

# The arrhythmogenic consequences of increasing late $I_{Na}$ in the cardiomyocyte

John C. Shryock<sup>1†</sup>, Yejia Song<sup>2</sup>, Sridharan Rajamani<sup>1</sup>, Charles Antzelevitch<sup>3</sup>, and Luiz Belardinelli<sup>1\*</sup>

<sup>1</sup>Department of Biology, Cardiovascular Therapeutic Area, Gilead Sciences, Foster City, CA, USA; <sup>2</sup>Division of Cardiology, College of Medicine, University of Florida, Gainesville, FL, USA; and <sup>3</sup>Masonic Medical Research Laboratory, Utica, NY, USA

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## Abstract

This review presents the roles of cardiac sodium channel  $Na_v1.5$  late current (late  $I_{Na}$ ) in generation of arrhythmic activity. The assumption of the authors is that proper  $Na^+$  channel function is necessary to the maintenance of the transmembrane electrochemical gradient of  $Na^+$  and regulation of cardiac electrical activity. Myocyte  $Na^+$  channels' openings during the brief action potential upstroke contribute to peak  $I_{Na}$  and initiate excitation–contraction coupling. Openings of  $Na^+$  channels outside the upstroke contribute to late  $I_{Na}$ , a depolarizing current that persists throughout the action potential plateau. The small, physiological late  $I_{Na}$  does not appear to be critical for normal electrical or contractile function in the heart. Late  $I_{Na}$  does, however, reduce the net repolarizing current, prolongs action potential duration, and increases cellular  $Na^+$  loading. An increase of late  $I_{Na}$ , due to acquired conditions (e.g. heart failure) or inherited  $Na^+$  channelopathies, facilitates the formation of early and delayed afterpolarizations and triggered arrhythmias, spontaneous diastolic depolarization, and cellular  $Ca^{2+}$  loading. These in turn increase the spatial and temporal dispersion of repolarization time and may lead to reentrant arrhythmias.

## Keywords

Heart • Late  $I_{Na}$  • Afterpotential • Arrhythmia • Reentry

## 1. Origins of cardiomyocyte late $I_{Na}$

Although the presence and potential importance of so-called non-inactivating  $Na^+$  current in myocytes was recognized as early as 1979,<sup>1,2</sup> the roles of this seemingly minor current in arrhythmogenesis were not identified until the demonstration that 'gain of function' mutations in the gene *SCN5A* enhance  $Na_v1.5$  late  $I_{Na}$  and cause the congenital long-QT syndrome type 3 (LQT3).<sup>3,4</sup> Pathological roles of late  $I_{Na}$  in the heart have been reviewed previously.<sup>5–18</sup>

After opening briefly (about 50  $\mu s$  at 35°C)<sup>19</sup> during the upstroke of the cardiac action potential (AP), individual  $Na^+$  channels usually inactivate and remain inactivated until repolarization of the cell membrane. Sodium channel openings after the upstroke create a small 'late' current that persists throughout the plateau of the AP. The amplitude of late  $I_{Na}$  is reported to be  $\leq 0.1\%$  of peak  $I_{Na}$  in isolated left-ventricular myocytes from the rat,<sup>20</sup> guinea pig,<sup>21</sup> and human heart.<sup>22</sup> Many  $Na^+$  channel mutations, pathological conditions, pharmacological agents and toxins delay or destabilize  $Na^+$  channel inactivation and increase late  $I_{Na}$ . The magnitude of late  $I_{Na}$  in cardiac myocytes may be increased by either acquired conditions such as heart failure,<sup>12,23–26</sup>

hypoxia/ischaemia,<sup>8,27–29</sup> inflammation,<sup>30</sup> oxidative stress,<sup>31</sup> and thyroid hormone,<sup>32</sup> (Table 1) or congenital (inherited) mutations in *SCN5A* and channel-interacting proteins that cause long-QT syndrome.<sup>3,4,15,33,34</sup> Several forms of cardiac  $Na^+$  channel dysfunction are direct causes of late  $I_{Na}$ : (i) delayed or failed inactivation of open channels (i.e. long openings); (ii) transient bursts of re-openings and scattered single late openings of channels that were in an unstable inactivated state; and (iii) fast recovery of channels from inactivation during non-equilibrium conditions, as during repolarization of the AP.<sup>19,20,22,35–38</sup> In addition, not all  $Na^+$  channels open during the AP upstroke, and those that do not open during peak  $I_{Na}$  are potentially available to open late. Lastly, within a 'window' of voltages that is sufficiently depolarized to cause activation of some  $Na^+$  channels but not so depolarized as to cause inactivation of all channels from the closed state, a small equilibrium  $Na^+$  current is theoretically present.<sup>1</sup> This  $Na^+$  window current is not typically referred to as late  $I_{Na}$ , and its significance is poorly understood. However, it is a potential cause of  $Na^+$  loading, especially in depolarized ischaemic myocardium.<sup>39</sup> Interestingly, the range of voltages for steady-state window current was shifted by tens of millivolts in a hyperpolarizing direction by membrane stretch.<sup>40</sup>

<sup>†</sup> Present address. 3420 SW 77th Street, Gainesville, FL 32608, USA, Email: johnshryock5@gmail.com.

\* Corresponding author. Tel: +1 650 524 3889; fax: +1 650 522 1654, Email: luiz.belardinelli@gilead.com

**Table 1** Conditions and agents that have been demonstrated to increase cardiac late  $I_{Na}$ 

Conditions/endogenous agents	Drugs and toxins
Activation of CaMKII	Aconitine
Activation of Fyn tyrosine kinase	ATX-II
Activation of PKC	Batrachotoxin
Angiotensin II	DPI 201–106 and analogues
Carbon monoxide	KB130015
2,3-Diphosphoglycerate	Ouabain (indirectly)
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	Pyrethroids (e.g. tefluthrin)
Hypoxia, ischaemia	Veratridine
Lysophosphatidylcholine	
Nitric oxide (NO)	<b>Diseases</b>
Palmitoyl-L-carnitine	Heart failure
Thyroid hormone T3	Hypertrophic cardiomyopathy

This may be partly responsible for a background Na<sup>+</sup> current that occurs close to the threshold voltage for Na<sup>+</sup> channel activation in myocytes.<sup>41</sup>

## 2. Myocyte late $I_{Na}$ , Na<sup>+</sup> and Ca<sup>2+</sup> homeostasis, and contractile function

The Na<sup>+</sup>/Ca<sup>2+</sup> exchanger (NCX) and voltage-gated Na<sup>+</sup> channels are major routes of Na<sup>+</sup> entry into cardiac myocytes.<sup>42</sup> Late  $I_{Na}$  constitutes perhaps one-half of Na<sup>+</sup> channel-mediated Na<sup>+</sup> entry in ventricular myocytes.<sup>43,44</sup> In normal ventricular myocardium at a heart rate of 60/min, late  $I_{Na}$ -mediated Na<sup>+</sup> influx during phase 2 of the AP plateau is estimated to be about 30% of total Na<sup>+</sup> influx through Na<sup>+</sup> channels.<sup>44</sup> Na<sup>+</sup> influx during phase 2 can be increased several-fold when late  $I_{Na}$  is enhanced by, for example, lysophosphatidylcholine and palmitoyl-L-carnitine (lipid metabolites that accumulate during ischaemia), or by H<sub>2</sub>O<sub>2</sub>, veratridine, or *SCN5A* mutations. Enhancement of late  $I_{Na}$  by five-fold during the AP plateau may double the total Na<sup>+</sup> influx into a myocyte during a cardiac cycle.<sup>44</sup> In this situation, Na<sup>+</sup> influx in phase 2 exceeds that during all other phases of the AP combined.<sup>44</sup>

The effect of an increase of late  $I_{Na}$  to raise the intracellular Na<sup>+</sup> concentration in cardiac myocytes is well documented. The late  $I_{Na}$  enhancer veratridine (0.1 μM) increased the intracellular Na<sup>+</sup> concentration in a sheep Purkinje fibre by 2.2 mM, accompanied by a 140% increase in twitch tension.<sup>45</sup> ATX-II (3 nM) enhanced late  $I_{Na}$  by four-fold in rat ventricular myocytes and increased the intracellular Na<sup>+</sup> concentration by 30%.<sup>46</sup> In rabbit myocytes exposed to ATX-II, a two-fold increase of late  $I_{Na}$  was associated with a four-fold increase of the intracellular Na<sup>+</sup> concentration.<sup>47</sup> The effect of an increase of late  $I_{Na}$  to increase the intracellular Na<sup>+</sup> concentration appears to be greater in rabbit than rat, as the resting Na<sup>+</sup> concentration and Na<sup>+</sup>/K<sup>+</sup> ATPase activity are lower in the rabbit.<sup>48,49</sup> Simulated-demand ischaemia (i.e. metabolic inhibition and pacing) of rabbit cardiac myocytes in the absence and presence of the late  $I_{Na}$  inhibitor ranolazine led to increases of the intracellular Na<sup>+</sup> concentration by 13 and 5 mM, respectively.<sup>50</sup> In myocytes from failing hearts, the intracellular Na<sup>+</sup> concentration is increased by 2–6 mM above normal.<sup>51–55</sup> This increase has been attributed to greater Na<sup>+</sup>

influx due to an enhanced late  $I_{Na}$ .<sup>12,23–26,47,51,56</sup> Increases of Na<sup>+</sup> window and/or background current potentially contribute to Na<sup>+</sup> influx more in the failing than in the normal heart, and are increased by veratridine and ATX-II as well, apparently due to effects of the latter toxins on the voltage dependence of Na<sup>+</sup> channel gating. To our knowledge, the effect of an LQT3 mutation in *SCN5A* on the intracellular Na<sup>+</sup> concentration has yet to be reported.

A late  $I_{Na}$ -induced increase of the intracellular Na<sup>+</sup> concentration alters contractile function. Studies of Purkinje fibres and papillary muscles demonstrate that elevation of the intracellular Na<sup>+</sup> concentration by 1–2 mM may cause twitch tension to increase acutely as much as 2.5-fold.<sup>43,45,55,57–59</sup> An increase of Na<sup>+</sup> concentration (generated by late  $I_{Na}$ ) in the t-tubule subsarcolemmal fuzzy space has been proposed to drive Ca<sup>2+</sup> entry via reverse mode NCX.<sup>56,60,61</sup> The direction of NCX-mediated ion fluxes is regulated by the electrochemical gradients of Na<sup>+</sup> and Ca<sup>2+</sup> and by the membrane potential. When intracellular Na<sup>+</sup> rises, forward mode (3 Na<sup>+</sup> in and 1 Ca<sup>2+</sup> out) NCX is reduced, whereas reverse mode (3 Na<sup>+</sup> out and 1 Ca<sup>2+</sup> in) NCX is increased.<sup>51,62</sup> Cohen *et al.*<sup>57</sup> calculated that an increase of the intracellular Na<sup>+</sup> concentration from 8 to 10 mM reduces the electrochemical driving force for NCX-mediated Ca<sup>2+</sup> efflux by half. An increase of late  $I_{Na}$  is associated with an increased diastolic Ca<sup>2+</sup> concentration in myocytes<sup>46,47,63</sup> and isolated hearts.<sup>64</sup> An increase of the intracellular Na<sup>+</sup> concentration in the hypertrophied/failing heart supports systolic function at low heart rates by increasing Ca<sup>2+</sup> influx via NCX.<sup>51</sup> However, it is associated with increases of diastolic Ca<sup>2+</sup>, diastolic contractile tension, and arrhythmias at higher heart rates.<sup>47,53–56,65</sup> Increases of late  $I_{Na}$  and intracellular Na<sup>+</sup> have been shown to raise tonic contractile force and myocardial wall stress in the intact heart.<sup>66–68</sup> The effects of an increase of late  $I_{Na}$  on AP duration and ion homeostasis in the guinea-pig ventricular myocyte have been modelled.<sup>69</sup> An increase of late  $I_{Na}$  from 0 to 0.2% of peak  $I_{Na}$  at a pacing rate of 1 Hz increased AP duration by nearly 2.2-fold and Na<sup>+</sup> and Ca<sup>2+</sup> concentrations in diastole by 34 and 52%, respectively, and was associated with spontaneous erratic releases of Ca<sup>2+</sup> from the sarcoplasmic reticulum.<sup>69</sup> An increase of late  $I_{Na}$  in myocytes isolated from failing human and dog hearts is also associated with spontaneous releases of sarcoplasmic reticular Ca<sup>2+</sup> during diastole.<sup>56</sup> Drug-induced inhibition of late  $I_{Na}$  has been shown to reduce Na<sup>+</sup>-dependent Ca<sup>2+</sup> loading and contractile dysfunction of cardiac myocytes from both normal and failing hearts,<sup>39,47,56,63–65</sup> and contractile dysfunction in the ischaemic heart.<sup>70,71</sup> One may conclude that an enhanced late  $I_{Na}$  can cause changes of Na<sup>+</sup> entry and the transmembrane Na<sup>+</sup> gradient that alter cardiac function.

The effects of inhibiting the normal, small, endogenous late  $I_{Na}$  have not been unequivocally demonstrated due to the lack of a selective blocker of the current. Lidocaine (20 μM) acutely reduced AP duration, twitch tension, and intracellular Na<sup>+</sup> concentration in sheep Purkinje fibres.<sup>43</sup> The reduction of twitch tension by lidocaine was due in roughly equal parts to the decrease in AP duration and the reduction of intracellular Na<sup>+</sup>.<sup>43</sup> Lidocaine reduces both peak and late  $I_{Na}$ , and reductions by the drug of contractile force and intracellular Na<sup>+</sup> concentration may be due to both actions. Because Na<sup>+</sup> channels become more inactivated as heart rate increases, a decrease in late  $I_{Na}$  at higher rates contributes to rate-dependent shortening of AP duration.<sup>72,73</sup> Results of a recent study of the selective late  $I_{Na}$  inhibitor GS967 indicate that reduction of endogenous late  $I_{Na}$  in rabbit hearts and isolated ventricular myocytes is associated with a decrease of AP duration, a small but non-significant decrease in intracellular Na<sup>+</sup>, and no change in Ca<sup>2+</sup>.<sup>74</sup>

### 3. Types and mechanisms of late $I_{Na}$ -induced arrhythmic activity

The detrimental electrical effects of an enhanced, pathological late  $I_{Na}$  are depicted in Figure 1, and include the following: (i) diastolic depolarization during phase 4 of the AP that may lead to spontaneous AP firing and abnormal automaticity, especially of myocytes that are relatively depolarized and have low resting  $K^+$  conductance (e.g. low  $I_{K1}$ ); (ii) an increase of AP duration, due to the depolarizing effect of an increased inward  $Na^+$  current during the AP plateau, and which may lead to early after-depolarizations (EADs) and triggered activity, as well as increased spatiotemporal differences of repolarization time, which promote reentrant electrical activity; and (iii) the indirect effects of a late  $I_{Na}$ -induced increase of  $Na^+$  entry to alter  $Ca^{2+}$  homeostasis in myocytes, which may lead to  $Ca^{2+}$  alternans and DADs. Acquired conditions and drugs that enhance late  $I_{Na}$  (Table 1) are associated with atrial tachyarrhythmias,<sup>75–78</sup> ventricular tachyarrhythmias including torsades de pointes (TdP),<sup>3,4,79,80</sup> afterpotentials (EADs, DADs), and triggered activity.<sup>23,26,76,81</sup> Patients with LQT3 are at a high risk for both ventricular arrhythmias and atrial fibrillation.<sup>3,4,15,82,83</sup> All three of the common mechanisms for tachyarrhythmias—abnormal automaticity, afterpotentials, and reentry—can occur as the result of an enhanced late  $I_{Na}$ .

### 4. Diastolic depolarization and abnormal automaticity

Spontaneous diastolic depolarization of a myocyte during phase 4 of the AP occurs normally in pacemaking cells of the central sinoatrial and compact atrioventricular nodes, but is rare in normal intact atrial and ventricular tissues. However, spontaneous diastolic depolarizations are often observed in isolated Purkinje fibres<sup>84</sup> and atrial tissue excised from diseased human<sup>85–87</sup> and animal<sup>88–90</sup> hearts, and are a cause of lethal arrhythmias in the infarcted heart.<sup>91</sup> The cause of diastolic depolarization in these cells—which are not normally involved in pace-making—is unclear. Non-inactivating  $Na^+$  current (window or background  $Na^+$  current) is observed in the threshold region for  $Na^+$  channel activation in Purkinje fibres.<sup>92</sup> This finding is consistent with reports that a slowly inactivating, lidocaine and tetrodotoxin (TTX)-sensitive  $Na^+$  current contributes to diastolic depolarization of cardiac Purkinje fibres.<sup>92–94</sup> In ventricular myocytes, late  $I_{Na}$  was shown to be present at voltages as negative as  $-70$  mV,<sup>95,96</sup> but spontaneous activity of ventricular myocytes in the intact heart appears to be rare in the absence of  $K^+$  channelopathies that decrease the resting potential. Spontaneous diastolic depolarization and the rate of AP firing of atrial myocytes can be increased and decreased by late  $I_{Na}$  enhancers and inhibitors, respectively.<sup>77</sup> Late  $I_{Na}$  was found to be present in atrial myocytes that undergo spontaneous diastolic depolarization, and ATX-II accelerated diastolic depolarization and induced rapid firing of APs in these cells.<sup>77</sup> The reactive oxygen species  $H_2O_2$  increases late  $I_{Na}$  and causes diastolic depolarization and rapid AP firing of isolated atrial myocytes (Figure 2).<sup>77</sup> Atrial myocyte diastolic depolarization and AP firing in the absence and presence of  $H_2O_2$  were reduced when late  $I_{Na}$  was inhibited using ranolazine or TTX.<sup>77</sup> Voltage-clamp studies of atrial myocytes demonstrated that an inward current is activated by a depolarizing ramp pulse and that the ramp-induced current is blocked by TTX and enhanced by ATX-II, consistent with its identification as late  $I_{Na}$ .<sup>77</sup> These findings suggest that late  $I_{Na}$  is a

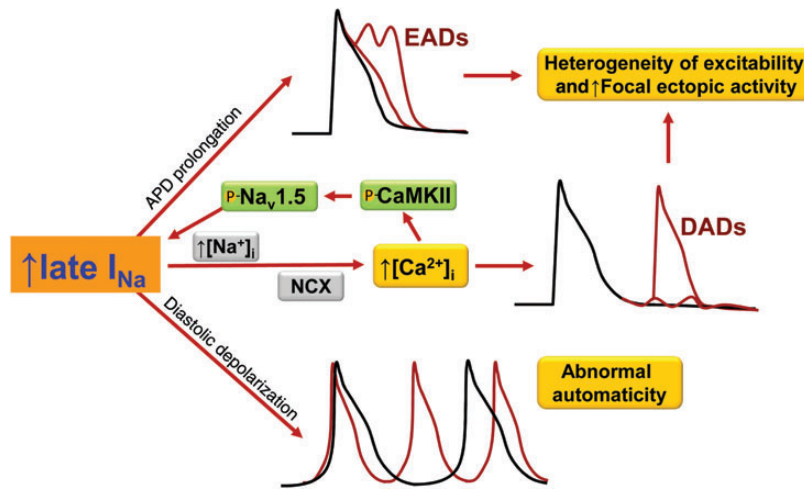
cause of spontaneous diastolic depolarization and abnormal automaticity that may contribute to arrhythmogenesis in atrial myocytes and Purkinje fibres.

### 5. AP prolongation and EADs

During the AP plateau, membrane resistance is high (i.e. ionic conductance is low). A modest increase of an inward current such as late  $I_{Na}$ , or reduction of an outward current such as  $I_{Kr}$ , can cause marked AP prolongation.<sup>81</sup> Late  $I_{Na}$  is documented to prolong the duration of the AP.<sup>21,81,97–99</sup> Tetrodotoxin and lidocaine inhibit late  $I_{Na}$  and reduce the duration of the AP in Purkinje fibres and ventricular myocytes.<sup>2,23,43,92,97,99</sup> These findings are consistent with the interpretation that late  $I_{Na}$  during the AP plateau reduces net repolarizing current (i.e. repolarization reserve<sup>100</sup>).

EAD are a primary mechanism of arrhythmic activity and there is considerable evidence that their occurrence is facilitated when late  $I_{Na}$  is enhanced and the AP is prolonged. AP prolongation provides time for L-type  $Ca^{2+}$  channels to recover from inactivation and re-activate.<sup>101–104</sup> The resulting  $Ca^{2+}$  'window' current may increase progressively over a range of voltages from  $-30$  to  $0$  mV to form the upstroke of an EAD.<sup>104,105</sup> Calcium influx leads to increases of subsarcolemmal  $Ca^{2+}$ ,  $Ca^{2+}$ -induced  $Ca^{2+}$  release, and  $Ca^{2+}$ /calmodulin-dependent protein kinase II (CaMKII) activity.<sup>104</sup> An increase of the subsarcolemmal  $Ca^{2+}$  concentration drives forward mode NCX. Forward mode NCX generates inward, depolarizing current that further contributes to AP prolongation.<sup>106–108</sup> Both forward mode NCX and late  $I_{Na}$  (which is increased by CaMKII-mediated  $Na^+$  channel phosphorylation) contribute inward current that may be sufficient to overcome outward repolarizing  $K^+$  currents and enable an EAD. Single and burst openings of  $Na^+$  channels are reported to occur at take-off voltages for EADs.<sup>24</sup> Indeed, modelling of Purkinje fibre electrophysiology indicates that late  $I_{Na}$  is the major inward current responsible for generation of the EAD in that cell.<sup>109</sup> Consistent with this hypothesis, the late  $I_{Na}$  enhancer anthopleurin-A increases the dispersion of repolarization and induces spontaneous tachyarrhythmias that are triggered by subendocardial Purkinje tissue in the dog heart.<sup>110</sup> The increase of late  $I_{Na}$  that is observed in myocytes isolated from failing human and dog hearts is associated with a prolonged AP duration, increased beat-to-beat variability of AP duration, and EADs.<sup>23,26</sup> Enhancers of late  $I_{Na}$  such as ATX-II (Figure 3) and  $H_2O_2$  cause EADs and TdP.<sup>31,65,79,81,111</sup> EADs are common in mice expressing the LQT3 mutant 'gain-of-function'  $Na^+$  channel  $\Delta KPQ$ .<sup>112</sup> In contrast, inhibitors of late  $I_{Na}$  reduce occurrences of EADs and TdP in the presence of  $H_2O_2$ <sup>31,63,113</sup> and  $I_{Kr}$  blockers.<sup>80,114,115</sup> Reentrant and multifocal ventricular fibrillation in aged rat isolated hearts can be induced by rapid pacing or treatment with  $H_2O_2$ ; the late  $I_{Na}$  inhibitor ranolazine suppressed EADs and the number of foci, and terminated ventricular fibrillation in these hearts.<sup>113</sup> Inhibition of late  $I_{Na}$  was recently reported to markedly shorten AP duration and halve the occurrence of EADs in myocytes isolated from patients with hypertrophic cardiomyopathy.<sup>116</sup>

The amplitude of late  $I_{Na}$  and its contribution to EAD formation depend on heart rate. Late  $I_{Na}$  is greater at slow than at fast heart rates<sup>56,72,117</sup> because an increased rate of  $Na^+$  channel opening increases channel inactivation and reduces late  $I_{Na}$ .<sup>56,72</sup> Reduction of late  $I_{Na}$  with increased rate contributes to the normal reverse-rate dependence of AP duration in Purkinje fibres,<sup>109</sup> rabbit hearts,<sup>72</sup> and myocytes isolated from failing human hearts.<sup>56</sup> Slow pacing rates facilitate long APs and increase late  $I_{Na}$  and occurrences of EADs and



**Figure 1** Mechanisms of late  $I_{Na}$ -induced arrhythmia: EADs, DADs, and spontaneous diastolic depolarization. Not shown, late  $I_{Na}$  increases spatio-temporal dispersion of repolarization and facilitates reentrant arrhythmic activity. NCX,  $Na^+/Ca^{2+}$  exchange; CaMKII,  $Ca^{2+}$ /calmodulin-dependent protein kinase II.

TdP.<sup>37,72,103</sup> The role of late  $I_{Na}$  to increase EAD formation and dispersion of repolarization is increased by heart rate slowing, and the pro-arrhythmic risk associated with an increased late  $I_{Na}$  is high when heart rate is low.<sup>37,118,119</sup>

A small increase of late  $I_{Na}$  that does not cause arrhythmic activity in the normal heart may do so in the heart with reduced repolarization reserve. Low concentrations of the late  $I_{Na}$  enhancer ATX-II increased the duration of the monophasic AP, but did not cause arrhythmias in rabbit isolated hearts.<sup>79,120</sup> However, late  $I_{Na}$  facilitated the induction of EADs by blockers of the rapid ( $I_{Kr}$ ) or slowly ( $I_{Ks}$ ) activating components of the delayed rectifier  $K^+$  current (Figure 4). When low concentrations of E-4031, amiodarone, cisapride, quinidine, moxifloxacin, or ziprasidone alone caused little or no arrhythmic activity in the isolated rabbit heart, combinations of these  $I_{Kr}$  blockers with ATX-II greatly increased AP duration and caused ventricular tachyarrhythmias.<sup>79,120–122</sup> Similarly, in guinea pig isolated ventricular myocytes, low concentrations of ATX-II, the  $I_{Kr}$  blocker E-4031, and the  $I_{Ks}$  blocker chromanol 293B individually caused small increases of AP duration.<sup>81</sup> However, combinations of ATX-II with either E-4031 or chromanol 293B markedly prolonged AP duration and induced EADs (Figure 4).<sup>81</sup> In patients, drugs that block  $I_K$  prolong the QT interval and may induce EADs.<sup>123</sup> However, not all patients exposed to these drugs develop arrhythmias. Genetic analysis revealed that susceptibility to drug-induced long QT syndromes is linked to *SCN5A* mutations (e.g. L1825P or Y1102) that enhance late  $I_{Na}$ .<sup>124,125</sup> Patients with 'silent'  $Na^+$  channel gene mutations had normal QT intervals, but developed long QT syndrome and TdP when given an  $I_{Kr}$  blocker such as cisapride or amiodarone.<sup>124,125</sup> An enhanced late  $I_{Na}$  is therefore a risk factor predisposing to EADs under both acquired (disease and drug-induced) and inherited (LQT1 and LQT2) pathological conditions. An ideal substrate for generation of EADs and TdP in the failing and/or hypertrophic heart is present when late  $I_{Na}$  is enhanced,<sup>26,56</sup> the inward-rectifier  $K^+$  current,  $I_{K1}$  is reduced,<sup>126,127</sup> NCX, diastolic  $Ca^{2+}$ , and sarcoplasmic reticular  $Ca^{2+}$  sparks are increased,<sup>128–131</sup> repolarizing  $K^+$  currents are reduced,<sup>123</sup> and spatial and temporal lability of repolarization is prominent.<sup>128,132</sup> Inhibition of late  $I_{Na}$  reduces EADs in ventricular myocytes isolated

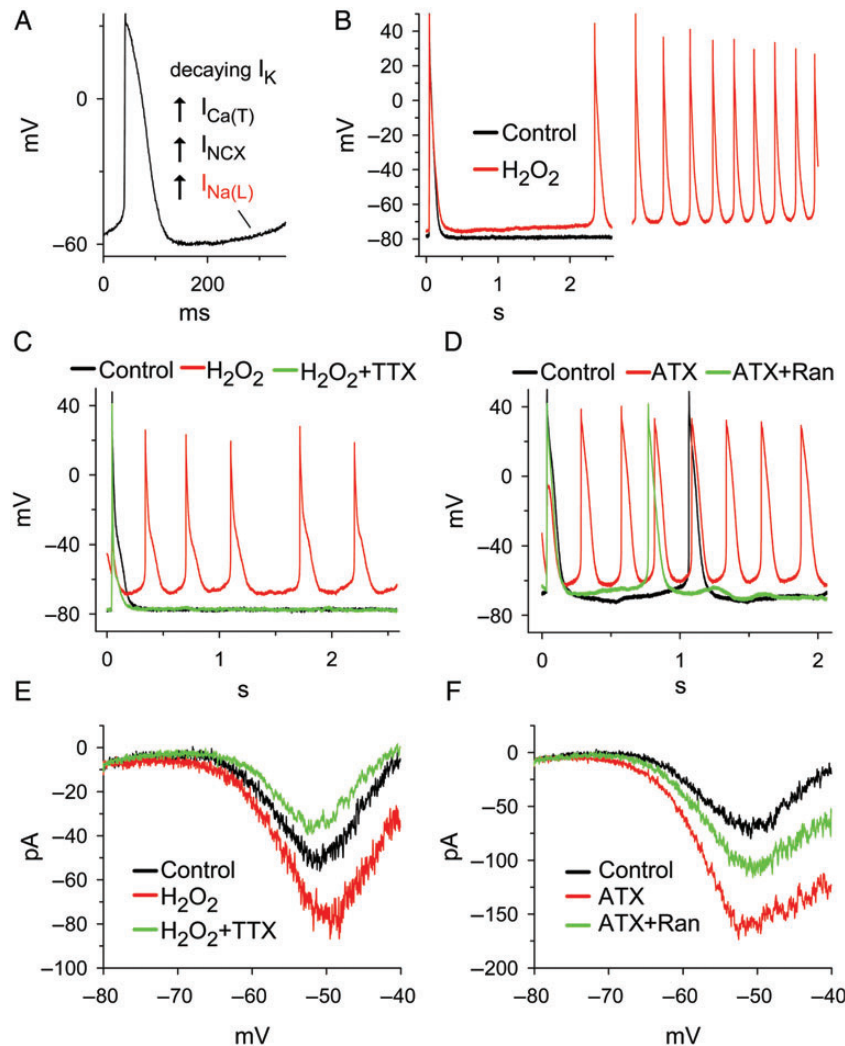
from failing and hypertrophic hearts<sup>22,26,116</sup> and in left atrial myocytes from hearts of rabbits with left-ventricular hypertrophy.<sup>78</sup> Interestingly, stem cell-derived cardiomyocytes generated from an LQT3 mouse model carrying the human  $\Delta$ KPQ  $Na_v1.5$  mutation recapitulate the typical pathophysiological  $\Delta$ KPQ phenotype, including APD prolongation and EAD development.<sup>133</sup>

The contribution of late  $I_{Na}$  to EAD formation in phase 3 of the ventricular AP is unclear. In phase 3, L-type  $Ca^{2+}$  channel activation and  $Ca^{2+}$  window current are negligible.<sup>62</sup> The more negative membrane potential during phase 3 relative to phase 2 favours  $Na^+$  influx. Therefore, increases of both late  $I_{Na}$  and inward  $Na^+/Ca^{2+}$  exchange current<sup>134–136</sup> may contribute to the upstroke of EADs during phase 3. Rapid recovery from inactivation and reactivation of  $Na^+$  channels is a potential cause of phase 3 EADs and triggered activity.<sup>38,137</sup> However, because repolarizing  $K^+$  currents during phase 3 are normally robust unless the extracellular  $[K^+]_o$  is reduced and  $I_{K1}$  is inhibited, it would appear that depolarizing currents must be large to elicit an EAD at this time. Depolarizing current flowing electrotonically from myocytes with long APs to those with shorter APs may contribute to initiation of phase 3 EADs in the intact heart.<sup>138</sup> Exacerbation of the large repolarization gradients that favour current flow between Purkinje fibres and M cells, on the one hand, and adjacent cells with shorter AP durations, on the other hand, would favour EAD formation<sup>110,139–141</sup> and reentrant arrhythmias<sup>141</sup> by this 'extrinsic' electrotonic mechanism.<sup>105</sup> Late  $I_{Na}$  is inherently greater in Purkinje fibres and M cells<sup>139,142</sup> than in other cells in the heart and contributes to AP prolongation and EAD formation in these cells. Enhancement of late  $I_{Na}$  enables reentrant AP propagation from these endocardial cells with long APs to repolarized myocardium.<sup>137</sup>

## 6. Intracellular $Na^+$ and $Ca^{2+}$ loading and DADs

Transient depolarizations of the cell membrane that follow repolarization of a previous AP are referred to as delayed after-depolarizations.



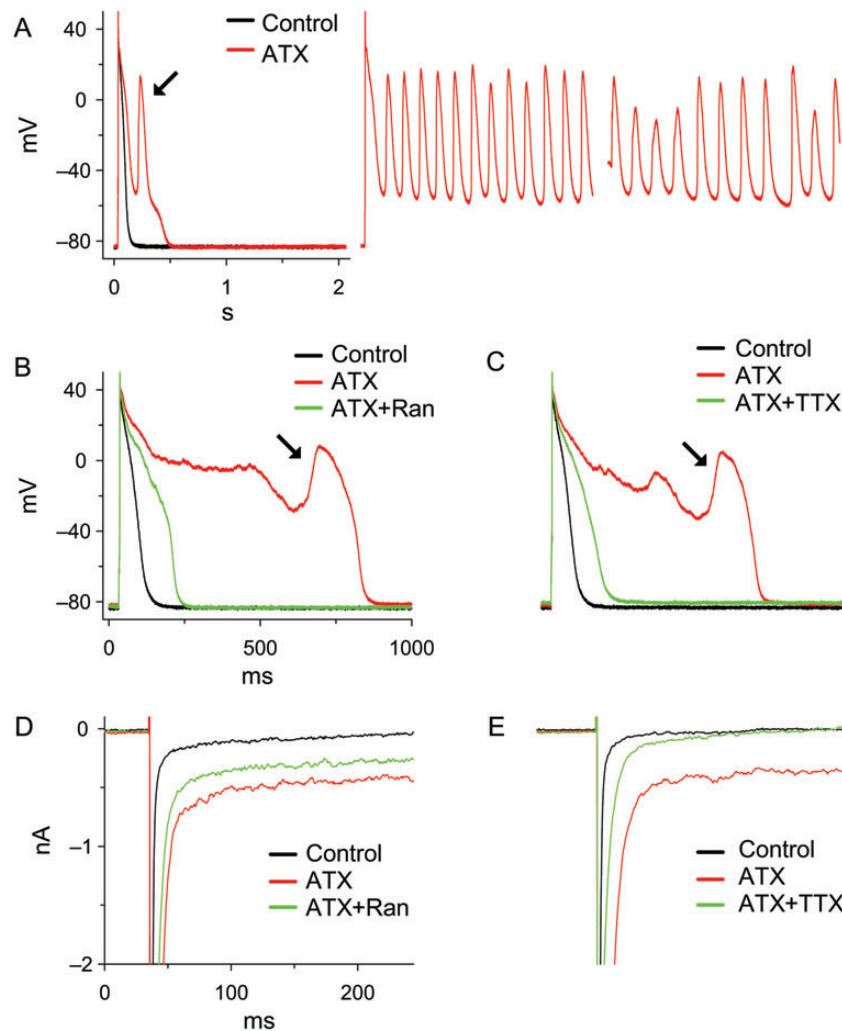


**Figure 2** Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ,  $50 \mu\text{M}$ ) and anemone toxin-II (ATX,  $5 \text{ nM}$ ) increase late  $I_{\text{Na}}$  and induce diastolic depolarization in guinea pig atrial myocytes. (A) Four proposed mechanisms for diastolic depolarization: decay of the delayed rectifier current,  $I_{\text{K}}$ ; an increase of T-type  $\text{Ca}^{2+}$  current,  $I_{\text{Ca(T)}}$ ; an increase of forward mode  $\text{Na}^+/\text{Ca}^{2+}$  exchange current,  $I_{\text{NCX}}$ ; and an increase of late  $I_{\text{Na}}$ . (B) Induction by  $\text{H}_2\text{O}_2$  of diastolic depolarization and rapid spontaneous firing in a quiescent atrial myocyte. (C)  $\text{H}_2\text{O}_2$ -induced spontaneous firing was terminated by tetrodotoxin (TTX,  $1 \mu\text{M}$ ). (D) Spontaneous action potential firing of an atrial myocyte was accelerated by ATX. The effect of ATX was attenuated by ranolazine (Ran,  $10 \mu\text{M}$ ). (E) Inward late  $I_{\text{Na}}$  in an atrial myocyte was activated by simulating diastolic depolarization using a ramp voltage clamp.  $\text{H}_2\text{O}_2$  increased whereas TTX decreased the amplitude of late  $I_{\text{Na}}$ . (F) ATX enhanced whereas Ran inhibited inward late  $I_{\text{Na}}$  activated by ramp voltage clamp pulses. pA, picoamperes; mV, millivolts.

DADs of Purkinje fibres have been recognized for >40 years as a mechanism of digitalis glycoside-induced arrhythmogenesis and non-reentrant triggered activity.<sup>143,144</sup> A transient inward current,  $I_{\text{Ti}}$ , was found to be responsible for the DAD,<sup>144–146</sup> and inward, forward mode NCX (i.e. entry of  $3\text{Na}^+$  with exit of  $1 \text{Ca}^{2+}$ ) was identified as the source of this current.<sup>145–148</sup>  $I_{\text{Ti}}$  and/or DADs have been observed in Purkinje,<sup>144</sup> ventricular,<sup>145–147</sup> atrial,<sup>76,149</sup> pulmonary vein sleeve,<sup>150,151</sup> superior vena cava,<sup>152</sup> and sinoatrial node<sup>153</sup> tissues. DADs are observed under conditions in which myocytes are relatively overloaded with  $\text{Ca}^{2+}$ , causing  $\text{Ca}^{2+}$  to be released from multiple sarcoplasmic reticulum sites into the cytoplasm during diastole;<sup>154</sup> this increase of cytoplasmic  $\text{Ca}^{2+}$  leads to aftercontractions and forward mode NCX that generates transient inward current and a DAD.<sup>101,144,147,148,155–157</sup> Events that promote a combination of an increase of the intracellular  $\text{Na}^+$  concentration, increased  $\text{Ca}^{2+}$  influx

(e.g. rapid pacing, catecholamines, block of  $I_{\text{Ks}}$ ), decreased  $\text{Ca}^{2+}$  efflux, opening of sarcoplasmic reticulum  $\text{Ca}^{2+}$  channels (i.e. ryanodine receptors), and reduced outward  $\text{K}^+$  current (e.g.  $I_{\text{K1}}$ ) during diastole act to facilitate DADs.

The role of late  $I_{\text{Na}}$  in DAD generation is not as a source of inward current, as that is provided by forward mode NCX, but rather to 'set the stage' by increasing cellular  $\text{Ca}^{2+}$  loading via reverse mode NCX (Figure 1). An increase of late  $I_{\text{Na}}$  can increase the intracellular, subsarcolemmal  $\text{Na}^+$  concentration, thereby increasing  $\text{Ca}^{2+}$  entry via reverse-mode NCX ( $3 \text{Na}^+$  out,  $1 \text{Ca}^{2+}$  in) during the AP plateau.<sup>45,47,48,56,158</sup> The contribution of late  $I_{\text{Na}}$  to  $\text{Na}^+$  and  $\text{Ca}^{2+}$  loading has been referred to as an intrinsic digitalis-like effect.<sup>12,26,159</sup> Like digitalis, late  $I_{\text{Na}}$ -mediated  $\text{Na}^+$  loading (i) may increase  $\text{Ca}^{2+}$  entry into the cell, and  $\text{Ca}^{2+}$  uptake by sarcoplasmic reticulum, (ii) increase diastolic  $\text{Ca}^{2+}$  and reduce the rate and extent of diastolic relaxation, and (iii) give rise to



**Figure 3** Anemone toxin-II (ATX, 10 nM) induced EADs and enhanced late  $I_{Na}$  in guinea pig atrial myocytes; these effects were attenuated by either ranolazine (Ran) or tetrodotoxin (TTX). (A) ATX induced EADs and sustained triggered activity. (B and C) ATX-induced EADs were suppressed by either 10  $\mu$ M Ran or 2  $\mu$ M TTX. (D and E) ATX increased the late  $I_{Na}$  activated by square voltage clamps from  $-90$  to  $-20$  mV in a ventricular myocyte. The effect of ATX was attenuated by either 10  $\mu$ M Ran or 10  $\mu$ M TTX. nA, nanoamperes; mV, millivolts.

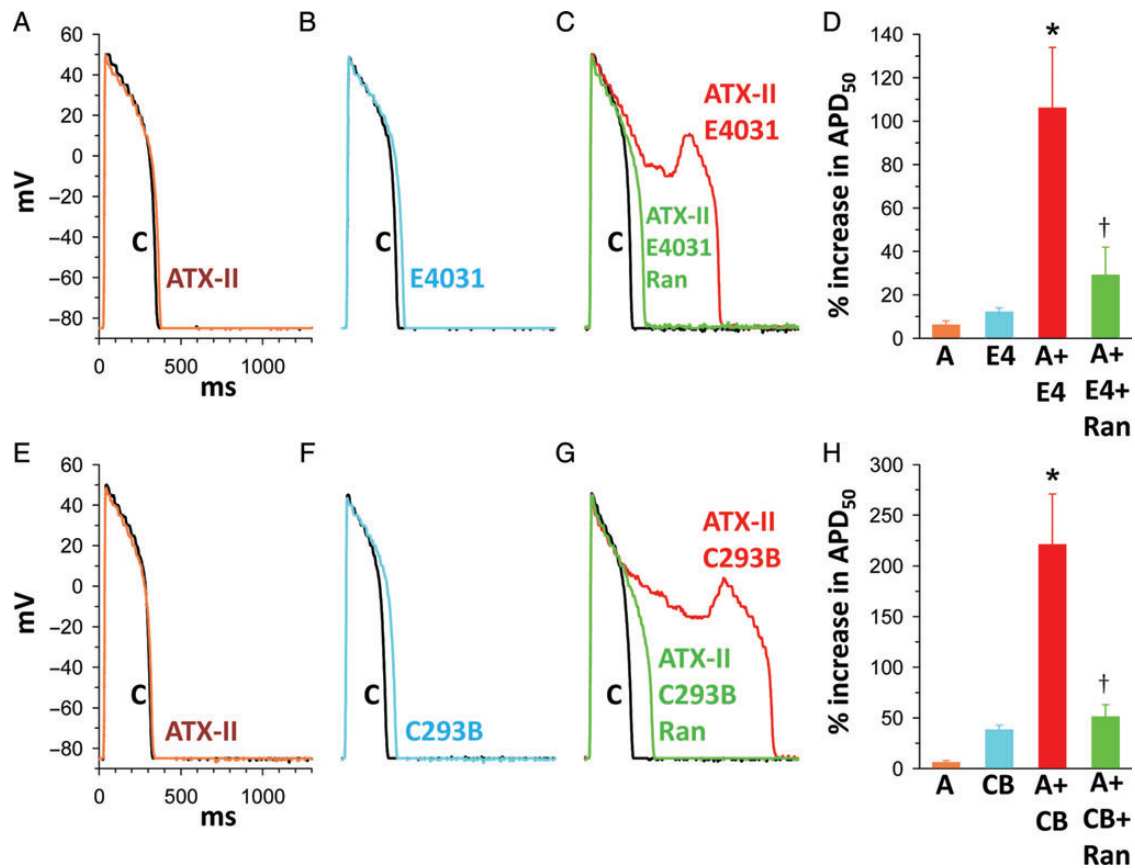
$Ca^{2+}$  release from the sarcoplasmic reticulum during diastole, and DAD formation (Figure 1).<sup>12,47,56,63,65</sup> An increase of late  $I_{Na}$  prolonged the  $Ca^{2+}$  transient and induced spontaneous  $Ca^{2+}$  waves during rapid pacing of rat isolated hearts.<sup>160</sup> Exposure of myocytes to late  $I_{Na}$  enhancers provokes DADs.<sup>27,63,76,161,162</sup> The transient inward current  $I_{TI}$  and both DADs and DAD-dependent triggered activity can be induced by ATX-II in guinea pig atrial myocytes.<sup>76</sup> DADs induced by cardiac glycosides or other interventions are suppressed by inhibitors of  $Na^+$  channels and late  $I_{Na}$ , including TTX, lidocaine, mexiletine, R56865, and ranolazine.<sup>63,65,76,162–164</sup> Inhibition of late  $I_{Na}$  has also been shown to decrease the incidence of DADs in studies of pulmonary vein and superior vena cava sleeves,<sup>152</sup> and in myocytes from hearts of patients with hypertrophic cardiomyopathy.<sup>116</sup> These findings implicate increased  $Na^+$  entry into myocytes via  $Na^+$  channel late  $I_{Na}$  as a cause of DADs. Inhibition of late  $I_{Na}$  is a means of reducing occurrences of DADs.

A positive feedback loop between the amplitude of late  $I_{Na}$  and the activity of CaMKII appears to contribute to DAD formation and arrhythmogenesis. An increase of late  $I_{Na}$  can lead to myocyte  $Ca^{2+}$  loading and

activation of CaMKII.<sup>46</sup> CaMKII phosphorylates sodium channel sites in the intracellular linker between domains 1 and 2, and this increases late  $I_{Na}$ .<sup>165–169</sup> CaMKII also phosphorylates cardiomyocyte ryanodine receptor II (RyR2), which increases RyR2 sensitivity to SR  $Ca^{2+}$ -induced opening.<sup>170</sup> This facilitates more frequent and larger releases of  $Ca^{2+}$  from the SR (i.e. sparks) during diastole,<sup>170</sup> and leads to  $Ca^{2+}$  waves and DAD generation.<sup>171</sup> Thus increases of late  $I_{Na}$  and activity of CaMKII increase SR  $Ca^{2+}$  loading and SR  $Ca^{2+}$  release, respectively; together they create a substrate for DAD-induced arrhythmias.

## 7. Late $I_{Na}$ , dispersion of repolarization, and reentry

The mechanism of reentrant arrhythmia involves unidirectional block and conduction around a circuit long enough to enable recovery of excitability at each point in the circuit before the wave of excitation returns.<sup>172</sup> Acquired or congenital conditions that exacerbate normal



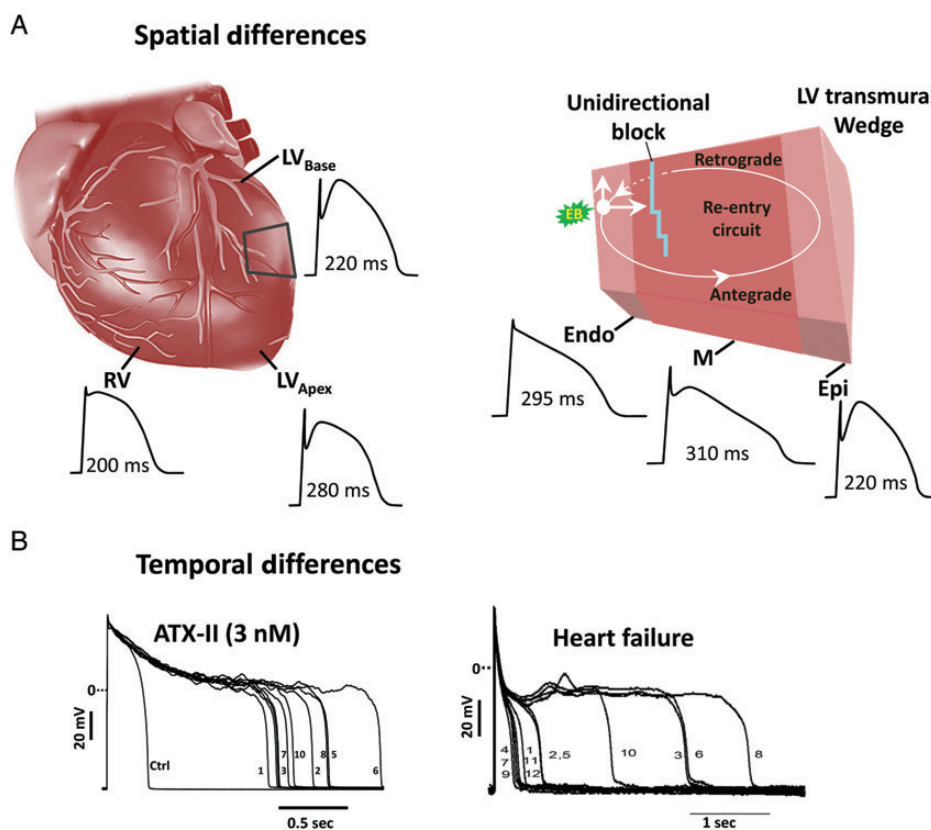
**Figure 4** Synergistic effects of the late  $I_{Na}$  enhancer ATX-II (3 nM) with either the  $I_{Kr}$  blocker E4031 (1  $\mu$ M) or the  $I_{Ks}$  blocker chromanol 293B (30  $\mu$ M) to prolong the action potential duration (APD) and induce early afterdepolarizations. The combined effects of ATX-II and E4031 or 293B were attenuated by ranolazine (10  $\mu$ M) (C and G). Data summarized in (D) and (H). Each bar represents the value (mean  $\pm$  SEM) of a percentage increase of APD<sub>50</sub>. \* $P$  < 0.05 vs. ATX-II, E4031, or 293B alone; † $P$  < 0.05 vs. ATX-II plus E4031 or ATX-II plus 293B. The duration of drug treatment was 3 min. C, control (absence of drug); A, ATX-II; E4, E-4031; C293B or CB, chromanol 293B; Ran, ranolazine.

regional differences in duration of the AP and the time sequence of repolarization in the heart create a substrate for reentry.<sup>137,173,174</sup> Electrical and/or structural heterogeneity of repolarization, excitability, and conduction among adjacent regions of myocardium are associated with EADs, TdP, and reentrant arrhythmic events.<sup>174–178</sup> Results of computational modelling studies indicate that increased spatial and temporal dispersion of AP duration and repolarization time act to increase susceptibility to reentrant arrhythmic activity.<sup>179,180</sup>

Late  $I_{Na}$  contributes to the regional differences of AP duration and repolarization in myocardium. Differences in the density of late  $I_{Na}$  in various cell types (i.e. Purkinje fibre, M cell > endo > epicardium)<sup>2,7,142</sup> contribute to transmural differences in AP duration (Figure 5). An increase of late  $I_{Na}$  can prolong APD more in some cells than others, thereby increasing dispersion of repolarization and providing a potential substrate for reentry. An ATX-II induced increase of late  $I_{Na}$  increases AP duration more in M and Purkinje cells than in epicardium, and increases transmural dispersion of repolarization time; these effects are attenuated by inhibition of late  $I_{Na}$  with mexiletine or tetrodotoxin.<sup>7,80,181,182</sup> Augmentation of late  $I_{Na}$  with ATX-II in canine left-ventricular wedge preparations leads to reentrant arrhythmias.<sup>80,182</sup> The late  $I_{Na}$  enhancers anthopleurin-A and veratridine also cause EADs and reentrant arrhythmias in intact isolated guinea pig and rabbit hearts, respectively.<sup>183,184</sup> Reduction of late  $I_{Na}$  decreased transmural dispersion of repolarization

and suppressed TdP in canine and rabbit experimental models of LQT1, LQT2, and LQT3 syndromes.<sup>16,79,80,115,185</sup>

An increase of late  $I_{Na}$  also increases the beat-to-beat (temporal) variability of AP duration and repolarization (Figure 5) in isolated myocytes<sup>23,81</sup> and intact hearts.<sup>115</sup> Late  $I_{Na}$  and repolarization variability are especially enhanced in myocytes isolated from failing hearts, and reduction of late  $I_{Na}$  reduces the beat-to-beat variability of AP duration in these cells.<sup>26,65</sup> Reduction of late  $I_{Na}$  by ranolazine decreased the beat-to-beat variability of repolarization caused by treatment of rabbit isolated hearts with the  $I_{Kr}$  blocker E-4031,<sup>115</sup> and the rate dependence of pacing-induced alternans of the beat-to-beat  $Ca^{2+}$  transient amplitude in rat isolated hearts treated with ATX-II.<sup>160</sup> The magnitude of T-wave alternans, which is believed to reflect the spatio-temporal heterogeneity of ventricular repolarization, was suppressed by ranolazine in intact pigs subjected to acute coronary stenosis.<sup>186</sup> During ventricular fibrillation, dynamic beat-to-beat heterogeneity of AP duration (i.e. alternans) is a major contributor to wave break, a process in which new waves of reentrant excitation are continually formed, thus sustaining fibrillation.<sup>187</sup> The effect of ranolazine to reduce beat-to-beat variability of AP duration may reduce wave break and reentrant activity,<sup>113</sup> and explain the many clinical and experimental findings that the drug reduces the incidence and duration of arrhythmias.<sup>7,9,13,14,16</sup>



**Figure 5** Spatial (A) and temporal (B) differences in the duration of the action potential (APD) create a substrate for reentrant arrhythmias. Reprinted from Belardinelli et al.<sup>140</sup> and Undrovinas et al.<sup>65</sup>

## 8. Models of late $I_{Na}$ -induced arrhythmogenesis

Experimental animal models for study of the arrhythmogenic effects of enhancing late  $I_{Na}$  include rabbit,<sup>120–122,184</sup> guinea pig,<sup>79,81</sup> and rodent isolated hearts,<sup>64,71,160</sup> intact pigs,<sup>186</sup> and dog isolated left-ventricular wedge preparations.<sup>80</sup> Late  $I_{Na}$  is enhanced in myocytes of dogs and humans with heart failure,<sup>23–26</sup> by toxins such as ATX-II,<sup>81,111</sup> veratridine,<sup>71</sup> and aconitine,<sup>159,161</sup> by ischaemia<sup>8,27–29,70</sup> and oxidative stress,<sup>31,50,63</sup> and by activation of various kinases including CaMKII<sup>165–169</sup> (Table 1). Among the inherited gain-of-function  $Na_v1.5$  channelopathies that are causes of LQT3, the best-studied is  $\Delta$ KPQ, a deletion of 3 amino acids in the putative inactivation gate of the  $Na^+$  channel.<sup>3,4,118</sup> Mice expressing heterozygous knock-in  $\Delta$ KPQ  $Na^+$  channels experience cardiac arrhythmias including TdP and have been used to investigate underlying mechanisms of late  $I_{Na}$ -associated arrhythmogenesis, including EADs, pause-induced DADs, AP prolongation with increased dispersion, and APD alternans.<sup>112,119,188–190</sup> Major limitations of rodent models of LQT3 as guides to an understanding of the consequences of increasing/decreasing late  $I_{Na}$  in human myocardium include the higher intracellular  $Na^+$  concentration in the rodent cardiomyocyte and therefore a reduced role of  $Na^+$  entry in the regulation of  $Ca^{2+}$  handling,<sup>48,49</sup> and the difficulty of assessing drug effects and channel function in hearts whose APs are so different in shape and duration as those in mouse and man. Both  $Na^+$  channel function and drugs actions are heart rate- and voltage-dependent, and  $Na^+$  channel drugs have non-specific

actions on other ion currents whose amplitude and roles differ in human and rodent hearts.

## 9. Conclusion and perspectives

Late  $I_{Na}$  is a small inward current that reduces repolarization reserve and prolongs the duration of the AP in cardiac myocytes. Physiological roles for late  $I_{Na}$ -mediated  $Na^+$  loading to contribute to the normal inotropic state and for late  $I_{Na}$ -induced AP prolongation to increase the effective refractory period and decrease reentry are theoretically possible but have not been adequately investigated. The amplitude of late  $I_{Na}$  is increased in many pathological conditions, where it contributes to atrial and ventricular arrhythmogenesis. An increase of late  $I_{Na}$  due to acquired or inherited  $Na^+$  channelopathies abnormally prolongs repolarization and increases the influx of  $Na^+$ , and via NCX,  $Ca^{2+}$  into the cell. Late  $I_{Na}$  and NCX-mediated  $Ca^{2+}$  loading increase diastolic force production. AP prolongation and  $Na^+/Ca^{2+}$  loading cause CaMKII activation and electrical instability. Enhancement of late  $I_{Na}$  may lead to automaticity, early and delayed afterdepolarizations, and  $Ca^{2+}$  and AP alternans that facilitate arrhythmias by triggered and reentrant mechanisms. Drugs that reduce late  $I_{Na}$  have been shown to reduce EADs, DADs,  $Ca^{2+}$  handling defects, and arrhythmias. Interactions between late  $I_{Na}$ , CaMKII, RyR2, and oxidative stress have been demonstrated, and their potential pathological roles in ischaemic heart disease, heart failure, and arrhythmias are subjects of current and future investigation.



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