channel blockers and indeed may get worse (see above) [68]. Clinical observation suggest serious questions regarding the safety of most calcium-channel blockers in patients with heart failure [71,72]. The exception seems to be with amlopidine that has been reported to reduce mortality in patients with non-ischemic heart failure [73]. Whether
these changes, bad or good, represent arrhythmia-related phenomena remains unknown [71,72].

4. Molecular pharmacology of calcium-channel blockers

Calcium currents were first recorded in cardiac myocytes which continue to provide a robust and highly useful experimental system allowing many key properties to be examined [22,74,75]. The characterization of calcium-channel function here has been significantly assisted using calcium-channel blockers [22]. Of interest is that whilst phenylalkylamines and benzothiazepines are the more effective antiarrhythmic agents dihydropyridines have been the more important for mechanistic studies (see above) [3].

For the purposes of the analysis of the molecular basis of the drug-ion channel interaction the calcium-channel is best considered as a pharmacological receptor with specific sites for the binding of activator and antagonist ligands [35]. The availability of cloned channels usually examined heterologously in combination with new molecular and electrophysiological assays has provided a great deal of information on the determinants of antiarrhythmic drug binding and action (Fig. 2) [22,76].

4.1. Calcium-channel blockers: drug–receptor interactions

The binding characteristics and, accordingly, the actions of the different groups of L-type calcium-channel blockers are qualitatively and quantitatively distinct [22,35,77]. These characteristics arise from state-dependent interactions with individual subclasses of channel receptor leading on to the observed pharmacological discrimination and relative selectivity of action [22]. Calcium-channel blockers interact at a set of allosterically linked sites on the α-subunit of the L-type calcium-channel localised in the pore-forming region of the L-type calcium-channel α1-subunit [35]. These sites have been identified by a combination of techniques that have included site-directed mutagenesis, photoaffinity labelling and chimeric α1-subunit construction [22,35].

Verapamil and diltiazem block channels mostly from the inside surface of the membrane by entering the channel preferentially when open [22,35,77]. In simple terms there is use-dependent block with benzothiazepines and phenylalkylamines but not with dihydropyridines [22]. This open-channel block with slow recovery of the channel results in pronounced frequency-dependency which contributes to the efficacy of verapamil and diltiazem with tachycardia [24]. State-dependent interactions contribute to our understanding of the antiarrhythmic action of the
phenylalkylamines and benzothiazepines in the atrio-ventricular node [24,61]. Diltiazem use-dependent block is qualitatively indistinguishable from that due to phenylalkylamines [34,78].

The selective modulation of T-type calcium-channels provided an attractive idea for drug design due to T-type channel localization in pacemaker regions [31]. The use of channel-specific drugs was thought likely to avoid some of the troublesome peripheral side-effects seen with L-type receptor blockers [31]. The benzimidazolyl-substituted teramine derivative, mibefradil, selectively inhibits T-type calcium-channels in vitro at concentrations that partially block L-type calcium-channels [31]. The drug shortened action potential duration without any reported important antiarrhythmic action [52]. Despite having promise as a cardioprotective agent [79] the drug had several problematic pharmacokinetic interactions and has now been withdrawn from the market [80].

4.2. Modulation of other ion channels by calcium-channel blockers

Calcium-channel blockers are not specific and have been reported to interact with a number of other ion channel species (see Table 1) [81] particularly voltage-gated potassium channels [82,83]. In recent studies transfection of HERG or KvLQT1/IsK coding sequences into various cell lines has demonstrated that verapamil is a potent antagonist of HERG-encoded channels occurring at clinically relevant concentrations (Fig. 3) [82,83]. The actual contribution of this inhibition to clinical effects remains however unknown.

5. Calcium-channel blockers and arrhythmias: current perspectives

Calcium-channel blockers do not reduce mortality but often decrease symptoms and improve the quality-of-life of patients with arrhythmias [2]. There have been great swings in the relative enthusiasm for antiarrhythmic drugs in the nearly three decades since Vaughan-Williams and Singh’s paper appeared [9,84]. Most strikingly, the large prospective Cardiac Arrhythmia Suppression Trial (CAST) demonstrating an increase in mortality in patients with ventricular premature beats post-myocardial infarction given flecainide, encainide or moricizine [11] indirectly increased calcium-channel blocker use. The fact that calcium-channel blockers were not implicated in proarrhythmic drug action left them relatively unscathed [2] and

<table>
<thead>
<tr>
<th>Drug</th>
<th>L-type calcium channels</th>
<th>Potassium channels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type (species)</td>
<td>IC_{50} (µM)</td>
<td>Cell type (species)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Atrial (rabbit)</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>Ventricular (GP)</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Ventricular (GP)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Ventricular (rat)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Ventricular (human)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Heterologous (α_{1}+β)</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D600</td>
<td>Atrial (frog)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diltiazem</td>
<td>Atrial (frog)</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Ventricular (GP)</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>Atrial (rabbit)</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Heterologous (α_{1}+β)</td>
<td>5.0</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Atrial (rabbit)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Ventricular (GP)</td>
<td>0.1–1.0</td>
</tr>
<tr>
<td></td>
<td>Atrial (frog)</td>
<td>0.2</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>Atrial (frog)</td>
<td>0.016</td>
</tr>
<tr>
<td>Amodipine</td>
<td>Ventricular (GP)</td>
<td>0.1–1.0</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>Atrial (rabbit)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Atrial (frog)</td>
<td>1.0</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>Ventricular (GP)</td>
<td>0.2–12</td>
</tr>
<tr>
<td></td>
<td>Heterologous (α_{1}+β)</td>
<td>1.4–4.9</td>
</tr>
</tbody>
</table>

A fourth class of anti-dysrhythmic action

A fourth class of anti-dysrhythmic action mechanism under these circumstances could invoke neurohumoral activation with abrupt changes in blood pressure leading to β-adrenergic enhanced influx of calcium through L-type calcium-channels [86]. How important such a mechanism will turn out to be is unclear.

Many observations show that calcium-channel blockers do not reduce mortality when given during or after myocardial infarction and they are not recommended as standard treatment [58,87]. The position of calcium-channel blockers in hypertension is currently unclear [19]. Taken together these data have led to several recommendations. Patients with coronary artery disease, for example, should not receive short-acting dihydropyridines although verapamil may be an alternative to β-blockers in those with unstable angina [17,88].

6. Calcium-channel blockers and arrhythmias: future prospects

There are several reasons for thinking that calcium-channel blocker use will increase in patients with arrhythmias. First, although the indications often differ they are probably relatively safe when compared with other direct ion channel modulating drugs [11,32,84]. Second, the large body of work on molecular binding and structural diversity is likely to generate ideas providing new drugs that may enhance the hitherto powerful combination of low toxicity, reasonable tolerability and relatively high efficacy of verapamil and diltiazem [23]. Such approaches may be useful for targeting drug-binding sites with other receptor- and tissue-specificities outside the cardiovascular system and currently untouched by the drugs discussed herein.

There remains the possibility of novel sources of new agents [89]. Third, there is now a considerable body of emerging data supporting the use of calcium-channel blockade to achieve useful atrial electrical re-modelling in the context of atrial fibrillation [90–93]. This is a potential strategic sea change: targeting the development of the arrhythmia substrate rather than trying to modulate the developed arrhythmia [25]. The idea comes for the already classic work showing electrical re-modelling in the goat atrial fibrillation model [94]. Intracellular calcium was felt to be a potential determinant of re-modelling and this contention is now supported by the observation of delayed recovery from electrical re-modelling with the administration of calcium gluconate [92] and reduced re-modelling with verapamil [90–93]. Further, it is now suggested from retrospective analysis that compounds that are likely to result in a lowered cytosolic calcium such as verapamil and beta-blockers reduce the risk of relapse following cardioversion (Fig. 4) [95]. Fourth and possibly more speculative is the potential exploitation of altered relationships between calcium influx via L-type calcium-channels and calcium release from the sarcoplasmic reticulum in heart failure [96–98]. Altered sparks and quarks may point

Fig. 3. Concentration-dependent blockade of HERG current by verapamil. (a) HERG current was evoked according to the voltage protocol (inset). Top trace, recording of tail current on repolarization to −40 mV in control conditions. Increasing concentrations of verapamil (0.1–10 μM) dose-dependently decreased the current and its corresponding tail. (b) Plot of the maximal amplitude of the tail current against time from the same cell as above during control condition and superfusion with increasing concentrations of verapamil. The blockade produced by verapamil was totally reversible within 2 min of washout (WO) with standard solution. (c) Verapamil induced a concentration-dependant blockade of HERG tail currents (mean±SEM, six cells). Data were best fitted with Hill equation (inset, chemical structure of verapamil). The EC_{50} value is 0.83 μM (Hill coefficient, 1.19). Reproduced with permission from Ref. [82].

despite a general decline in antiarrhythmic drug use [84] the use of calcium-channel blockers increased after the publication of CAST [85]. In the National Ambulatory Medical Care Survey the percentage of patients with atrial fibrillation taking calcium-channel blockers was 12.1% in 1989–1991 and 21.1% in 1992–1993 [85].

More recently the fortunes of some drug formulations in this class have not been so good. The recent much discussed meta-analysis of patients with coronary artery disease but without clinical arrhythmias demonstrated a dose-dependent increased risk of total mortality when short-acting nifedipine was compared to diuretics or beta-blockers [18]. Albeit indirect a potential proarrhythmic
to entirely novel therapeutic options [97]. These targets could conceivably have relevance to the management of arrhythmias possibly achieved via the modulation of intracellular calcium signalling [99] in addition to receptor blockade.

Finally, it seems possible that calcium-channel blockade may be usefully incorporated into the mechanism of action of new agents designed to achieve multichannel inhibition as has been raised by amiodarone [24,25]. In a meta-analysis amiodarone use has been associated with reduced mortality in high-risk patients following myocardial infarction [100]. The calcium antagonist component may also reduce the likelihood of torsade de pointes which is so evident with other class III compounds [66].

7. Concluding comments

Molecular characterization of cardiac ion channels has sharpened thinking about antiarrhythmic agents and provides an illustration of how new knowledge can break boundaries instead of encouraging exclusivity. Accordingly restricted classes are now regarded as less important and discussions focus on drug action [53,55]. But are calcium-channel blockers in a class of their own as suggested by Vaughan-Williams and Singh? They certainly have a different spectrum of targets, usually hit different vulnerable parameters, and possibly most importantly have lesser clinical risk when compared to those drugs that have principal actions against sodium and potassium channels [50].

Whatever one thinks about classifications we would suggest that whether in a class of their own or not, calcium-channel blockers have an excellent future used either as mono- or as adjunctive-antiarrhythmic therapy. Their future place in other indications such as hypertension is being addressed prospectively and these data will also be relevant [19,101,102]. There remains of course much basic and clinical research to clarify mechanisms and ensure optimum use.

References

A fourth class of anti-dysrhythmic action


