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Changes in action potentials and intracellular ionic homeostasis in a ventricular cell model related to a persistent sodium current in SCN5A mutations underlying LQT3.

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Abstract

In LQT3 patients, SCN5A mutations induce ultraslow inactivation of a small fraction of the hNav1.5 current i.e. persistent Na⁺ current (I_{pNa}). We explored the time course of effects of such a change on the intracellular ionic homeostasis in a model of guinea-pig cardiac ventricular cell (Pasek et al., 2007b, this issue). Sudden addition of I_{pNa} prevented action potential (AP) repolarization when its conductance (g_{pNa}) exceeded 0.12% of the maximal conductance of fast I_{Na} (g_{Na}). With g_{pNa} at 0.1% g_{Na}, the AP duration at 90% repolarization (APD_{90}) was initially lengthened to 2.6 fold that in control. Under regular stimulation at 1 Hz it shortened progressively to 1.37 fold control APD_{90}, and intracellular [Na⁺], increased by 6% with a time constant of 106 sec. Further increasing g_{pNa} to 0.2% g_{Na} caused an immediate increase in APD_{90} to 5.7 fold that in control, which decreased to 2.2 fold that in control in 30 sec stimulation at 1 Hz. At this time diastolic [Na⁺]; and [Ca^{2+}]i were respectively 34% and 52% higher than in control and spontaneous erratic SR Ca release occurred.

In the presence of I_{pNa} causing 46% lengthening of APD_{90}, the model cell displayed arrhythmogenic behaviour when external [K⁺] was lowered to 5 mM from an initial value at 5.4 mM. By contrast, when K⁺ currents I_{Kr} and I_{Ks} were lowered in the model cell to produce the same lengthening of APD_{90}, no proarrhythmic behaviour was observed, even when external [K⁺] was lowered to 2.5 mM.

Keywords: Sudden death – mutation – SCN5A – long QT syndrome – model
Introduction

Mechanisms by which mutation-induced alterations of the hNav1.5 current predispose LQT3 patients to arrhythmias have been explored from an electrical point of view (Bennett et al., 1995; Clancy et al., 1999; Clancy et al., 2003; Tian et al., 2004; Henry et al., 2004; Berecki et al., 2006). However, only few works have addressed possible changes in ionic homeostasis related to increased persistent or late Na\(^+\) current (Priori et al., 1996; Bito et al., 2006; Noble and Noble, 2006). In particular, the presence of a persistent Na\(^+\) current (\(I_{pNa}\)) during the action potential might cause elevation of intracellular [Na\(^+\)], a condition that predisposes to calcium overload and arrhythmogenesis (Levi et al., 1997; Noble and Noble, 2006; see also Maltsev and Undrovinas, 2007 in this volume). We explored whether this effect would be quantitatively important in a computer model of cardiac ventricular myocyte. We also compared the immediate and delayed effects of introducing a persistent Na\(^+\) current. Finally, we compared the alterations of ionic homeostasis in two modifications of the model producing equivalent AP prolongation as in symptomatic LQT (addition of a persistent Na\(^+\) current or decreased K\(^+\) currents) and their response to manoeuvres likely to aggravate sodium overload: tachycardia and hypokalemia. We discussed the relevance of the effects produced by the model to clinical situations. Part of this work was reported in abstract form (Christé et al., 2005; Christé et al., 2006).
Methods

A ventricular cell model that includes ionic currents, intracellular ionic homeostasis and a single
compartment tubular system (Pasek et al., 2003) was modified to closely match the properties of
guinea-pig ventricular cells at 37°C as described in Pasek et al., (2007b), a companion paper in this
issue. This model was written and integrated under the MATLAB environment (The MathWorks). A
persistent Na⁺ current (termed I_pNa in the rest of the text) was formulated according to the description
of Sakmann et al., (2000), see equations A7 to A10 in Appendix 1 to the paper by Pasek et al.,
(2007b) in this issue. The conductance of this current in control conditions was 0.0053 mS/cm².

In the first series of modelling attempts (figures 1, 2 and 3), the model was implemented with a
transient outward (I_o) current (as in Pasek et al. 2003) to approach the ion current conditions of a
human myocyte. The immediate and long term effects of introducing a persistent I_pNa were explored
in these conditions.

In the second series of model challenges (figs 4 and 5), three versions of our model (Pasek et al.,
2007b, this issue) were used. The unmodified version of the model is named the Cont model. In the
first modification (named +I_pNa model) of the model Cont, the maximal conductance of I_pNa (g_pNa)
was set to 0.02 mS/cm² to simulate of a LQT3 condition. In a second modification (named -I_k model)
of model Cont, the maximal conductance for both I_kr and I_kp was reduced by 67%, to simulate a
LQT1/LQT2 condition. Both modified models and the control model were run for at least 600 s cell
life time to ensure that all variables had reached steady-state. For examination of the susceptibility of
each modified model to generate arrhythmias in lowered extracellular [K⁺]e or increased frequency,
each model was run for 300 s at 1 Hz and at 3 Hz, and in each modified [K⁺]e condition at 1 Hz.
Results

The control AP shown in figure 1 was recorded after 630 s cell lifetime of stimulation at 1 Hz. We then tested the addition of different amounts of \( I_{\text{pNa}} \) current. When the conductance of \( I_{\text{pNa}} \) was increased by 0.3 mS/cm\(^2\) (which represents 1% of the maximal fast \( I_{\text{Na}} \) conductance that is 30 mS/cm\(^2\), and is within the range of fractions of late \( I_{\text{Na}} \) produced by most 'gain of function' mutations of SCN5A), the first AP failed to repolarize (not shown, but see Undrovinas et al., 1999). The total conductance of \( I_{\text{pNa}} \) had to be less than 0.035 mS/cm\(^2\) (0.12% of maximal fast \( I_{\text{Na}} \) conductance) for the model cell AP to repolarize. As depicted in figure 1A, after increasing \( I_{\text{pNa}} \) conductance to 0.03 mS/cm\(^2\), the first AP at 1 Hz showed a lengthened AP duration at 90% repolarisation (APD\(_{90}\)) that was 2.6 times that in control. Remarkably, however, as regular stimulation at 1 Hz proceeded, APD\(_{90}\) shortened to a steady-state value 1.37 times that in control, and this change was complete in 150 s. From this state, further increase of \( I_{\text{pNa}} \) conductance to 0.06 mS/cm\(^2\) did not compromise AP repolarization (Fig 1B). The next AP in this new condition displayed a APD\(_{90}\) 5.7 times that in control. There also appeared small voltage oscillations late during the AP plateau (see below). Subsequently, and as formerly, APD\(_{90}\) decreased down to 2.2 fold that in control in 30 sec stimulation at 1 Hz. There appeared small voltage oscillations at regular intervals (delayed after depolarisations (see below).

Thus the model cell tends to compensate for a large part but not all of the lengthening of AP duration caused by the increased sustained sodium inward current. After increasing \( g_{\text{pNa}} \) to 0.03 mS/cm\(^2\), the repolarizing currents that most prominently increased and account for shorter duration of AP at steady state in figure 1A are \( I_{\text{Nak}} \) (the electrogenic sodium-potassium pump current), \( I_{\text{NaCa}} \) (the electrogenic sodium-calcium exchanger current) and \( I_{\text{KNa}} \) (the sodium activated potassium current). Remarkably, the \( I_{\text{Ks}} \) current that was prominent during the plateau of the first AP is greatly decreased during the AP at steady state under 1Hz stimulation (not shown). After further increasing \( g_{\text{pNa}} \) to 0.06 mS/cm\(^2\), the extra outward currents that increased after progressive APD shortening were \( I_{\text{Nak}} \) and \( I_{\text{KNa}} \) whereas the Na-K pump current did not increase further (not shown).

We next considered the most remarkable changes in ionic currents and intracellular Na\(^+\) and Ca\(^{2+}\) handling upon suddenly increasing \( g_{\text{pNa}} \) to 0.06 mS/cm\(^2\) (figure 2A), as such a sudden increase in \( I_{\text{pNa}} \) might occur in case of drugs altering \( I_{\text{Na}} \) inactivation (e.g.: anthopleura toxin Priori et al., 1996;
amiodarone (Bito et al., 2006), but also under respiratory acidosis (Plant et al., 2006) or acute hypoxia (Ju et al., 1996; Fearon et al., 2004). As shown in figure 2A, small early after-depolarisations (EADs) are present during the plateau of the AP (arrows). These are not accompanied with reactivation of the Ca current (second panel from top in fig 2A), but are caused by spontaneous Ca release from the SR as indicated by sudden changes in [Ca$^{2+}$] in the dyadic subspace ([Ca$^{2+}$]$_{sub}$), which caused [Ca$^{2+}$] transients. The variations in the Na-Ca exchanger current ($I_{Na,Ca}$) during the plateau of the AP are closely related to changes in [Ca$^{2+}$].

After regular stimulation at 1 Hz for 30 s, the AP had significantly shortened (figure 2B). At this time there developed an overload of the intracellular compartment with Na$^{+}$ and of the SR with Ca$^{2+}$ (see figure 3). Under this condition, delayed after-depolarisations (DADs) were present, concomitant with sharp increases in [Ca$^{2+}$]$_{sub}$ and intracellular Ca$^{2+}$ transients. Inward changes in the Na-Ca exchanger current closely followed the changes in [Ca$^{2+}$].

To further understand the build up of Na$^{+}$ overload, we compared action potentials ($V_m$, figure 3A) and $I_{pNa}$ (figure 3B) in control and after stabilization at 1 Hz with addition of 0.03 and 0.06 mS/cm$^2$ $g_{pNa}$ It is readily visible that the amount of Na$^{+}$ ions entering during an AP (i.e. the area under the $I_{pNa}$ trace) is greatly enhanced due the extra Na$^{+}$ ions carried by the added $I_{pNa}$, but also due to AP prolongation. The time integral of the $I_{pNa}$ current was 8.26 that in control after addition of 0.03 mS/cm$^2$ $g_{pNa}$, 39% of which was carried during the additional AP duration versus control. After further increasing $g_{pNa}$ to 0.06 mS/cm$^2$, the integral was further increased 3.2 fold (reaching 26.5 times that in control) and 73% of this was carried during additional AP duration versus control.

Intracellular [Na$^{+}$] and [Ca$^{2+}$] at end of diastole are shown respectively in panels C and D of figure 3. The progressive overload of the intracellular space with Na$^{+}$ ([Na$^{+}$], in figure 3C) was modest after increasing $g_{pNa}$ to 0.03 mS/cm$^2$ (gain of less than 1 mM from 15.4 mM to 16.3 mM) whereas after further increasing $g_{pNa}$ to 0.06 mS/cm$^2$, [Na$^{+}$], raised by 4.4 mM to reach 20.67 mM at steady-state. In both cases, the relaxation of intracellular Na$^{+}$ was slow (time constant of 106 s and 75 s respectively for the first and second increases in [Na$^{+}$], in figure 3C, indicating that the overload builds up from beat to beat. As shown in figure 3D, each of these progressive increases in [Na$^{+}$], was accompanied by a parallel rise in end diastolic [Ca$^{2+}$], from 171 nM to 200 nM during the first period. By 30 s during the second period, at the time when the AP in figure 3A ($g_{pNa} = 0.06$, dotted line) was recorded, [Ca$^{2+}$], was 260 nM. Shortly thereafter, [Ca$^{2+}$] started to display large sudden increases (fig 4D) that occurred again later, eventually reaching values persistently beyond 1 µM, which would
have been deleterious to a real cardiac cell. Thus the model cardiac cell could stabilize to a new steady-state with moderate sodium overload when the increase in $I_{pNa}$ was moderate, whereas a larger $I_{pNa}$ would cause the cell to enter a deleterious $Na^+$ and $Ca^{2+}$ overload.

We so far have explored three situations: i) an instantaneous increase in $I_{pNa}$, that is likely to be directly arrhythmogenic, due to a large increase in AP duration leading to EADs, ii) a situation with moderate $Na^+$ and $Ca^{2+}$ overload, in which AP lengthening has largely recessed and that may represent a stable adapted state, iii) a third condition when $I_{pNa}$ was further increased might relate to a deleterious situation in which the cell may suffer from large $Ca^{2+}$ overload.

In the next series of simulations, we explored situation ii), that might correspond to a stable symptomatic LQT3 condition that does not show arrhythmogenic behaviour per se but in which AP prolongation and $Na^+$ and $Ca^{2+}$ overload would predispose to lethal arrhythmias triggered by other proarrhythmic events (e.g.: bradycardia, QT prolonging drugs, Wu et al., 2006). Two features might favour the output of proarrhythmic events: the prolonged AP duration was reported to be proarrhythmogenic in LQT3 patients, especially at low cardiac frequencies and this has been documented in animal and computer models of LQT3 (Bennett et al., 1995; Clancy et al., 1999; Clancy et al., 2003; Tian et al., 2004; Henry et al., 2004; Berecki et al., 2006). The second factor: intracellular $Na^+$ overload, has received less attention. Two manoeuvres likely to further increase $Na^+$ overload are an increase in cardiac frequency (Pieske et al., 2002) and any action likely to block the Na-K pump, e.g. hypokalaemia, that is a known risk factor of sudden cardiac death as it increases AP duration and the incidence of EADs but also aggravates $Na^+$ overload.

We thus investigated the response of two modifications of our model causing similar AP prolongation, the first one due to an increase of $I_{pNa}$, the second to the partial block of $K^+$ currents $I_{ks}$ and $I_{kr}$.

As described in the methods, a first variant (model $+I_{pNa}$) was created, that included a $I_{pNa}$ set at 0.02 mS/cm$^2$ (i.e. 0.07 % of peak fast $g_{Na}$) to produce an APD$_{90}$ prolongation by 46%, comparable to that seen in symptomatic patients with LQT3 syndrome (Modell et al., 2006). The properties of this model are compared with another model (model $-I_{ks}$), with decreased repolarizing currents $I_{kr}$ and $I_{ks}$, that achieved a similar AP prolongation at 1 Hz. The steady state APs in these two modified models are superimposed with the AP from the control model. Fig. 4, left column shows the time-course of changes in several variables during one cycle at 1 Hz stimulation frequency once the model reached dynamic stability (after 300 s cell lifetime at 1 Hz).
The APD₉₀ was similar in both modified models (i.e. 223 ms for model +IₚNa and 222 ms for model -Iₖ). This represented a 46 % prolongation of APD₉₀ versus 153 ms in the Cont model (fig. 4a). This compares well with QTc lengthening in LQT3 subjects (Modell et al., 2006).

Fig. 4b shows the magnitudes of the sum of Iₖ, and Iₖ₄ (in Cont and -Iₖ models) and of IₚNa in +IₚNa model. Intracellular Na⁺ concentration ([Na⁺], 10.95 mM in Cont model - fig. 4c) increased to 12.1 mM (+10.5 %) in the +IₚNa model whereas it decreased to 9.95 mM (-9.1 %) in the -Iₖ model. In the +IₚNa model, the intracellular Ca²⁺ transient ([Ca²⁺]ᵢ, fig. 4d) was larger than in Cont model (peak value 1.08 μM versus 0.77 μM in Cont model i.e. +40 %) as well as the end-diastolic intracellular Ca²⁺ value (0.11 μM versus 0.09 μM in Cont model i.e. +30 %). In the -Iₖ model, these values were slightly lowered to respectively 0.73 μM (-5 %) and 0.08 μM (-8%). Correspondingly, the Ca²⁺ concentration in the uptake compartment of the SR ([Ca²⁺]SRap, fig. 4e) in +IₚNa model was higher than in Cont model by 58 % at peak and 43 % at the end of diastole whereas in the -Iₖ model, these values were, respectively, 10 % and 7 % lower than in Cont model.

Thus at a stimulation rate of 1 Hz, the presence of a persistent Na⁺ current caused increase [Na⁺]ᵢ and [Ca²⁺]ᵢ, whereas the model with decreased K⁺ currents, although producing similar lengthening of AP duration, caused a slight decrease of these concentrations. Next, we tested the response of these models to a high stimulation rate (3 Hz, fig. 4 right column). The respective decrease in APD₉₀ was 36 ms in the Cont model, 72 ms in the +IₚNa model and 44 ms in the -Iₖ model. The AP remained prolonged versus control in both modified models (fig. 4f), but this was less for the +IₚNa model (+30 %) than for the -Iₖ model (+53 %). These changes are similar to those in guinea pig ventricular myocytes exposed to either anthopleurin A (to enhance the late Na current) or to doxetilide (to block the potassium current Iₖ, when the stimulation frequency was increased from 1 Hz to 2.5 Hz(Priori et al., 1996). The time course of modified currents is shown in fig. 4g. For both modified models, the deviations of [Na⁺]ᵢ, (fig. 4h), [Ca²⁺]ᵢ, (fig. 4i) and [Ca²⁺]SRap (fig. 4j) from levels in the Cont model were all amplified by the higher stimulation frequency versus those at 1 Hz. The model with persistent Na⁺ current thus showed a tendency to overload the cell with Na⁺ and Ca²⁺ that is enhanced at higher rates of stimulation whereas the model with decreased K⁺ currents showed an opposite tendency.

Hypokalaemia is a condition reported to trigger arrhythmias in LQT patients. We tested the response of each model to gradual stepwise lowering of external K⁺ concentration ([K⁺]ₑ) from the control value of 5.4 mM. The results are summarized in fig. 5. The -Iₖ model could stand lowering of
[K⁺]ₑ to 2.5 mM (fig. 5a) similarly to the Cont model (fig. 5b). Several values of the maximal conductance for IₚNa were used in the +I_pNa model and the model run for 600 s in normal [K⁺]ₑ. Then, the simulations with gradually lowered [K⁺]ₑ were run again. The results show that when gₚNa was set to 0.01 mS/cm², the model could stand [K⁺]ₑ as low as 2.5 mM and keep regular beating (fig. 5c), whereas when gₚNa was set to 0.018 mS/cm², the model became arrhythmic at 4.5 mM (fig. 5d). Model +I_pNa with gₚNa at 0.02 mS/cm² displayed arrhythmic behaviour at 5 mM (fig. 5e).

Thus the propensity to develop arrhythmic behaviour in response to lowered extracellular [K⁺] was directly related to the increased persistent sodium current and to its magnitude. It is noteworthy that even a quite low gₚNa (0.06 % of peak gₚNa) is enough to enable proarrhythmic behaviour with a rather mild degree of hypokalaemia: [K⁺]ₑ lower than control by less than 1 mM. In contrast, the -I_k model did not show more propensity than the Cont model to develop after-depolarisations under lowered [K⁺]ₑ.

If these results with the +I_pNa model would hold in LQT3 subjects, even pacing the heart (e.g. at 1 Hz) to protect from bradycardia-induced arrhythmias might not prevent a proarrhythmic action of hypokalaemia.
Discussion

*Compared effects of immediate versus steady-state effects of inclusion of $I_{pNa}$.*

Immediate effects of inclusion of a persistent Na current are pertinent to several situations that may occur in clinical settings. First some drugs provoke the appearance of long lived re-openings of fast Na channels that support a persistent $I_{Na}$ (Priori et al., 1996; Bito et al., 2006). Furthermore, intracellular acidosis combined with a underlying SCN5A pH-sensitising mutation (Plant et al., 2006), acute thyroid hormone exposure (Wang et al., 2003) and acute hypoxia (Ju et al., 1996; Fearon et al., 2004) may all trigger the appearance of a persistent $I_{Na}$. As analysed in figures 1 and 2, these changes may prove directly arrhythmogenic, through early after-depolarisations (EADs) appearing as a consequence of a dramatic prolongation of AP plateau. This implies that the first minutes after exposure would represent a critical window for immediate arrhythmogenic consequences and associated risk of sudden death.

Should ventricular cells resist this initial AP prolongation, AP duration may rapidly shorten as a consequence of raised intracellular [Na$^+$] and increased repolarizing currents (figures 1 and 3) and this might be considered as a salvaging influence of Na$^+$ overload combined with an intact Na-K pump activity.

This second situation may be more pertinent to mimic the presence of a persistent $I_{Na}$ as due to the expression of mutated SCN5A genes. Indeed, the inclusion of modified Na channels into the sarcolemmal membrane through translation and protein synthesis would in this case be progressive along time, leaving way to adaptation to the new condition, with changes in intracellular homeostasis, as illustrated here, and chronically elevated intracellular Na$^+$ and Ca$^{2+}$ concentrations. These changes may not directly trigger arrhythmias but create a background rendering the cell more responsive to proarrhythmogenic manoeuvres, e.g.: bradycardia, drug-induced decrease in $I_{kr}$ or $I_{Ks}$, and any manoeuvres likely to further increase $I_{pNa}$ (see above). In addition, the present modelling points out to the increased susceptibility of a model cell to conditions favouring Na$^+$ overload: hypokalaemia that is a known trigger of arrhythmias causing sudden death. Another condition is tachycardia, that is known to cause increased [Na$^+$], and does so more in the model cell with persistent Na current (fig 4). However, consistent with a increased ability of high heart rate to shorten the QT interval in LQT3, the APD$_{90}$ at 3 Hz showed twice larger shortening in the model cell with increased $I_{pNa}$. This may represent another positive consequence of Na$^+$ overload.
Our model results stress the importance of the lengthening of APD in maintaining \( \text{Na}^+ \) overload (fig 3 and related analysis), as a large part of the \( \text{Na}^+ \) inflow occurs during the late part of the AP that is in excess of the duration of control AP. As a consequence, therapeutic actions aiming at shortening AP duration without reducing \( I_{\text{pNa}} \) might be efficient in reducing \( \text{Na}^+ \) overload. However, selective inhibition if \( I_{\text{pNa}} \) would altogether correct the long QT condition and the associated \( \text{Na}^+ \) overload. Among such therapeutic agents, mexiletine, an efficient blocker of late Na current has proven efficient in preventing torsades de pointes associated with LQT3. However, mexiletine can also act as a chemical chaperone and has been found able to rescue SCN5A/M1766L mutated channels from defective trafficking (Valdivia et al., 2002). An adverse effect might occur if such an action results in an increase of the late Na current. This view is supported by recent evidence of restoration by mexiletine of normal trafficking of the trafficking defective SCN5A/T353I mutant with increased late Na current (Pfahnl et al. 2007). Such pharmacological rescue likely takes part to induction of acquired LQTs with cisapride in the case of the SCN5A/L1825P mutation (Liu et al. 2005). A recent therapeutic approach with a potent preferential blocker of late \( I_{\text{Na}} \) current through SCN5A channels, Ranolazine (Zygmont et al., 2002; Noble and Noble, 2006, Maltsev and Undrovinas, 2007 in this volume) may not have such a potential adverse effect.

**Compared ionic homeostasis changes with increased \( I_{\text{pNa}} \) current or with decreased \( I_C \) currents**

The magnitude of the persistent current that was introduced in the \( +I_{\text{pNa}} \) model caused an AP prolongation by 46%, which is in the range reported for the QTc lengthening in LQT3 syndrome (Modell et al., 2006). For so doing, \( g_{\text{pNa}} \) was set to a value of 0.02 mS/cm² which represents 0.066% of the maximal conductance for the fast Na⁺ current. The magnitude of the persistent current for exogenously expressed mutated hNav1.5 channels amounted 1 to 6% of the peak Na⁺ current (Baroudi et al., 2000; Bezzina et al., 2001; Veldkamp et al., 2003; Chang et al., 2004). Thus only a small part of fast Na⁺ channels (1.1% to 6.6%) would need to be mutated to account for a fraction of 0.066% persistent Na⁺ current. This is consistent with the heterozygous presence of most of such mutations (Veldkamp et al., 2003), allowing a mixed population of mutated and wild-type channels to coexist in cardiac cells of subjects bearing such mutations. It also entails that the mutated channel genes need not attain a high level of expression for causing a LQT3 phenotype. A consequence is that whenever the efficiency of expression of the mutated channels would be low, the magnitude of the persistent current may well be enough to create a state of predisposition of the heart to arrhythmogenic behaviour.
Here we obtained indication that the presence of a persistent Na\(^+\) current causes intracellular overload in Na\(^+\) and Ca\(^{2+}\) (fig 4). In contrast, a decrease in Na\(^+\) and Ca\(^{2+}\) load (versus control condition) was seen when a similar AP prolongation was caused by a decrease in K\(^+\) currents I\(_K\) and I\(_{Ka}\) (fig 4). This suggests that the proarrhythmogenic background created by these two alterations in ionic currents is different, and that LQT3 with persistent Na\(^+\) current makes the cell more sensitive to an aggravation of Na\(^+\) and Ca\(^{2+}\) overload. This is supported by the arrhythmogenicity of [K\(^+\)]\(_e\) lowering in the +I\(_pNa\) model that was not seen in the -I\(_K\) model (fig 5). However, when g\(_{pNa}\) was decreased by 50% in the +I\(_pNa\) model, it became resistant even to a low [K\(^+\)]\(_e\) of 2.5 mM, as Cont and -I\(_K\) models (fig 5). Thus there is a threshold magnitude of I\(_pNa\) below which the higher sensitivity to low [K\(^+\)]\(_e\) did not appear.

Limitations of the model

It may be noted that the change introduced in our model to account for a persistent Na\(^+\) current is not an exact image of the gain of function induced in the fast hNav1.5 current by most of the mutations causing LQT3 phenotype (Bezzina et al., 2001; Veldkamp et al., 2003). In particular, voltage shifts of the steady-state inactivation versus voltage relation and changes in the kinetics and/or voltage dependence of fast inactivation of the hNav1.5 current were not taken into account here. This was purposely done to explore the effects of a persistent Na\(^+\) current per se on ionic homeostasis. Nevertheless, the complete set of changes for a given mutation should be explored to test whether the effects outlined here are altered. Another simplification is that the equations used here for the persistent Na\(^+\) current do not account for the ultra-slow inactivation present in the persistent current of mutated channels (Bennett et al., 1995; Baroudi et al., 2000; Chang et al., 2004). However, as the time constant of this inactivation was several fold longer than the duration of APs in the present model, the magnitude of the persistent Na\(^+\) current should be negligibly altered within the duration of an AP.

The model that we used includes a representation of the transverse-axial tubular system (TATS) and the related distributions of ion transfer mechanisms (Pasek et al., 2007b). We used this model because, in this respect, it is a likely more accurate description of a ventricular cardiac myocyte than previous models (see review by Pasek et al., 2007a, in this issue). We have shown in a former study that the presence of the TATS would retard the proarrhythmic effect of progressive hypokalaemia (Pasek et al., 2002). However, the possible effects of the presence of the transverse axial tubular system (TATS) in our model were not explored here.
The basic frequency of the model at 1 Hz was in the upper range of heart rates reported for LQT3 subjects that show bradycardia related to the persistent $I_{\text{Na}}$ in sinus node cells (Veldkamp et al., 2003).

The present results were obtained in a model of guinea-pig ventricular cell and may not readily hold for a human cardiac cell. In particular, the frequency-related increase in $[\text{Na}^+]$, reported for control human cardiomyocytes (Pieske et al., 2003), was not seen in our model when frequency was increased from 1 to 3 Hz.

In our model, a single type of cell was modelled whereas in the real ventricular wall three main types of cells are present: epicardial, endocardial and mid-myocardial cells (Antzelevitch et al., 2001). There are differences between these cell types in the expression of the persistent $I_{\text{Na}}$ current (Sakmann et al., 2000; Zygmunt et al., 2001) of the fast $I_{\text{Na}}$ (Li et al., 2002; Antzelevitch et al., 2006) and of other currents (Antzelevitch et al., 2001). As a result, these cells have different AP durations and should respond differently to the presence of an equal amount of persistent $I_{\text{Na}}$ current arising from mutated Na$^+$ channels. Furthermore, intracellular ionic homeostasis also varies among these cell types, in particular, intracellular $[\text{Na}^+]$ at rest is different as well as the activity of the Na-K pump (Gao et al., 2005), so that the incidence of a given amount of $I_{\text{pNa}}$ shall differ also for this reason. However, the expression of the mutated channels may also be different among cell types. In the syncitial tissue, the differences in action potential durations between ventricular cells subtypes may be damped owing to electrotonic interaction (Conrath et al., 2004; Henry et al., 2004). This may not be the case for differences in $[\text{Na}^+]$, owing to the small equivalent length constant of transcellular Na$^+$ diffusion (Gao et al., 2005). These features should obviously be taken into account in a more rigorous modelling of the effects of persistent $I_{\text{Na}}$ on each of these cell types, and on the syncitial tissue.
Conclusions

The persistence of quite a small fraction of the peak $I_{Na}$ current (1/1000th) is enough to sizably lengthen APs in a ventricular GP myocyte model. This is relevant to the triggering of arrhythmias by an acute increase of a persistent $I_{Na}$ current (drug, hypoxia, acidosis), but probably not to the LQT3 condition, in which the gain of function of mutated SCN5A channels would appear progressively over at least several hours.

A persistent $I_{Na}$ directly contributes to raise intracellular [Na$^+$] and thereby favours intracellular [Ca$^{2+}$] overload, in addition to the AP lengthening per se. When the cell can adapt to this condition, a stable state of proarrhythmogenic condition is met. This is probably a closer image of the stable state of a LQT3 condition, that predisposes the cell to arrhythmogenic behaviour with additional conditions such as hypokalaemia.

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References


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Legends to figures

Figure 1: Changes in APs after increasing $I_{pNa}$. A: From steady-state at 1 Hz (SS Control, continuous line), $g_{pNa}$ was increased from 0.0053 to 0.03 mS/cm$^2$, the model was then run at 1 Hz. The first subsequent AP (1st AP, dotted line) and the AP at steady-state after 600 s (SS AP, dashed line) are superimposed. B: $g_{pNa}$ was subsequently increased to 0.06 mS/cm$^2$, the first (1st AP 0.06, dotted line) and steady-state (SS AP 0.06, dashed line) APs are superimposed with the control AP (grey trace) and the steady-state AP with 0.03 mS/cm$^2$ $g_{pNa}$ (SS AP 0.03).

Figure 2: From upper to lower panels in A and B: action potentials (Vm), L-type Ca current ($I_{Ca}$), Na-Ca exchange current ($I_{NaCa}$), Ca$^{2+}$ concentrations in the dyadic subspace ([Ca$^{2+}]_{m}$) and the intracellular space ([Ca$^{2+}]_{i}$). A) When the persistent current was suddenly increased from 0.03 mS/cm$^2$ to 0.06 mS/cm$^2$. B) After 30 seconds regular stimulation under this new condition. Arrows in the top panel in A denote EADs. In the second panel from top in A and B, the $I_{Ca}$ trace was shown with its full magnitude (vertical scale on the left) and also with a 40 fold magnification (denoted x40, vertical scale on the right).

Figure 3: Progressive changes in intracellular [Na$^+$] ([Na$^+$], panel C) and intracellular [Ca$^{2+}$] ([Ca$^{2+}$], panel D) that accompany the stabilization towards the shortened APs represented in panel A. The traces of $I_{pNa}$ corresponding to APs in A are shown in panel B.

Figure 4. Output data from models Cont (continuous lines), $+I_{pNa}$ (doted lines) and $-I_K$ (dashed lines) after 600 s of regular stimulation at 1 Hz (left column) or at 3 Hz (right column) in the I-clamp mode. Upper row: action potentials (Vm), second row show the sum of $I_{Ks}$ and $I_{Kr}$ currents in the Cont model and in the $-I_K$ model, whereas the dotted line shows the $I_{Nap}$ current in model $+I_{Nap}$ only.

Figure 5. APs in low [K$^+$]e at 1 Hz stimulation frequency: a: in the Cont model after 300 s in 2.5 mM [K$^+$]e. b: in the $-I_K$ model after 300 s in 2.5 mM [K$^+$]e. c: in the $+I_{pNa}$ model with $g_{pNa}=0.01$ mS/cm$^2$ after 300 s in 2.5 mM [K$^+$]e. d: in the $+I_{pNa}$ model with $g_{pNa}=0.018$ mS/cm$^2$ during the first 4 s of simulation in 4.5 mM [K$^+$]e, after 300 s regular activity in 5.0 mM [K$^+$]e (not shown). e: in the $+I_{pNa}$ model with $g_{pNa}=0.02$ mS/cm$^2$ during the first 4 s of simulation at 5.0 mM [K$^+$]e, applied from steady-state at 5.4 mM [K$^+$]e (not shown).
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