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REVIEW : FRONTIERS IN PHARMACOLOGY

The cardiac persistent sodium current: an appealing therapeutic target?

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The sodium current in the heart is not a single current with a mono-exponential decay but rather a mixture of currents with different kinetics. It is not clear whether these arise from distinct populations of channels, or from modulation of a single population. A very slowly inactivating component, [$_{I}$Na(P)] $I_{Na(P)}$ is usually about 1% of the size of the peak transient current [$I_{Na(T)}$], but is enhanced by hypoxia. It contributes to Na^+ loading and cellular damage in ischaemia and re-perfusion, and perhaps to ischaemic arrhythmias. Class I antiarrhythmic agents such as flecainide, lidocaine and mexiletine generally block $I_{NA(P)}$ more potently than block of $I_{Na(T)}$ and have been used clinically to treat LQT3 syndrome, which arises because mutations in SCN5A produce defective inactivation of the cardiac sodium channel. The same approach may be useful in some pathological situations, such as ischaemic arrhythmias or diastolic dysfunction, and newer agents are being developed with this goal. For example, ranolazine blocks $I_{Na(P)}$ about 10 times more potently than $I_{Na(T)}$ and has shown promise in the treatment of angina. Alternatively, the combination of $I_{Na(P)}$ block with K^+ channel block may provide protection from the induction of Torsades de Pointe when these agents are used to treat atrial arrhythmias (eg Vernakalant). In all of these scenarios, an understanding of the role of $I_{Na(P)}$ in cardiac pathophysiology, the mechanisms by which it may affect cardiac electrophysiology and the potential side effects of blocking $I_{Na(P)}$ in the heart and elsewhere will become increasingly important.
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Abbreviations: $I_{Na(P)}$, persistent sodium current; $I_{Na(T)}$, transient sodium current

Background

The existence of a persistent component of the cardiac sodium current was suggested almost 30 years ago. Tetrodotoxin (TTX) was shown to shorten action potential in Purkinje fibres at concentrations (3.3×10^{-8} M), which had little or no effect on upstroke (10^{-6} M) (Coraboeuf *et al*., 1979). A slow TTX-sensitive current was subsequently shown directly by voltage clamp (Gintant *et al*., 1984; Carmeliet, 1987a), and the channels responsible for several time components of I_{Na} inactivation shown by patch clamp (Patlak and Ortiz, 1985; Kiyosue and Arita, 1989). Similar observations have been made in rat ventricle cells (Kirsch and Brown, 1989), dog Purkinje fibres (Fozzard *et al*., 1987) and mouse (Bohle and Benndorf, 1995) and rabbit (Grant and Starmer, 1987) ventricle cells and human ventricle cells (Maltsev and Undrovinas, 2006). More recently, approaches such as voltage ramps (Clancy *et al*., 2003) or 'synthetic' action potential clamp protocols (Magyar *et al*., 2004) and a 'dynamic action potential clamp' technique (Berecki *et al*., 2006), have been developed which can directly demonstrate the magnitude of $I_{Na(P)}$ or the channel activity during the action potential and the influence of channel kinetics on this.

The potential of this persistent sodium current ($I_{Na(P)}$) as a therapeutic target was initially highlighted by reports that mutations in Na^+ channels that produced a slowing of inactivation were responsible for some serious clinical arrhythmias (Bennett *et al*., 1995; Marban, 2002), and by demonstrations that $I_{Na(P)}$ was enhanced in hypoxia (Hammarstrom and Gage, 1998, 2002). Later work has shown that $I_{Na(P)}$, is important in myocardial ischaemic damage (or, more accurately, ischaemia–reperfusion damage) and some types of arrhythmias. Recently, compounds which block this current have reached the later stages of clinical development: ranolazine (Ranexa; Cardiovascular Therapeutics) was approved by the US FDA for the treatment of chronic angina in January 2006 and Vernakalant (RSD1235-Cardiome) is undergoing phase III trials for the treatment of atrial fibrillation. Both block $I_{Na(P)}$ to some extent, although both have other actions also, which makes ascribing their clinical effectiveness to block of $I_{Na(P)}$ somewhat contentious.

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Properties of the persistent sodium current

Biophysically, $I_{Na(P)}$ has similar properties to $I_{Na(T)}$ except that the inactivation component is missing or greatly slowed. Hence, at potentials more positive than resting potential the transient current inactivates but $I_{Na(P)}$ does not. $I_{Na(P)}$ deactivates very rapidly on repolarization (Saint *et al.*, 1992) and has slightly different activation properties to $I_{Na(T)}$ at the macroscopic level. In rat ventricular cells $I_{Na(P)}$ has a V_{50} for activation about 20 mV more negative than $I_{Na(T)}$ (V_{50} of –52 and −34 mV) (Saint *et al.*, 1992). $I_{Na(P)}$ in cardiac cells has the same ionic selectivity as $I_{Na(T)}$ and the single channels have the same conductance, mean open time and reversal potential as the transient channel (Gintant *et al.*, 1984). $I_{Na(P)}$ is not a 'window' current since it can be recorded at very positive membrane potentials (Saint *et al.*, 1992). $I_{Na(P)}$ should also not be confused with the background or 'leak' sodium current, which is TTX insensitive and not voltage dependent (Spindler *et al.*, 1998). $I_{Na(P)}$ usually has a magnitude of about 1% of the transient component; in rat myocytes: Saint *et al.* (1992) reported peak $I_{Na(T)}$ of 53 nA and $I_{Na(P)}$ of about 300 pA at 140 ms after a step depolarization to –40 mV. In guinea-pig ventricular myocytes, Kiyosue and Arita (1989) reported a value of 12–50 pA for the sustained I_{Na} at −40 mV (26 ± 14 pA, mean ± s.d., $n = 5$). Although it is small, since it is non-inactivating the amount of charge (and sodium) that can be carried by it over the time course of an action potential can be of the same order as that carried by the transient current. Block of $I_{Na(P)}$ can therefore considerably reduce the sodium load on the cell.

$I_{Na(P)}$ is more sensitive to block by agents such as lidocaine and TTX than is $I_{Na(T)}$ (Nilius *et al.*, 1987; Josephson and Sperelakis, 1989; Ju *et al.*, 1992; Saint *et al.*, 1992). Channels which have been modified by the action of toxins to slow or disable inactivation, or which have mutations affecting inactivation, have similar enhanced susceptibility to block by TTX, lidocaine and related drugs such as flecainide (Liu *et al.*, 2002) and mexiletine (Sicouri *et al.*, 1997).

Mutations in SCN5A leading to LQT3

$I_{Na(P)}$ is implicated in many genetic abnormalities leading to arrhythmias, such as some forms of long Q-T and Brugada syndrome (Rook *et al.*, 1999; Baroudi and Chahine, 2000; Deschenes *et al.*, 2000; Makita *et al.*, 2000; Huang *et al.*, 2006). A bewildering array of mutations in SCN5A (the cardiac sodium channel) have now been documented (for a review see Tan *et al.*, 2003), most of which lead to a gain of function in the channel in LQT3 (Clancy and Kass, 2005) but a loss in function in Brugada syndrome (Brugada and Brugada, 1992). LQT3 mutations in SCN5A generally lead to an enhanced action potential shortening with increased rate, and this is the mechanism for the pause-induced Torsades seen with these mutations (Viswanathan and Rudy, 1999; Oginosawa *et al.*, 2005; Fredj *et al.*, 2006a). Block of $I_{Na(P)}$ may be a useful therapeutic intervention in some LQT3 cases. For example, chronic low-dose flecainide significantly shortened the QTc interval in LQT-3 subjects with the DeltaKPQ mutation (Moss *et al.*, 2005), mexiletine was used to treat a patient with SCN5A single-nucleotide substitution from arginine to glutamine at position 1623 (R1623Q) (Miura *et al.*, 2003) and lidocaine has been used clinically to differentiate LOT3 from Herg mutations (Schwartz *et al.*, 1995). However, the detailed pharmacology of LQT3 is beyond the scope of this review.

Enhanced $I_{Na(P)}$ with toxins

There are a variety of toxins and other agents which can interact with the sodium channel and produce persistent activation (Wang and Wang, 2003). By and large the toxins affect inactivation of the channel, or shift the voltage dependence of activation and so produce enhanced persistent currents. Hence, many of the toxins have been used to mimic the effect of enhanced $I_{Na(P)}$ seen in LQT3 (Sicouri *et al.*, 1997). However, it should be borne in mind that modulation with toxins is not necessarily a perfect mimic of the modulation of I_{Na} that may occur in ischaemia or hypoxia, in the same way that mutations of the channel in LQT3 do not reproduce the details of enhanced $I_{Na(P)}$.

Hypoxia and $I_{Na(P)}$

Perhaps, a closer mimic to the modulation of 'native' $I_{Na(P)}$ may be obtained by use of compounds which alter the redox state of the channel. DPI [(±)-4-(3-(4-(diphenylmethyl)-1-piperazinyl)-2-hydroxyperoxy)-1*H*-indole-2-carbonitrile: Sandoz] appears to switch the channel to a bursting mode (Nilius, 1987). Importantly, hypoxia enhances $I_{Na(P)}$ (Ju *et al.*, 1996b). Somewhat paradoxically, hydrogen peroxide also enhances $I_{Na(P)}$ (Song *et al.*, 2006) while 1 mM reduced glutathione can reverse the effects of hypoxia (Wang *et al.*, 2007). (It has been suggested that the redox effects may be mediated by the action of a closely associated regulatory protein, which may explain the apparently paradoxical effects of redox agents; Hammarstrom and Gage, 1999).

Is the persistent current carried by a distinct channel type?

Apart from the mutations in SCN5A or modification of sodium channels by toxins, in which the underlying mechanisms producing $I_{Na(P)}$ are clear, there are two possible ways in which a non-inactivating component of I_{Na} might arise: (1) there could be only one isoform of the channel, but a proportion of the channels could at any given time display different inactivation kinetics because of, for example, modification by phosphorylation, or the action of a regulatory subunit and (2) there could be different isoforms of the channel present, each with different inactivation kinetics.

The first of these possibilities is given credence by the observation that a persistent component of I_{Na} is present even when a single isoform of the Na^+ channel is expressed in heterologous systems (Fearon and Brown, 2004). There have been reports of the sodium channel undergoing changes in gating mode, where 'burst' mode or non-inactivating modes can arise spontaneously (Patlak and Ortiz, 1985; Bohle *et al.*, 2002). Since the slow Na^+ channels

had the same conductance, reversal potential and TTX sensitivity as fast Na^+ channels in the same cells, Patlak and Ortiz argued that the fast and the slow currents result from a single class of Na^+ channels with two or more kinetic modes. Presumably, a small subpopulation of channels in a cell can be in these modes at any given time, giving rise to a persistent current at the macroscopic level. This model for the genesis of $I_{Na(P)}$ has the appeal of simplicity, but seems, at first sight, difficult to reconcile with the apparent different sensitivity to blocking agents such as TTX and lidocaine of $I_{Na(P)}$ and $I_{Na(T)}$.

The obvious way to account for this different pharmacology is to propose that $I_{Na(P)}$ is due to a distinct channel. The Na^+ channel has many different isoforms; it has long been known that cardiac channels are substantially different from neuronal channels in their kinetics and sensitivity to TTX. The molecular basis of this diversity of sodium channels is now well understood (Leffler *et al*., 2005). The dominant form of the channel in the heart is Nav 1.5 or Nav 1.1, with associated β-1 or β-2 subunits. Of course, there is no reason why a given cell should not express a mixture of isoforms, and this could give rise to mixed Na^+ currents. Indeed, as long ago as 1985 it was suggested that the slowly inactivating current (in sensorimotor neurones) was due to a different channel, based on block by QX-314 (Stafstrom *et al*., 1985). If $I_{Na(P)}$ in cardiac cells does arise from a different isoform of the channel, this channel is apparently identical to the $I_{Na(T)}$ channel in conductance, mean open time and selectivity (Ju *et al*., 1992), suggesting that the only difference is in kinetic properties and apparent affinity of some blockers.

Its important to realize, though, that this difference in pharmacology (at the macroscopic level) is not inconsistent with $I_{Na(T)}$ and $I_{Na(P)}$ arising from one population of channels. It has been suggested that the kinetics of the channel can interact with the use-dependence of blockers such as TTX or lidocaine to produce an apparent preferential block (Carmeliet, 1987b; Josephson and Sperelakis, 1989; Saint, 2006). A similar mechanism has been proposed for flecainide (Liu *et al*., 2002) and may hold for other similar types of blockers. Theoretical models of channel block by mexiletine and lidocaine predict an enhanced block of LQT3, or burst mode, channels compared to the transient channels, because of their different kinetics, not because of intrinsic differences in the channels themselves (Clancy *et al*., 2007).

Although $I_{Na(P)}$ is small compared to $I_{Na(T)}$ under most conditions, there are situations in which $I_{Na(P)}$ is enhanced and which have physiological (or pathophysiological) implications; for example reactive oxygen species, such as H_2O_2, activate $I_{Na(P)}$ (Meng and Nie, 2004; Ma *et al*., 2005; Song *et al*., 2006), as does NO (Hammarstrom and Gage, 1999; Ahern *et al*., 2000), some phospholipids (Undrovinas *et al*., 1992) and hypoxia (Ju *et al*., 1996b; Hammarstrom and Gage, 2002; Saint, 2006). While the mechanisms by which these operate is not fully understood, it's likely that they mimic some of the mechanisms that operate *in vivo* (for example in ischaemia). Its not known whether these interventions produce their effects by enhancing the activity of a susceptible subpopulation of distinct channels, or whether they are converting some $I_{Na(T)}$ channels into $I_{Na(P)}$ channels.

In summary, the jury is still out on whether the co-presence of $I_{Na(T)}$ and $I_{Na(P)}$ is a consequence of distinct channel isoforms being co-expressed, or merely due to a kinetically distinct subpopulation of an otherwise heterogeneous isoform. Either hypothesis is consistent with the data available at present.

The physiological role of the persistent sodium current

The physiological role of $I_{Na(P)}$ in the heart is unclear. It certainly contributes to the action potential plateau, as demonstrated by the effect of low doses of TTX to shorten cardiac action potentials noted above, although this effect does not seem large enough to produce contractile changes under physiological conditions (Brette and Orchard, 2006). Some groups have reported a gradient of expression of $I_{Na(P)}$ across the ventricular wall; in guinea-pig, mid-myocardial cells had a smaller $I_{Na(P)}$ ($0.23 \pm 0.02\,pA\,pF^{-1}$) than epi- or endo-cardial (0.40 ± 0.04 and $0.36 \pm 0.03\,pA\,pF^{-1}$ respectively) and computer modelling predicted that this could lead to action potential heterogeneity (Sakmann *et al*., 2000). These results were not consistent with those of Main *et al*. (1998), who observed that $0.1\,\mu mol\,l^{-1}$ TTX shortens APD_{90} in guinea-pigs by about 36% in subendocardial myocytes, 16% in mid-myocardial myocytes, and 20% in subepicardial myocytes. In myocytes isolated from different regions of the dog heart, late sodium current density at 0 mV was 47% greater in mid-myocardial cells and averaged $-0.532 \pm 0.058\,pA\,pF^{-1}$ in endocardial, $-0.463 \pm 0.068\,pA\,pF^{-1}$ in epicardial and $-0.785 \pm 0.070\,pA\,pF^{-1}$ in mid-myocardial cells (Zygmunt *et al*., 2001). Although the gradient of I_{Na} is thought to be important in the pro-and anti-arrhythmic effect of class 1 agents, particularly in models of LQT3, the existence of a distinct population of mid-myocardial cells ('M cells') has not been verified in other species, for example, pig (Rodriguez-Sinovas *et al*., 1997); rabbit (Idriss and Wolf, 2004). Their existence and possible physiological significance in human is unclear (Drouin *et al*., 1995; Antzelevitch *et al*., 1999; Taggart *et al*., 2003).

Therapeutic potential of $I_{Na(P)}$

As noted above, block of $I_{Na(P)}$ may be a worthwhile therapeutic intervention in some LQT3 syndromes. A far more common clinical application may arise from the observation that $I_{Na(P)}$ may be upregulated in cardiac hypertrophy and failure, and may contribute to the action potential prolongation in these conditions. In addition, enhanced $I_{Na(P)}$ is almost certainly involved in reperfusion injury, some arrhythmias and possibly other disease states.

Protection in ischaemia

When myocardial tissue becomes ischaemic intracellular pH falls due to lactic acid production. Normally the increased $[H^+]i$ is extruded via the sodium–hydrogen exchanger, NHE-1. There is some evidence that NHE is inhibited during

ischaemia (Xiao and Allen, 2000), which results in an uncontrolled increase in $[H^+]_i$. Upon re-perfusion the inhibition of NHE is removed and consequently it acts to produce a large extrusion of H^+ at the expense of allowing a large Na^+ influx. The increased [Na]i reduces the electrochemical energy gradient available to the Na–Ca exchanger (NCX), leading to decreased Ca^{2+} extrusion from the cell or, in extreme cases, reversal of Na–Ca exchange; either situation leads to Ca^{2+} overload (the 'coupled exchanger' hypothesis (Lazdunski *et al*., 1985)) (Figure 1).

In animal experiments, NHE blockers are effective at ameliorating re-perfusion damage (Hartmann and Decking, 1999), although clinical trials have been disappointing (Theroux *et al*., 2000; Zeymer *et al*., 2001). An important clue as to their failure may lie in the observation that in animal models block of NHE does not provide complete protection, and protection can equally and synergistically be provided by blocking voltage-dependent sodium channels, for example with TTX (Eigel and Hadley, 1999) (Figure 2).

Hence, influx of Na^+ through $I_{Na(P)}$ channels contributes at least part of the $[Na^+]_i$ load in ischaemia. This effect is likely to be linked to the fact that $I_{Na(P)}$ is enhanced by hypoxia (Ju *et al*., 1996b; Hammarstrom and Gage, 2002; Fearon and Brown, 2004; Saint, 2006). Hence, agents which block $I_{Na(P)}$ should be protective in ischaemia, and this seems to be true (Haigney *et al*., 1994; Belardinelli *et al*., 2006). Indeed, over 10 years ago, Le Grand *et al*. (1995) showed that low concentrations of TTX (320 nM) can alleviate the contractile dysfunction caused by ischaemia in isolated guinea-pig heart, and suggested that this would be a useful target for therapeutic intervention.

Diastolic dysfunction in ischaemia. Post-ischaemic rat myocardium displays an elevated diastolic calcium (to about 0.5 μM from control values of about 0.3 μM) accompanied by an increased LVEDP (to about 80 mm Hg from control of about 10 mm Hg) and a prolonged calcium transient (Meissner and Morgan, 1995). These observations have subsequently generally been confirmed (for example, Saini and Dhalla, 2005), although other groups have disputed whether the increased intracellular calcium is directly related to the increased diastolic tension (Eberli *et al*., 2000), pointing out that the situation is complicated by changes in myofilament calcium sensitivity. Since it appears to be a persisting defect in Ca^{2+} handling that is induced by ischaemia and reperfusion, rather than a persisting enhancement of $I_{Na(P)}$ itself, its likely that blockers of $I_{Na(P)}$ may be useful during reperfusion, but not post-reperfusion. This is still a matter to be resolved.

Protection against arrhythmias

Since $I_{Na(P)}$ carries a depolarizing current, it tends to prolong the action potential. Normally this contributes only slightly to the plateau of the AP but, in conditions where repolarization is defective, $I_{Na(P)}$ may produce arrhythmias. Even if $I_{Na(P)}$ channel activity is constant during the plateau, as the action potential starts to repolarize the driving force for Na^+ increases, and so $I_{Na(P)}$ also increases. If repolarizing currents are less powerful than normal (because of drug-induced block of K^+ channels, for example), the increase in $I_{Na(P)}$ can produce a regenerative depolarization and early afterdepolarization (EAD) (Noble and Noble, 2006). A similar effect will also occur if $I_{Na(P)}$ is increased more than usual, for example by hypoxia or by LQT3 mutations. Hence, block of $I_{Na(P)}$ should be antiarrhythmic in these situations.

A longer term effect of $I_{Na(P)}$ on arrhythmias is related to the coupled exchanger scheme noted above. Increased Ca^{2+} loading increases SR calcium to the point where the SR 'overflows' and produces spontaneous oscillatory Ca^{2+} release, which activates NCX. Since NCX is electrogenic (Kang and Hilgemann, 2004) under these conditions it can produce membrane currents powerful enough to generate

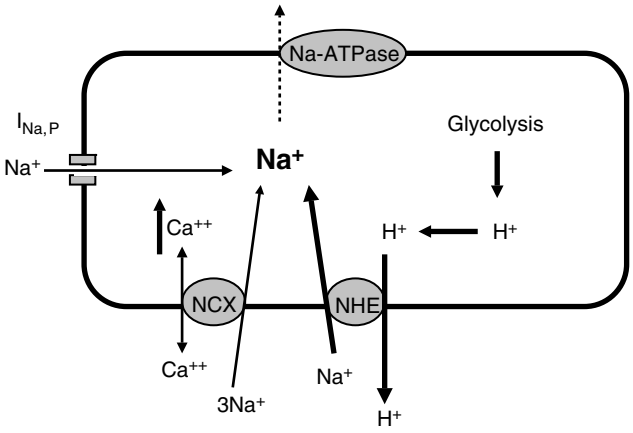

Figure 1 Diagram of coupled exchanger model. Ischaemia or hypoxia results in enhanced glycolysis, producing lactate and H^+. The H^+ ions are pumped out of the cell by NHE1 in exchange for sodium influx. Na efflux by the Na-KATPase is reduced because of low ATP. Additional Na^+ influx occurs via $I_{Na(P)}$. The Na^+ load results in reduction or reversal of NCX, leading to calcium overload (Note that NCX is shown here in reverse mode. From Saint, 2006.).

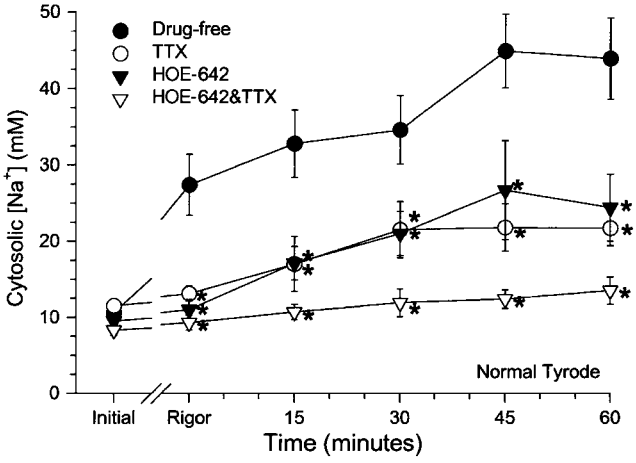

Figure 2 Fluorescence measurements of cytosolic $[Na^+]$ were made in isolated guinea-pig ventricular myocytes [loaded with SBFI-AM] exposed to anoxia beginning at the 'initial' time point and continuing until 60 min post-rigor contracture. Either HOE-642 (10 μM) or TTX (30 μM) reduced the anoxic Na^+ load by about 50% while a combination of both almost eliminated Na^+ load. Each time point represents mean six s.e. of eight cells. $*P = 0.05$ versus drug-free value at same time point. From Eigel and Hadley (1999).

arrhythmias. This appears to be the mechanism behind delayed afterdepolarizations (DADs) (Verkerk *et al.*, 2000) and some EADs (Szabo *et al.*, 1994). Following the logic above, blocking $I_{Na(P)}$ should be effective in preventing arrhythmias of these types by reducing $[Na^+]_i$ load, and the consequent $[Ca^{2+}]_i$ load, with the caveat that the block must be specific; some coincidental block of $I_{Na(T)}$ would be expected to be pro-arrhythmic due to slowing of conduction velocity facilitating re-entry.

There may be other pathological conditions where block of $I_{Na(P)}$ might provide an appealing antiarrhythmic target. It has been well documented that arrhythmias are more common in heart failure and hypertrophy. The mechanism is unclear, but it has recently been suggested that $I_{Na(P)}$ contributes to these arrhythmias (Maltsev and Undrovinas, 2006). Block of $I_{Na(P)}$ in myocytes from dogs with failure indeed appears to be antiarrhythmic (Undrovinas *et al.*, 2006), although this result must be interpreted with the reservation that it is difficult to extrapolate effects in isolated cells to the level of the whole heart. A number of experiments on ventricular samples from human heart failure patients have shown that $I_{Na(P)}$ is upregulated in failure (Undrovinas *et al.*, 1999; Maltsev *et al.*, 2001), although this is by no means universally accepted (Goldman and Balke, 2002). If $I_{Na(P)}$ is indeed upregulated in failure, this would provide an appealing therapeutic target.

Blockers of $I_{Na(P)}$

Ranolazine

Ranolazine (Ranexa; Cardiovascular Therapeutics) was approved by the US FDA for the treatment of chronic angina in January 2006 (Abrams *et al.*, 2006). Ranolazine has been shown to improve exercise performance and increase the time to angina/ischaemia in at least five trials (Chaitman, 2006). Interestingly, ranolazine seems to produce an additional improvement when added to existing antianginal agents (diltiazem, amlodipine and atenolol) (Morrow *et al.*, 2006). Patient recruitment into a Phase III trial for ranolazine in non-S-T segment elevation acute coronary syndrome [(MERLIN)-TIMI] was started in October 2004 and the results reported in early 2007 (see below).

The proposed mechanism of action of ranolazine to produce this anti-anginal effect is block of $I_{Na(P)}$. However, ranolazine was not developed as an $I_{Na(P)}$ blocker, and initially it was thought that ranolazine acted by shifting metabolic substrate utilization from fatty acids to glucose (McCormack *et al.*, 1996, 1998; Sabbah *et al.*, 2002). Ranolazine also attenuates α1 and β1 adrenergic receptor-mediated responses and has 5-HT1A receptor agonist activity (Allely *et al.*, 1993). This sympatholytic action may underlie some of the bradycardia seen with ranolazine in animal models, although the β-agonist effect is weak and there is an additional bradycardic effect not related to β-receptor block (Letienne *et al.*, 2001).

Because of concerns about the discrepancy between the EC_{50} for these effects and the therapeutic plasma level, attention has latterly turned to the electrophysiological actions of Ranolazine. In addition to block of $I_{Na(P)}$ ($EC_{50} = 5.9\,\mu M$), ranolazine inhibits a number of other ionic currents, including IK_r (12 μM), late I_{Ca} (50 μM), peak I_{Ca} (296 μM), $I_{(Na–Ca)}$ (91 μM) and IK_s (17% at 30 μM) (Antzelevitch *et al.*, 2004). Importantly, the EC_{50} for block of $I_{Na(P)}$ can vary from 5 to 21 μM, depending on the voltage protocol and frequency. In addition, a simple statement of the EC_{50} for block of IK_r, late I_{Ca} and $I_{Na(P)}$ does not give a true reflection of the degree of block in the therapeutic range—because of the different apparent Hill coefficients, each of these currents is blocked to a similar extent (by about 25%) in the therapeutic range of 2–6 μM (Antzelevitch *et al.*, 2004) (Block of $I_{Na(T)}$ was not specifically reported, but little block occurs at 10 μM (inferred from reduction in dv/dt_{max}). In two different mutations of SCN5A, ranolazine was about 10 times more potent to block $I_{Na(P)}$ than $I_{Na(T)}$ (Fredj *et al.*, 2006b) (Figure 3).

Hence, although ranolazine blocks $I_{Na(P)}$ with high potency, it blocks other currents just as well in the therapeutic range. Prediction of the effects of block of these currents at the tissue level is not a trivial exercise, since it depends on the relative size of each of the currents at each phase of the action potential. For example, ranolazine prolongs APD_{90} in guinea-pig isolated perfused hearts (Wu *et al.*, 2004), prolongs APD in canine epicardial cells but produces a bi-phasic effect on M cells (prolongation at low concentration, shortening at high), and shortens canine Purkinje fibre APD (Antzelevitch *et al.*, 2004).

Consistent with other $I_{Na(P)}$ blockers, ranolazine is protective in a rabbit model of ischaemia–reperfusion (Hale *et al.*, 2006), suggesting that it may be a $I_{Na(P)}$ blocker in this situation (although an earlier study in dogs found no reduction in infarct size (Black *et al.*, 1994)). Direct evidence that this is the mechanism of protection has been sparse, although a recent study comparing the effect of ranolazine on Ca^{2+} accumulation and ventricular dysfunction in rat heart produced by either ATX-II or ischaemia–reperfusion did directly show that ranolazine produced a reduction in diastolic Ca^{2+} accumulation and an improvement in function in both situations (Fraser *et al.*, 2006). However, the proposal that ranolazine is effective in angina by blocking $I_{Na(P)}$ seems to be based on the logic that if

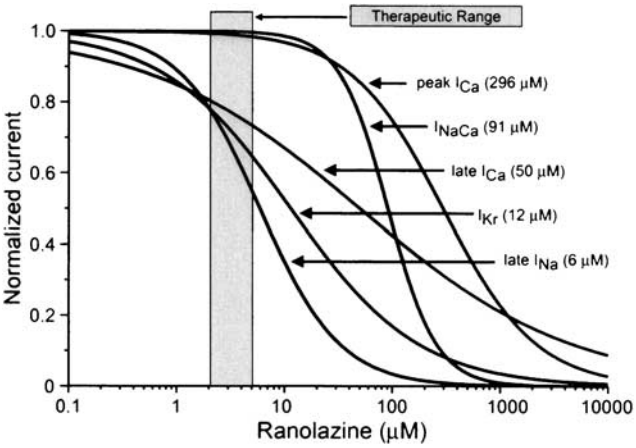

Figure 3 Summary of potency of ranolazine to block various currents. From Antzelevitch *et al.* (2004) (Circulation).

ranolazine blocks $I_{Na(P)}$ in reperfusion experiments and is effective in angina, then $I_{Na(P)}$ must be implicated in angina. This is something of a circular argument, and needs more investigation. The difficulty is that neither the levels of hypoxia produced in angina or the degree to which $I_{Na(P)}$ is increased by this are well understood—the levels of hypoxia in ischaemia–reperfusion experiments, or those used to enhance $I_{Na(P)}$ *in vitro* are comparatively severe (pO_2 around 10–20 mm Hg) and it seems unlikely that pO_2 can fall to this level in angina, particularly non-ST-segment-elevation angina. There may of course be synergistic effects: it is possible that formation of reactive oxygen species, release of palmitoyl carnitine or lysophosphatidylcholine may occur in angina and these agents have been shown to increase $I_{Na(P)}$ (Undrovinas *et al.*, 1992; Wu and Corr, 1994; Ward and Giles, 1997), but whether this actually occurs in angina is again unknown. In this case it is perhaps unsurprising that the results of the MERLIN-TIMI trial showed that ranolazine provided no improvement in the primary efficacy endpoint (a composite of cardiac death, myocardial infarction or recurrent ischaemia), although recurrent ischaemia was reduced.

QTc prolongation requiring a reduction in the dose of intravenous drug occurred in 31 patients (0.9%) receiving ranolazine, compared with 10 patients (0.3%) receiving placebo. There was actually a significant reduction in arrhythmias detected on Holter monitoring during the first 7 days of treatment. The authors stated that the results of the MERLIN trial 'do not support the use of ranolazine for acute management of ACS or as disease-modifying therapy for secondary prevention of cardiovascular death or MI.'

Hence, although there is clinical evidence that ranolazine improves ventricular function in angina (Hayashida *et al.*, 1994; Rousseau *et al.*, 2005) the idea that it does this via $I_{Na(P)}$ block is unproven and it may be that its other pharmacological actions underlie this effect. Indeed, block of $I_{Na(P)}$ was being proposed only as a potential mechanism of action of ranolazine as late as 2004 (Chaitman, 2004).

Nevertheless, it may be that this complexity of action is an advantage; it has been noted that because of these mixed electrophysiological actions, ranolazine, even though it may prolong QT in some situations, does not produce large disturbances in *trans*-mural dispersion of repolarization, and hence is less arrhythmogenic (particularly for Torsade de pointes) than comparable agents (Antzelevitch and Belardinelli, 2006).

Vernakalant (RSD1235)

Vernakalant (Cardiome Ltd, Vancouver, BC, Canada) has been reported to be a blocker of $_{INa(P)}$ (Orth *et al.*, 2006). It is in Phase III trials in intravenous form for reversion of atrial fibrillation (Roy *et al.*, 2004) and the oral form is in Phase II trials. As with ranolazine, Vernakalant was not initially developed as an $I_{Na(P)}$ blocker, but as an atrial-specific K^+ channel blocker (Kv1.5, or IK_{ur}). In rats, ischaemic arrhythmias induced by coronary artery occlusion were inhibited with an ED_{50} of 1.5 μmol kg^{-1} per min (infusion rate), while 4–8 μmol kg^{-1} per min gave complete protection (Fedida *et al.*, 2005). Unfortunately, it was not stated whether there was any reduction in the size of the ischaemic zone with Vernakalant. In channels expressed in HEK cells the same paper reported block of K^+ channels with IC_{50} values of 13 μM on hKv1.5 (1 Hz) versus 38 and 30 μM on Kv4.2 and Kv4.3, respectively, and 21 μM on hERG channels. Sodium channel block was less potent ($IC_{50} = 43$ μM at 1 Hz), although the potency increased with high frequency ($IC_{50} = 40$ μM at 0.25 Hz, to an $IC_{50} = 9$ μM at 20 Hz), and with depolarization (107 μM at −120 mV to 31 μM at −60 mV (1 Hz)). This paper did not examine $I_{Na(P)}$ block, but this was subsequently investigated by the same group in 2006 (Orth *et al.*, 2006). Although it blocked $I_{Na(P)}$, Vernakalant showed almost no selectivity for $I_{Na(P)}$ over $I_{Na(T)}$: 30 μM blocked $I_{Na(P)}$ by 70 ± 4% and $I_{Na(T)}$ by 61 ± 4%, a difference much less than lidocaine in the same experiments (Orth *et al.*, 2006). Again, these data were obtained with Nav1.5 channels expressed in HEK cells. This lack of block of $I_{Na(P)}$ and a more potent block of K^+ channels is consistent with Vernakalant's effects on action potential duration: in isolated rat myocytes the AP was prolonged approximately 75% over control by 10 μM, and at 30 μM APD increased approximately threefold, with prominent elevation of the AP plateau. Studies in patients with atrial fibrillation have shown that QRS duration is increased by 10% by Vernakalant at peak plasma levels of 5.8 μg ml^{-1} (or ~15 μM) (Roy *et al.*, 2004). In the Phase I trial a dose of 5 mg kg^{-1} produced plasma levels of 4 μg ml^{-1} (about 11 μM), heart rate increased from 61 ± 11 to 70 ± 11 b.p.m., PR interval increased from 169 ± 24 to 184 ± 15 and QRS from 88 ± 8 to 100 ± 5. Slight QT prolongation was seen, from 384 ± 14 to 419 ± 6. These prolongations of QRS and QTc were not seen in the Phase II trial, compared to placebo at up to 3 mg kg^{-1} (about 16 μM). In Phase III, Vernakalant has shown promising conversion rates (Fedida, 2007). Hence, it is likely that Vernakalant's antiarrhythmic actions stem from block of K^+ channels, with an additional effect of frequency- and depolarization-dependent block of $I_{Na(T)}$. $I_{Na(P)}$ is unlikely to play a direct role, although it is possible that block of $I_{Na(P)}$ counters to some extent the AP prolonging effect in the ventricle, and hence makes Vernakalant less likely to induce Torsades de Pointe.

Merlin TIMI-36 outcomes

Endpoint	*Ranolazine (%)*	*Placebo (%)*	*HR (95% CI)*	P
Primary endpoint	21.8	23.5	0.92 (0.83–1.02)	0.11
CV death/MI/recurrent ischaemia	18.7	19.2	0.96 (0.86–1.08)	0.50
CV death/MI	10.4	10.5	0.99 (0.85–1.15)	0.87
Recurrent ischaemia	13.9	16.1	0.87 (0.76–0.99)	0.03*

Riluzole

Riluzole is a neuroprotective agent which can protect the brain from focal and global ischaemia (Bae *et al*., 2000). The mechanism by which it does this is uncertain, but it is thought to be due to a reduction of glutamate release (Doble, 1996) or possibly by activation of tandem pore K^+ channels (Duprat *et al*., 2000). Recently, it has been suggested that Riluzole blocks $I_{Na(P)}$ in neurones (St John *et al*., 2007) and it may be that this is how it confers protection. Data on the effects of Riluzole in cardiac tissue are sparse, but it appears that it does not have antiarrhythmic effects (Mestre *et al*., 2000).

Other compounds

There are other compounds purported to block $I_{Na(P)}$ which are in early development as antiarrhythmic or anti-ischaemic agents. AZD7009 (AstraZeneca) is a compound under development for the treatment of atrial fibrillation (Crijns *et al*., 2006). It is proposed to be atrial-selective, in much the same way as Vernakalant. Its antifibrillatory action is probably due to block of IK_r, IK_ur and I_{to}, but it has less pro-arrhythmic effect than would be expected from this. It has been proposed, in much the same way as for Vernalkalant, that this is due to a concomitant block of $I_{Na(P)}$. A recent study examined block of $I_{Na(P)}$ in CHO cells expressing hNav1.5. AZD7009 blocked $I_{Na(P)}$ with an EC_{50} of $11 \pm 2\,\mu M$, but showed little selectivity over $I_{Na(P)}$ ($16 \pm 2.2\,\mu M$). Similar results were found in rabbit atrial and ventricular myocytes. AZD7009 also counteracted the AP prolongation and EAD formation in Purkinje fibres induced by E-4031 (Persson *et al*., 2007).

R56865 (Janssen Pharmaceuticals, Beerse, Belgium) has been shown to block the late I_{Na} induced by veratridine (Le Grand *et al*., 1998) and it was proposed that this is the basis for its protective effects against ischaemic damage (Ravens and Himmel, 1999). A more recent paper cast doubt on this interpretation, showing that R56865 did not prevent sodium overload during ischaemia (Hartmann and Decking, 2003), suggesting that its protective effect may be due to some other action (for example it was suggested some time ago that R56865 blocked calcium release from the SR; Ichikawa *et al*., 1994). Development of R56865 has apparently not progressed past the preclinical stage. KC12291 (Solvay Pharmaceuticals, Daix, France) is an anti-ischaemic compound thought to act by blocking $I_{Na(P)}$ and thus preventing sodium overload. (Decking *et al*., 1998; Tamareille *et al*., 2002; John *et al*., 2004). KC12291 is still in the early stages of development.

Some caveats about $I_{Na(P)}$ block

Although the heart expresses mainly Nav 1.5 (the more TTX-insensitive Na^+ channel), there is also considerable expression of the more TTX-sensitive Nav 1.1, 1.2 and 1.3 (Brette and Orchard, 2006), particularly in the Purkinje fibres and bundles of His (Haufe *et al*., 2005). This should sound a note of caution for attempts to develop blockers of $I_{Na(P)}$—such a block may lead to a greater than expected disruption of the conducting system of the heart.

In addition, $I_{Na(P)}$ appears to play a role in pacemaking in cells in the SA node (Muramatsu *et al*., 1996; Ju *et al*., 1996a). Consistent with this, KC12291 and R56865, which more selectively block $I_{Na(P)}$ than TTX or lidocaine, have a much greater bradycardic effect in rat heart (Letienne *et al*., 2006). There is evidence that ranolazine is similarly bradycardic in animal models (Letienne *et al*., 2001) and small decreases in heart rate have been reported in the MARISA and CARISA trials, although subsequent trials have not raised concerns in this area. Vernakalant also appears to be bradycardic in humans, but this interpretation is complicated by the number of patients reverting from AF being included in the data (Roy *et al*., 2004). Paradoxically, LQT3 patients are often bradycardic (Veldkamp *et al*., 2003) while TTX has been reported to not be bradycardic in rabbit (Muramatsu *et al*., 1996).

In addition to being present in the heart, $I_{Na(P)}$ is widely expressed in the CNS, where it seems to underlie repetitive or bursting behaviour for example (Rybak *et al*., 2003). Hence, a specific $I_{Na(P)}$ blocker may have CNS effects. Of course, the presence of similar channels in the CNS and the heart has not prevented the development of antiarrhythmic agents blocking those channels (for example lidocaine). In this context, the blood–brain barrier can be an ally.

It was shown almost 20 years ago that a very specific blocker of $I_{Na(P)}$, that is low dose TTX, is a good anti-arrhythmic agent (Abraham *et al*., 1989). Clearly TTX is not a good candidate for an antiarrhythmic or ischaemia-protective agent. It remains to be seen whether a truly specific blocker of $I_{Na(P)}$ will be.

Future directions

$I_{Na(P)}$ blockers certainly show promise in protecting myocardium from ischaemia/reperfusion damage. However, it appears that in post-ischaemic myocardium, the contractile dysfunction is likely due to impaired calcium handling (due to irreversible damage produced during reperfusion) not due to alterations of $I_{Na(P)}$. The idea that $I_{Na(P)}$ may be enhanced in post-ischaemic (or 'stunned') myocardium appears not to have been fully tested, and would be a worthwhile investigation. If it is enhanced, then blockers of $I_{Na(P)}$ may be useful after reperfusion has occurred, rather than only prior to reperfusion or during ischaemia. Better data on the degree to which $I_{Na(P)}$ may be involved in angina is also needed.

Paradoxically, targeting of $I_{Na(P)}$ as a strategy for ischaemic protection may be best served by development of highly specific blockers, whereas for anti-arrhythmic efficacy, the development of deliberately non-specific channel blockers may be more fruitful, that is agents that have a clever combination of block of different ion channels, with attendant frequency dependence, to produce the desired electrophysiological profile. In these agents, block of $I_{Na(P)}$ would be only one of the suite of blocking actions used to tailor the electrophysiological response. This approach (perhaps serendipitously) seems to have worked well in the

case of Vernakalant and Ranolazine, and similar compounds may be on the horizon.

Conflict of interest

The authors state no conflict of interest.

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