Effects of a Persistent Sodium Current Through Mutated hNav1.5 Sodium Channels on Intracellular Ionic Homeostasis in a Ventricular Cell Model

G Christe[1], L Restier[2], M Chahine[3], P Chevalier[2], M Pasek[4]

[1]INSERM E0219, CEA Grenoble, Grenoble, France
[2]Hôpital Cardiologique, Lyon, France
[3]Hôpital Laval, Sainte-Foy, Quebec, Canada
[4]Institute of Thermomechanics, Czech Academy of Sciences, Brno, Czech Republic

Abstract

In LQT3 patients, SCN5A mutations were found that lead to a small fraction of persistent hNav1.5 current. We explored the effects of such a change on the intracellular ionic homeostasis in a model of guinea-pig cardiac ventricular cell. At steady-state under 1 Hz stimulation, the presence of a persistent Na^+ current (I_{Nap}) with g_{Nap} 0.02 ms/cm^2 led to a prolongation of the action potential from 153 ms (control) to 223 ms and an increase of $[Na^+]_i$, diastolic and systolic $[Ca^{2+}]_i$ and $[Ca^{2+}]_{SRup}$ by 10 %, 30 %, 40 % and 43 %, respectively. These changes were larger at 3 Hz. Such intracellular Na^+ and Ca^{2+} overload was not found when the action potential prolongation (to 222 ms at 1 Hz) was due to decreased I_{Kr} and I_{Ks} currents. The model with I_{Nap} became arrhythmogenic when $[K^+]_e$ was lowered from 5.4 to 5.0 mM, whereas control and low K^+ current models did not produce arrhythmias even when $[K^+]_e$ was 2.5 mM.

1. 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 [1-3]. However, a possible contribution of altered ionic homeostasis was not explored quantitatively. In particular, the presence of a persistent Na^+ current (I_{Nap}) during the action potential might cause elevation of intracellular $[Na^+]$, a condition that predisposes to calcium overload and arrhythmogenesis. We explored whether this effect would be quantitatively important in a computer model of guinea pig ventricular myocyte. Two modifications of the model causing equivalent prolongation of the action potential were compared. In the first modified model, a persistent Na^+ current was introduced, to mimic a mutation-induced LQT3 condition. In the other modification, a decrease in I_{Kr} and I_{Ks} currents was introduced to cause a similar prolongation of the action potential. The two models were compared in their ability to cause changes in intracellular ionic homeostasis. We further evaluated the response of these modified models to an increase in heart rate that favors Ca^{2+} overload and to hypokalaemia, that may trigger arrhythmias in LQT patients.

2. Methods

A ventricular cell model that includes ionic currents, intracellular ionic homeostasis and a single compartment tubular system [4] was modified to closely match the properties of guinea-pig ventricular cells at 37°C (briefly described in [5] - this volume). This model was written and integrated under the MATLAB environment.

In the control version of the model (named *Cont*), I_{Nap} was not present and both the fast and slow outwardly rectifying potassium currents (I_{Kr} and I_{Ks}) were formulated with a maximal conductance of 0.8 mS/cm^2 (which is for $[K^+]_e$=5.4 mM for I_{Kr} and for $[Ca^{2+}]_i$=32.5 nM for I_{Ks}).

In the first modification of the model (named $+I_{Nap}$), a persistent Na^+ current was introduced in the form:

$$I_{\mathrm{Nap}} = g_{\mathrm{Nap}}\, k_{\mathrm{Nap}} (V_{\mathrm{m}} - E_{\mathrm{Nap}})$$

with $$k_{\mathrm{Nap}} = \frac{1}{1+e^{-(V_{\mathrm{m}}+54)/8}}$$

and $$E_{\mathrm{Nap}} = \frac{RT}{F} \frac{\left[\mathrm{Na}^+\right]_{\mathrm{e}} + 0.12\left[\mathrm{K}^+\right]_{\mathrm{e}}}{\left[\mathrm{Na}^+\right]_{\mathrm{i}} + 0.12\left[\mathrm{K}^+\right]_{\mathrm{i}}}.$$

The maximal conductance g_{Nap} was set to 0.02 mS/cm^2.

In a second modification (named *-IK*) of model *Cont*, the maximal conductance for both I_{Kr} and I_{Ks} was reduced by 67% the same value of 0.25 mS/cm^2. Both modified models and the control model were run for at least 600s cell life time to ensure that all variables had reached steady-state. For examination of susceptibility of each model to generate arrhythmias in lowered extracellular

$[K^+]_e$, each model was run for 300 s in each condition.

3. Results

Fig. 1, 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.

Intracellular Na^+ concentration ($[Na^+]_i$, 10.95 mM in *Cont* model - fig. 1c) increased to 12.1 mM (+10.5 %) in the $+INa_p$ model whereas it decreased to 9.95 mM (-9.1 %) in the *-IK* model. In the $+INa_p$ model, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$, fig. 1d) was larger than in *Cont* model in both its peak value (1.08 µM vs 0.77 µM

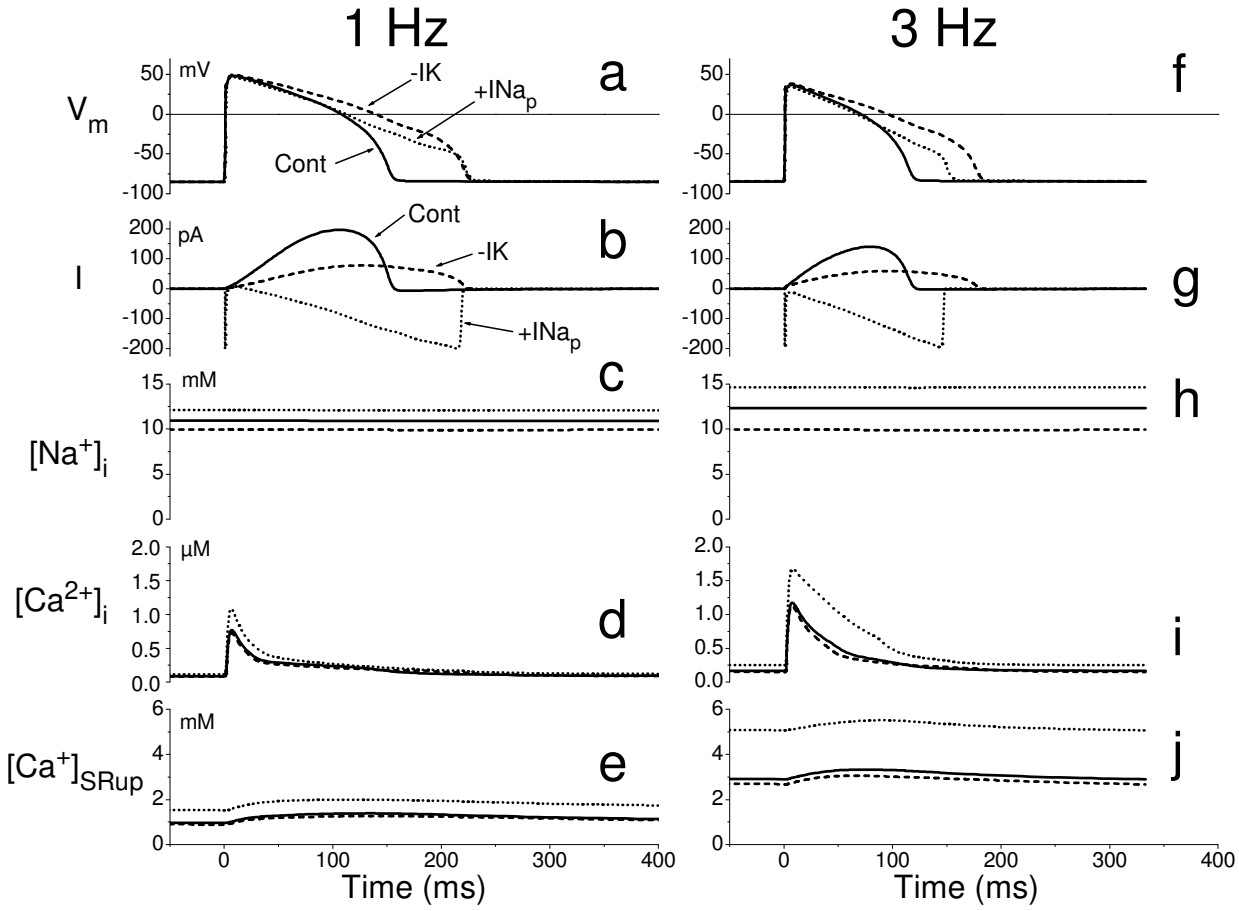

Figure 1. Ouput data from models *Cont*, $+INa_p$ and *-IK* after 600 s of regular stimulation at 1 Hz (left column) or at 3 Hz (right column) in the I-clamp mode. Continuous lines refer to the *Cont* model, dotted lines to the $+INa_p$ model and dashed lines to the *-IK* model.

The action potential duration at 90% repolarization (APD_{90}) was similar in both modified models (i.e. 223 ms for model $+INa_p$ and 222 ms for model *-IK*). This represented a 46 % prolongation of APD_{90} versus 153 ms in the *Cont* model (fig. 1a).

Fig.1b shows the magnitudes of the sum of I_{Kr} and I_{Ks} (in *Cont* and *-IK* models) and of I_{Nap} in $+INa_p$ model. in *Cont* model i.e. +40 %) and in its end-diastolic value (0.11 µM vs 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 Ca^{2+} concentration in the uptake compartment of the SR ($[Ca^{2+}]_{SRup}$, fig. 1e) in $+INa_p$ 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 $[Ca^{2+}]_i$, whereas the model with decreased K^+ currents, although

producing similar lengthening of action potential duration, caused a slight decrease. Next, we tested the response of these models to a high stimulation rate (3 Hz, fig. 1 right column). The action potential remained prolonged versus control in both modified models (fig. 1f), but this was less for the $+INa_p$ model (+30 %) than for the *-IK* model (+53 %). The time course of modified currents is reported in fig. 1g. For both modified models, the deviations of $[Na^+]_i$ (fig. 1h), $[Ca^{2+}]_i$ (fig. 1i) and $[Ca^{2+}]_{SRup}$ (fig. 1j) 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 Ca^{2+} that is enhanced at higher rates of stimulation.

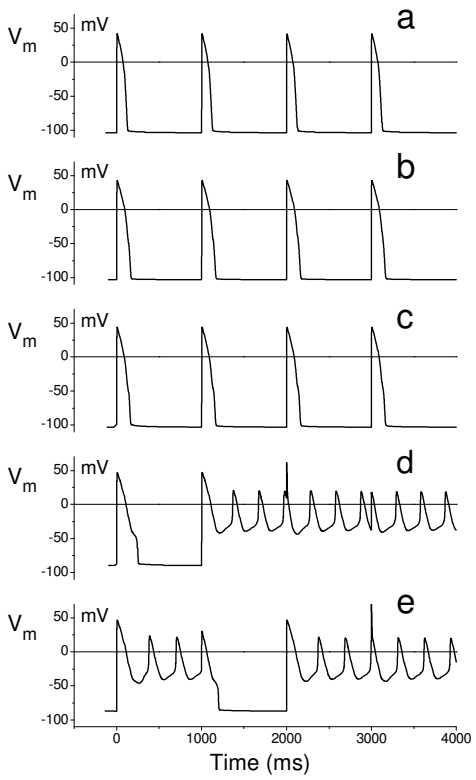

Figure 2. Action potentials in low $[K^+]_e$ at 1 Hz stimulation frequency. **a**: the *Cont* model after 300 s in 2.5 mM $[K^+]_e$. **b**: the *-IK* model after 300 s in 2.5 mM $[K^+]_e$. **c**: the $+INa_p$ model with gNa_p=0.01 mS/cm^2 after 300 s in 2.5 mM $[K^+]_e$. **d**: the $+INa_p$ model with gNa_p=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**: the *+INap* model with gNa_p=0.02 mS/cm^2 during the first 4 s of simulation at 5.0 mM $[K^+]_e$.

Hypokalaemia is a condition reported to trigger arrhythmias in LQT3 patients. We tested the response of each model to gradual lowering of external K^+ concentration ($[K^+]_e$) from the control value of 5.4 mM. The results are summarized in fig. 2. The *Cont* model could stand lowering of $[K^+]_e$ to 2.5 mM (fig. 2a) as well as the *-IK* model (fig. 2b). Model $+INa_p$ displayed arrhythmic behavior at 5 mM (fig. 2e). Then, intermediate values of the maximal conductance for I_{Nap} were used in the $+INa_p$ model and the model run for 600 s in normal $[K^+]_e$. After that the simulations with gradually lowered $[K^+]_e$ were run again. The results showed that when g_{Nap} was lowered to 0.018 mS/cm^2, the model became arrhythmic at 4.5 mM (fig. 2d) whereas when g_{Nap} was lowered to 0.01 mS/cm^2, the model could stand $[K^+]_e$ as low as 2.5 mM and keep regular beating (fig. 2c).

4. Discussion and conclusions

The magnitude of the persistent current that was introduced in the $+INa_p$ model caused an action potential prolongation by 46 %, which is in the range reported for the QTc lengthening in LQT3 syndrome. For so doing, g_{Nap} was set to a value of 0.02 mS/cm2 which represents 0.066 % of the maximal conductance for the fast Na^+ current (I_{Na}). The magnitude of the persistent current for exogenously expressed mutated hNav1.5 channels amounted 1 to 6 % of the peak Na^+ current [6,7]. Thus only a small part of fast Na^+ channels (1.1 % to 6.6 %) would need to be mutated to account for the fraction of 0.066 % persistent Na^+ current. This is consistent with the heterozygous presence of such mutations, allowing a mixed population of mutated and wild-type channels to coexist in cardiac cells of patients bearing such mutations. It also entails that the mutated channel genes need not attain a high level of expression for causing a LQT3 phenotype.

Here we obtained indication that the presence of a persistent Na^+ current causes intracellular overload in Na^+ and Ca^{2+}. In contrast, a opposite change in Na^+ and Ca^{2+} load was seen when a similar action potential prolongation was caused by a 68 % decrease in K^+ currents I_{Kr} and I_{Ks}. 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 $+INa_p$ model that was not seen in the *-IK* model. However, when g_{Nap} was halved in the $+INa_p$ model, it became resistant even to a low $[K^+]_e$ of 2.5 mM, as *Cont* and *-IK* models. Thus there is a threshold magnitude of I_{Nap} below which the higher sensitivity to low $[K^+]_e$ did not appear.

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 change induced in the fast hNav1.5 current by mutations causing LQT3 phenotype [1,6,7]. In particular, the voltage shifts of the steady-state inactivation versus voltage relation and the accelerated 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 [1,6,7]. However, as the time constant of this inactivation was several fold longer than the duration of action potentials in the present model, the magnitude of the persistent Na^+ current should be negligibly altered within the duration of an action potential.

The possible effects of the presence of the transverse axial tubular system (TATS) in our model were not explored here. We have shown in a former study that the TATS exerts a protective effect in retarding the proarrhythmic effect of progressive hypokalaemia [8]. Such an effect should not alter the conclusions from the present work.

These results were obtained in a model of guinea-pig ventricular cell and may not readily hold for a human cardiac cell. However, they suggest to consider the possibility that intracellular Na^+ and Ca^{2+} overload may contribute to predisposition of LQT3 patients to syncope.

Acknowledgements

This work was supported by a grant to GC from the Fédération des Maladies Orphelines, Paris and by the project AV0Z 20760514 from the Institute of Thermo-mechanics of Czech Academy of Sciences.

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Address for correspondence

Georges Christé, Present address:
UMR5123,
Univ. Claude Bernard - Lyon1,
F 69622 Villeurbanne Cedex, France
E-mail address: christe@lyon.inserm.fr