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Quantification of repolarization reserve to understand inter-patient variability in the response to pro-arrhythmic drugs: a computational analysis

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Abstract

Background—"Repolarization reserve" is frequently invoked to explain why potentially pro-arrhythmic drugs cause, across a population, a range of changes to cardiac action potentials (APs). The mechanisms underlying this inter-individual variability, however, are not understood quantitatively.

Objective—We performed a novel analysis of mathematical models of ventricular myocytes to quantify repolarization reserve and gain insight into the factors responsible for variability in the response to pro-arrhythmic drugs.

Methods and Results—In several models of human or canine ventricular myocytes, variability was simulated by randomizing model parameters and running repeated simulations. With each randomly-selected set of parameters, APs before and after simulated 75% block of the rapid delayed rectifier current (I_{Kr}) were calculated. Multivariable regression was performed to determine how much each model parameter attenuated or exacerbated the AP prolongation caused by the I_{Kr} -blocking drug. Simulations with a human ventricular myocyte model suggest that drug response is influenced most strongly by: 1) the density of I_{Kr} ; 2) the density of slow delayed rectifier current I_{Ks} ; 3) the voltage-dependence of I_{Kr} inactivation; 4) the density of L-type Ca^{2+} current and 5) the kinetics of I_{Ks} activation. The analysis also identified mechanisms underlying non-intuitive behavior, such as ionic currents that prolong baseline APs but decrease drug-induced AP prolongation. Finally, the simulations provided quantitative insight into conditions that aggravate the drug response, such as silent ion channel mutations and heart failure.

Conclusions—These modeling results provide the first thorough quantification of repolarization reserve and improve our understanding of inter-individual variability in adverse drug reactions.

Keywords

arrhythmia; systems biology; long QT syndrome; ventricular tachycardia; modeling

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Conflicts of Interest: None

INTRODUCTION

Increased risk of ventricular arrhythmia is a major side effect of many drugs, including both anti-arrhythmics and drugs intended for other purposes.^{1,2} Although rare, these can prove fatal, and avoiding them is of paramount importance in drug development.¹⁻³ Electrophysiological studies have demonstrated that pro-arrhythmic drugs block the K^+ channel responsible for the rapid delayed rectifier current (I_{Kr}), colloquially known as HERG. HERG block lengthens APs in cardiac myocytes and QT intervals on electrocardiograms. Drug-induced QT prolongation, although acknowledged to be an imperfect predictor,^{4,5} is therefore considered a reasonable surrogate for increased arrhythmia risk. All pro-arrhythmic drugs withdrawn from the market lengthen the QT interval in patients and in experimental models.²

Inter-individual variability greatly complicates our understanding of drug-induced arrhythmias. "Dangerous" drugs cause arrhythmias in only a small minority of patients, and the extent of QT prolongation may vary widely among a population exposed to identical doses of a given drug.^{2,6} The concept of "repolarization reserve,"⁷ or an individual's excess capacity for membrane repolarization, has been invoked by several groups to explain experimental results.⁸⁻¹² Repolarization reserve remains, however, an essentially qualitative concept.¹³ Modeling studies, although invaluable for understanding the complexity of cardiac electrical activity¹⁴ and arrhythmia risk, have thus far only considered differences between a healthy myocyte and one affected by a mutation, a drug, or a disease-causing insult^{15,16} and have not addressed the challenges posed by heterogeneity across a population.

Here we have used a recently-developed computational methodology^{17,18} to understand inter-patient variability in drug-induced QT prolongation. This has allowed us to quantify, to our knowledge for the first time, how the electrophysiological characteristics of a simulated ventricular myocyte influence the cell's response to a HERG-blocking drug. The analysis generates unexpected predictions regarding which factors are most important, thereby suggesting future experiments. Furthermore, our results quantify reduced repolarization reserve in disease and establish a rigorous, quantitative framework for understanding the factors underlying the potentially pro-arrhythmic effects of drugs.

METHODS

The goal of this study was to understand possible causes of inter-individual variability in the response to HERG-blocking drugs. Mathematical modeling was combined with multivariable regression techniques^{17,18} to correlate cellular electrophysiological parameters with AP properties measured before and after block (75%) of the rapid delayed rectifier current I_{Kr} . This technique was used with the ventricular myocyte models developed by: (1) ten Tusscher et al¹⁹ (TNNP) (2) Fox, McHarg, and Gilmour,²⁰ (3) Hund & Rudy,²¹ (4) Kurata et al,²² and (5) Grandi, Pasqualini, and Bers.²³ Results obtained with the TNNP model are presented in the greatest detail because this human ventricular model is well-established and contains a manageable number of parameters. A complete description of the procedure is provided in the Supplemental Materials; a few relevant details are mentioned here.

Parameter randomization followed by multivariable regression, as described elsewhere,^{17,18} was performed. With each model, three classes of parameters were examined: 1) parameters that describe maximal ionic conductances or rates of ion transport (G's and K's); 2) "p-values" that influence the kinetics of ion channel gating; 3) voltage shifts (V's) that control the voltage dependence of ion channel activation or inactivation. For a particular ionic

current, an increase in G makes that current uniformly larger, an increase in p causes gating (either activation or inactivation) to be slower, and an increase in V shifts activation or inactivation to more positive membrane potentials.

A model's dependence on parameters is expressed in terms of matrix multiplication: changes in outputs ($\mathbf{Y}$) can be approximated as the change in parameters ($\mathbf{X}$) times a matrix of parameter sensitivities $\mathbf{B}$, i.e. $\hat{\mathbf{Y}} = \mathbf{XB} \approx \mathbf{Y}$. Additional methodological details are provided in the Supplemental Materials and published studies.^{17,18}

RESULTS

We employed mathematical models to understand variability in the response to HERG-blocking drugs using a technique of parameter randomization followed by multivariable regression described in the Methods section and elsewhere.^{17,18} Figs. 1A and 1B respectively illustrate the procedure and show an especially relevant result obtained with the TNNP model.¹⁹ Two sets of randomly-chosen parameters (Trials 97 and 270) result in baseline APDs extremely similar to that produced by the control set of parameters (Trial 1). However, these changes in parameters greatly influence the response of the model myocytes to HERG blockade. Compared with the AP prolongation produced by HERG block in the control myocyte (23.7 ms), Trial #97 showed a smaller response ($\Delta\text{APD} = 15.9$ ms) whereas Trial #270 exhibited a more dramatic response ($\Delta\text{APD} = 45.3$ ms). Thus, variations in parameters can influence both the behavior of the unperturbed model^{17,18,24} and the response of the model myocyte to drugs.

The response of the population of 300 model cells is illustrated in Figure 2. Fig. 2A shows that ΔAPD in the population of models varies over a wide range (8.4 to 53.9 ms). Fig. 2B illustrates that in the TNNP model, ΔAPD produced by HERG blockade is uncorrelated with the baseline APD before HERG blockade, similar to results observed clinically.⁶ Thus, model myocytes that display an especially deleterious response to HERG block cannot be identified simply from their baseline APs.

The multivariable regression procedure^{17,18} computes a matrix $\mathbf{B}$ that relates the input matrix of parameters $\mathbf{X}$ to the matrix of outputs $\mathbf{Y}$, i.e. $\mathbf{Y} \approx \mathbf{XB}$. Thus, parameter sensitivities in $\mathbf{B}$ indicate how much changing a parameter affects a model output. Fig. 3A displays the values in $\mathbf{B}$ corresponding to ΔAPD ; i.e. the length of each bar shows how much that parameter influences the cell's response to HERG block, and parameters are ranked from the most to the least important. Green and red bars indicate parameters for which an increase in the parameter increases or decreases ΔAPD , respectively. Fig. 3A therefore provides a comprehensive, systems-level understanding of how the model cell's electrophysiological properties influence its repolarization reserve. Some results are expected based on prior knowledge. For instance, important parameters include: the density of I_{K_r} itself (G_{K_r} ; rank 1), the density of I_{K_s} (G_{K_s} , rank 2), and the kinetics of I_{K_s} activation (p_{K_s} , rank 5). These are consistent with the obvious notion that I_{K_r} density will influence the response to I_{K_r} block, and with the conventional wisdom that I_{K_s} provides the main reserve current that allows HERG block to be tolerated.^{25,26} Conversely, parameters related to fast Na^+ current (I_{Na}) and transient outward K^+ current (I_{to}), which activate at the beginning of the AP and are considered unimportant for repolarization, rank relatively low. One advantage of the present strategy, however, is that the unbiased analysis can identify surprising behaviors. For instance, the voltage dependence of HERG inactivation (V_{xr2}) is the third most important contributor whereas the voltage dependence of HERG activation (V_{xr1}) is the least important parameter. Fig. 3B shows additional simulations that confirm these predictions. A +20 mV shift in V_{xr1} has virtually no effect on the response to HERG block whereas a +20 mV shift in V_{xr2} greatly increases ΔAPD produced by HERG block (44.8 ms).

To identify additional counterintuitive findings, we plotted the regression coefficients for APD versus the corresponding regression coefficients for Δ APD (Fig. 4A). With this representation, parameters located in the 2nd and 4th quadrants have opposing effects on APD and Δ APD and are therefore somewhat surprising. For instance, an increase in G_{Ca} , located in the 4th quadrant, will prolong the baseline APD but improve the response of the cell to HERG block by reducing Δ APD. Additional simulations were performed to determine the mechanism underlying this paradoxical result. As shown in Fig. 4B, Increasing G_{Ca} does indeed lead to greater APD but reduced Δ APD, as predicted by the regression model. This occurs because greater L-type Ca^{2+} current elevates the AP plateau, which leads to increased activation of I_{Ks} . Larger I_{Ks} during the plateau makes the cell less susceptible to block of I_{Kr} , and Δ APD is consequently smaller. To express the idea of counterintuitive results in more quantitative terms, we calculated the absolute difference between the parameter sensitivities corresponding to APD and Δ APD. The 5 parameters for which this quantity was greatest, indicating that a parameter influences the baseline AP and the drug response differentially, are listed in Table 1. Thus, the regression method can identify surprising model behaviors that can be understood in greater detail through more mechanistic simulations.

We extended our regression method to four other ventricular myocyte models.^{20–23} After performing, with each model, an analysis similar to that shown in Fig. 3A (Figs. S1–S4), we ranked the parameters in each model from most to least important. The ranks of the common parameters were then averaged to derive a consensus list of the 10 that were consistently important in determining repolarization reserve (Table 2). Besides the density of HERG itself (G_{Kr}), important parameters included G_{K1} , G_{Ca} , G_{Ks} , and K_{NaK} , the density of the Na^+-K^+ pump. The appearance on the list of G_{Kr} , G_{Ks} and G_{K1} , the three main K^+ currents that contribute to repolarization, makes sense physiologically. For instance, it has been demonstrated that I_{Ks} can limit AP prolongation when this occurs by other means such as I_{Kr} or I_{K1} block.^{25,26} Similarly, block of I_{K1} has been shown to influence the response to HERG-blocking drugs,²⁵ and has been hypothesized in simulations to play an important role in the development of early afterdepolarizations.²⁷ In contrast, the role of the Na^+-K^+ pump in determining repolarization reserve has been less well-explored and therefore suggests a potential avenue for future research.

The matrix framework of our approach allows one to understand complicated phenotypes, in which several electrophysiological properties are altered, as superimposed combinations of the individual effects. Since $\mathbf{Y} \approx \mathbf{X} \cdot \mathbf{B}$, the approximate change in outputs in a given cell ($\hat{\mathbf{Y}}$) can be computed as the vector describing that cell's parameter changes ($\mathbf{x}$) multiplied by the matrix of parameter sensitivities $\mathbf{B}$. Figure 5 shows examples of how this framework can provide insight. To compute an element of $\hat{\mathbf{y}}$, corresponding for instance to Δ APD in a particular cell, one multiplies each change in a parameter (element of $\mathbf{x}$) by the corresponding element of $\mathbf{B}$, then sums the individual products to calculate the overall response. For instance, G_{Ca} and G_{Ks} parameter sensitivities have opposite signs with regards to how they influence APD but the same sign with respect to Δ APD. This means that if G_{Ca} or G_{Ks} are increased or decreased in parallel, the effects on APD will offset one another but the effects on Δ APD will be additive. Thus, in the TNNP model, a cell with decreased G_{Ca} and G_{Ks} exhibits an especially dramatic increase in APD after HERG block, even if the baseline APD is essentially normal (Fig. 5A). We can similarly understand clinically relevant responses to HERG blockade through matrix multiplication. For instance, Yang et al identified a silent mutation in KCNQ1, the gene encoding the α -subunit of I_{Ks} , that causes a single amino acid substitution (R583C) and predisposes individuals to drug-induced arrhythmia.²⁸ It was not known, however, if the increased susceptibility was due primarily to the decrease in G_{Ks} or to the positive shift in the voltage-dependence of activation (parameter V_{xs} in the model). The regression model predicts that these two changes

contribute roughly equally to the increased Δ APD (Fig. 5B). Similarly, we can simulate heart failure (HF) by changing four model parameters, as done previously by Winslow et al.²⁹ Fig. 5C illustrates how each change contributes to reduced repolarization reserve in HF, as simulated with the TNNP model. The regression model predicts that even though the decrease in G_{to} is large, this does not alter repolarization reserve because this parameter's regression coefficient is near zero. In contrast, the small decrease in G_{K1} markedly decreases repolarization reserve due to a larger value in **B**. Changes in SERCA and NCX contribute mildly to the increased Δ APD.

DISCUSSION

Summary of Results

Repolarization reserve^{7,13} is generally considered the main reason for inter-patient variability in the response to HERG block.² This phrase expresses the idea that several ionic currents are involved in the repolarization phase of the AP, and thus a defect in any single channel or accessory protein can potentially be compensated by the others. A corollary is that the effect of blocking any particular current (e.g. I_{Kr}) depends not just on the density of that channel but also on the other ionic currents in the cell membrane. Although this concept is intuitive and intellectually satisfying, it has remained essentially qualitative. This study is, to our knowledge, the first attempt to quantify repolarization reserve by comprehensively evaluating how dozens of parameters in a mathematical model influence the APD prolongation produced by HERG blockade.

To accomplish this goal, we reproduced the variability observed in populations using a computational technique^{17,18} that generates large sets of simulated data, akin to those that might be measured using microarrays, whereby expression of each ion channel transcript will vary between individuals. Applying multivariable regression to this pseudo-dataset quantified the contribution of each channel property to the AP prolongation produced by HERG block. In addition to providing the first thorough quantification of repolarization reserve, this approach also allowed us to: (1) identify counterintuitive contributors (Fig. 4 and Table 1); and, (2) understand the individual contributions of multiple factors in complex phenotypes (Fig. 5).

Relation to prior work

Several prior studies, both experimental^{8–12} and computational^{16,27,30,31} have examined how changes in particular ionic currents may augment or reduce repolarization reserve. For instance, Tsuji et al showed that chronic tachypacing of rabbit hearts led to a decrease in G_{Ks} , and the effects of I_{Kr} block with E-4031 were more severe in these hearts.¹⁰ Analogously, Suzuki et al examined APD as a function of G_{Kr} and G_{Ks} in a computational study, and showed that I_{Kr} block could be tolerated when G_{Ks} was large.³⁰ Alterations in L-type Ca^{2+} current⁹ and late Na^{+} current¹¹ have also been shown to influence repolarization reserve. Our study extends these ideas by providing a more global view.

The comprehensive view provided by our computational approach offers advantages compared with the traditional strategy that perturbs ionic currents individually. Most important, the method is unbiased: instead of only examining those currents the investigator presumes to be important, the method simultaneously provides information about all model parameters that control ionic current characteristics (Fig. 3A). In addition, the parameter sensitivities computed in this analysis are quantitative and experimentally testable. Each bar in Fig. 3A represents a precise prediction, e.g. reducing G_{Ks} by 50% leads to a 34% increase in the response to HERG block. Most predictions related to conductances can be tested using combined pharmacology, whereas those related to channel kinetics and voltage dependences

can be addressed using novel technologies such as dynamic clamp.³² Ongoing work in our laboratory is aimed at performing these tests.

Clinical Relevance

Because variability between individuals is one of the significant unresolved issues that hinders our understanding of drug-induced arrhythmias,^{2,3} these results may have clinical relevance. At least within the context of model cells, the analysis allows us to understand in quantitative terms why HERG block leads to minimal AP prolongation in some cells but dramatic effects in others. As genomic data from individual patients become increasingly available, extensions of these ideas will help us to predict which individuals will respond poorly to particular drugs. Fig. 5B, for instance, predicts that the two consequences of a particular silent mutation (R583C amino acid substitution caused by C1747T nucleotide change in KCNQ1²⁸), a decrease in G_{Ks} and a positive voltage shift in I_{Ks} gating (positive V_{Ks}), contribute roughly equally to the phenotype of enhanced AP prolongation. Additional identified silent mutations and polymorphisms can be understood similarly. For instance, Itoh et al recently reported two novel mutations in KCNQ1.³³ The analysis predicts that the large decrease in G_{Ks} produced by the R213C mutation would have a larger effect on AP prolongation than the relatively small positive voltage shift produced by the R243H mutation. An interesting avenue for future work would be to comprehensively evaluate the relative quantitative effects of a large number of polymorphisms.³⁴

In addition, analysis such as that presented should assist in the development of therapies aimed at preventing arrhythmias by increasing repolarization reserve,¹³ since the results indicate which ionic currents represent the best targets. For instance, Fig. 3A and Table 2 suggest that increasing G_{Ks} might effectively reduce ΔAPD produced by HERG block whereas agents targeting transient outward current (G_{to}) or Na^+-Ca^{2+} exchange current (K_{NCX}) would be significantly less effective.

Limitations

It should be noted that although drug-induced QT prolongation is generally considered an appropriate surrogate for increased arrhythmia risk,¹⁻³ several investigators have suggested that other markers such as transmural dispersion of repolarization and AP triangulation might more effectively predict pharmacological pro-arrhythmia.^{4,5} Although we have only considered APD_{90} and ΔAPD in the present study, it is important to emphasize that our randomization/regression procedure is broadly applicable, and any output that can be extracted from the simulation results can be analyzed using this technique. Future work can address this limitation by investigating how the effects of model parameters on ΔAPD differ from effects on alternative pro-arrhythmia markers such as triangulation, transmural dispersion, rate dependence, and the propensity to develop afterdepolarizations. Such studies are likely to provide insight into why AP prolongation is frequently, but not always, a strong indicator of arrhythmia risk.

Another important limitation concerns the inherent differences between competing electrophysiological models (see Supplementary Figures S1–S4).^{17,35} As a result of these differences, a prediction generated by any particular model should not be considered universally applicable. For example, the paradoxical contribution of G_{Ca} observed in the TNNP model (Fig. 4) was not seen in other models. We mitigated against this limitation by analyzing several models and generating a consensus list (Table 2) reflecting predictions that are consistent across several models. A third limitation is that our approach can only be used to understand changes in channel densities or kinetic properties relative to the control values in a cell considered typical. At present, this framework cannot address the effects of channels that are absent in healthy cells but get "switched on" during disease progression.

Finally, our simulations were performed with 75% reduction of the HERG channel conductance. Whether the results remain the same with different degrees of HERG block, or different mechanisms of HERG block (i.e. voltage-dependent) remains an interesting question for future work.

CONCLUSIONS

Our study provides, to the best of our knowledge, the first thorough quantification of repolarization reserve. In doing so, we were able to not only confirm that the biological context within which the HERG channel is placed matters, but also to specify the extent to which other channels contribute to the response to HERG block. Such an understanding is critical to determine why different individuals exhibit divergent responses to the same drug. Since the pharmaceutical industry has been compelled to withdraw numerous drugs from the pipeline and even from the market due to unanticipated pro-arrhythmic effects, our method may eventually provide a low cost approach for characterizing pro-arrhythmic effects in terms of risks to specific segments of the population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Non-standard Abbreviations

AP	Action Potential
APD	Action Potential Duration
ECG	Electrocardiogram
HERG	Human Ether-a-go-go Related Gene.
HF	Heart Failure
LQTS	Long QT Syndrome
NCX	Sodium Calcium Exchanger
SERCA	Sarco/Endoplasmic Reticulum Ca ²⁺ ATPase
TNNP	ten Tusscher-Noble-Noble-Panfilov (model)

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Figure 1. Multivariable regression to quantify repolarization reserve

(**A**) Model parameters (**X**), simulation results (**Y**) and parameter sensitivities (**B**) are collected in matrices with the indicated structures and relationships. Values in matrices are represented using the indicated color table. (**B**) Three trials with similar baseline APDs and responses to HERG block (75% reduction in G_{K_r}) that are either typical ($\Delta\text{APD}=23.7$ ms, top), smaller than normal ($\Delta\text{APD}=15.9$ ms, middle), or larger than normal ($\Delta\text{APD}=45.3$ ms, bottom).

Figure 2. Cell-to-cell variation in the response to HERG block
(A) Distribution of ΔAPD from a set of 300 trials performed with the TNNP model, illustrating sample-to-sample variability in the drug response. (B) Baseline APD versus ΔAPD in simulations with the TNNP model shows a lack of correlation in this model.

Figure 3. Results of multivariable regression

(A) Elements of the matrix **B**, indicating how each model parameter influences ΔAPD , are plotted in descending order of importance. Green bars indicate parameters for which an increase leads to greater ΔAPD whereas red bars indicate parameters for which an increase leads to smaller ΔAPD . (B) Model validation of interesting regression analysis predictions. Voltage dependence of I_{Kr} activation (V_{xr1}) is the least important parameter whereas voltage dependence of I_{Kr} inactivation (V_{xr2}) is the third most important contributor. Simulations show APs before and after HERG block, shifting either V_{xr1} or V_{xr2} by +20 mV.

Figure 4. Counterintuitive contributors to HERG block

(A) Regression coefficients for APD (abscissa) plotted versus regression coefficients for ΔAPD (ordinate). (B) Increasing L-type Ca^{2+} current (G_{Ca}) by 3 times increases baseline APD but decreases ΔAPD (top). This occurs because increased G_{Ca} leads to an elevated plateau potential (middle) and increased activation of I_{Ks} (bottom).

Figure 5. Matrix multiplication to understand complex phenotypes

When multiple parameters change in a particular condition, the net effect on ΔAPD can be calculated as the matrix product of the change in parameters and the relevant parameter sensitivities. In all panels, values in matrices are represented using the red blue color table shown at the top left. Images are scaled from -0.6 to $+0.6$. **(A)** Parallel changes in G_{Ca} and G_{Ks} lead to no net change in APD but additive changes in ΔAPD . $\Delta \text{APD} = 55 \text{ ms}$ when G_{Ca} and G_{Ks} are decreased by 40% and 60% respectively. **(B)** Changes in I_{Ks} associated with a silent mutation in $KCNQ1^{28}$ that leads to both decreased conductance and activation at more positive potentials. The changes in G_{Ks} and V_{Ks} contribute roughly equally to the increase in ΔAPD . **(C)** Simulation of decreased repolarization reserve in heart failure. Changes in four model parameters were simulated (as per Winslow et al²⁹). Each change is multiplied by its corresponding parameter sensitivity, and the overall change in ΔAPD can be calculated as the sum of these products.

Table 1

Ranked list of TNNP model parameters that showed the greatest difference in the magnitude between the parameter sensitivities corresponding to APD and Δ APD

Rank	Parameter	Definition
1	G_{Kr}	Maximal rapid delayed rectifier conductance
2	p_f	L-type Ca^{2+} current voltage-dependent inactivation time constant
3	G_{Ca}	Maximal L-type Ca^{2+} current conductance
4	V_{xr2}	Rapid delayed rectifier K^+ current inactivation
5	p_{xs}	Slow delayed rectifier activation time constant

Table 2

Consensus list of model parameters that were consistently important in the determining repolarization reserve across several mathematical models.

Rank	Parameter	Definition
1	G_{K1}	Maximal inward rectifier conductance
1	G_{Kr}	Maximal rapid delayed rectifier conductance
3	V_{K1}	Inward rectifier K^+ current voltage-dependent rectification
4	G_{Ca}	Maximal L-type Ca^{2+} current conductance
5	G_{Ks}	Maximal slow delayed rectifier conductance
6	V_d	L-type Ca^{2+} current activation
7	K_{NaK}	Maximal Na^+ - K^+ pump current
8	V_f	L-type Ca^{2+} current voltage-dependent inactivation
9	p_{xs}	Slow delayed rectifier activation time constant
10	K_{NCX}	Maximal Na^+ - Ca^{2+} exchange current