Exploring the Role of pH in Modulating Effects of Lidocaine in Virtual Ischemic Tissue

Karen Cardona*, Beatriz Trénor*, Germán Moltó$, Miguel Martínez%, José María Ferrero (Jr)*, Frank Starmer#, Javier Saiz*

*Instituto de Investigación Interuniversitario en Bioingeniería y Tecnología Orientada al Ser Humano, Universidad Politécnica de Valencia (I3BH), Spain
$Departamento de Sistemas Informáticos y Computación, Universidad Politécnica de Valencia, Spain
%Instituto Universitario de Automática e Informática Industrial, Universidad Politécnica de Valencia, Spain
#Biostatistics and Bioinformatics, Duke University, Singapore

Running head: Simulation of lidocaine effects in ischemia

Contact information: Javier Saiz, Ph.D.
Instituto de Investigación Interuniversitario en Bioingeniería y Tecnología Orientada al Ser Humano (I3BH)
Universidad Politécnica de Valencia
Camino de Vera s/n
46022 Valencia
SPAIN
Tel: +34 96 387 75 18 (Ext. 67016)
Fax: +34 96 387 76 09
email: jsaiz@gbio.i3bh.es
ABSTRACT

Lidocaine is a class I antiarrhythmic drug that blocks sodium channels and exists both as neutral and charged forms at a physiological pH. In this work, a mathematical model of pH and the frequency-modulated effects of lidocaine has been developed and incorporated into the Luo-Rudy model of ventricular action potential. We studied the effects of lidocaine on sodium current, maximum upstroke velocity, and conduction velocity (CV); and demonstrated that a decrease of these parameters was dependent on pH, frequency, and concentration. We also tested the action of lidocaine under pathological conditions. Specifically, we investigated its effects on conduction block under acute regional ischemia. Our results in one-dimensional fiber simulations show the reduction of the window of block in the presence of lidocaine, thereby highlighting the role of reduced CV and safe conduction. This reduction may be related to the antifibrillatory effects of the drug by hampering wavefront fragmentation. In bi-dimensional acute ischemic tissue, lidocaine increased the vulnerable window for reentry and exerted proarrhythmic effects. In conclusion, the present simulation study uses a newly formulated model of lidocaine, which considers pH and frequency-modulation, and reveals the mechanisms by which lidocaine facilitates the onset of reentries. This study also helps to increase our understanding of the potential antifibrillatory effects of the drug.

Keywords: antiarrhythmic drug, sodium channel, guarded receptor theory, rate-dependence, ischemia.
INTRODUCTION

Lidocaine is a class I antiarrhythmic drug that exerts its effect by blocking inward sodium current ($I_{Na}$) in a use-dependent manner, and is more effective for high stimulation frequencies (11). The block of $I_{Na}$ by lidocaine leads to a decrease in the maximal action potential ($dV/dt_{\text{max}}$) (16; 37) and conduction velocity ($CV$) (1).

Lidocaine is a tertiary amine and at a physiological pH the drug exists both as neutral and charged forms (5; 24). Lidocaine is ionized with a $pK_a$ value in the range of 7.5 to 8.2 to give an equilibrium mixture of charged and neutral forms. The charged form predominates at low pH (6.4) due to the protonation of the neutral form with hydrogen molecules. Experimental data have confirmed that lidocaine is more effective and recovery from block is slowed (5; 35; 57) under acidosis. Therefore, the pH value plays an extremely important role in the effects of lidocaine.

The substantial fall in external pH during some pathologies, such as myocardial ischemia, changes the kinetics of the drug binding to the sodium ($Na^+$) channels and may partially explain the heightened depressant effect of this drug during myocardial ischemia (13; 54).

Currently, lidocaine is one of the most controversial pharmacological agents among the antiarrhythmic drugs. The mechanism by which lidocaine suppresses or exerts arrhythmic effects is still not well understood. Experimental studies have shown that the reduction of excitability provoked by lidocaine promotes proarrhythmic effects, especially under ischemic conditions. This is because the level of external pH decreases and the action of lidocaine on the $Na^+$ channel increases, leading to a higher dispersion of conduction in the tissue (46; 60). However, other studies support the antifibrillatory effects of lidocaine in hampering wavefront fragmentation (7; 62). By modeling the effects of lidocaine on
sodium current, computer simulations can be conducted at cellular, tissue, and organ levels. The resultant studies will definitely shed light into the mechanisms of action of this drug and its role in arrhythmogenic processes. The goal of the present work is to study the effect of pH on the action of lidocaine in ventricular myocardium under normal and pathological conditions. A mathematical model of the effect of lidocaine on the Na\(^+\) channel is proposed and incorporated into a ventricular action potential model. The effects of lidocaine on AP characteristics for different pH values and concentrations of the drug are analyzed. Furthermore, a theoretical investigation focusing on the mechanism by which lidocaine exerts controversial effects under ischemic conditions is presented.

**METHODS**

**Formulation of lidocaine model**

To formulate the model of the effects of lidocaine on Na\(^+\) channels, it is important to consider that drugs such as lidocaine have two specific accesses to the Na\(^+\) channel receptor, i.e. through hydrophobic and hydrophilic pathways, depending on the local pH (26). The model of lidocaine-I\(_{Na}\) interaction formulated in this work takes into account the modulatory effect of pH on the Na\(^+\) channel block. The mathematical model is based on the guarded receptor theory (GRT) proposed by Starmer (48). In our formulation, three main processes have been considered: the hydrophobic pathway, the hydrophilic pathway, and coupling between blocked channels by charged and neutral forms using a proton exchange process.

It is widely known that the hydrophobic pathway is available at all times to lipid-soluble molecules (35). Several experimental studies developed in conditions in which the neutral drug form was present in a higher proportion have suggested that the use-dependent block
93 of Na⁺ channels in cardiac tissue represents a balance between block development during depolarization (activated state) and incomplete recovery after repolarization (inactivated state) (11; 23). On the other hand, Bean et al. found an initial blockade produced by lidocaine when a voltage clamp protocol was applied, suggesting that the drug was bound in the resting state, although the binding was weaker than in inactivated or activated states (3). According to this experimental evidence, we have assumed that the neutral form can bind and unbind in all states of the Na⁺ channel: resting (R), activated (A) and inactivated (I), as shown in Figure 1. This figure represents the kinetic diagram of the complete model, and Equation (1) mathematically describes the interaction of the neutral form with Na⁺ channels.

\[
\frac{db_N}{dt} = \left[ m^3hj\cdot k_A + m^3(1-hj)\cdot k_I + \left(1-m^3\right)\cdot kp \right] \cdot D_N \cdot (1-b_N - b_C) - \\
\left[ m^3hj\cdot l_A + m^3(1-hj)\cdot l_I + \left(1-m^3\right)\cdot l_R \right] \cdot b_N + l_p \cdot b_C - kp[H^+] \cdot b_N
\]

(1)

\(b_N\) stands for the fraction of channels blocked by the neutral form. The association and dissociation rate constants for the different states (activated, inactivated, and resting) are: \(k_A, k_I, k_R, l_A, l_I, l_R\), respectively. Additionally, we have taken into account the rate constants \(k_p\) and \(l_p\) to determine the proton exchange process.

Regarding the charged form, different authors (14; 61) suggest that interaction with the channel only takes place in the activated state. Indeed, Moorman et al. (35) suggested that the charged drug form can bind through the aqueous side of the channel pore during diastole, when inactivation gates (h and j) are open. On the other hand, Liu et al. (33) found a restricted access to the inner pore receptor in the resting and inactivated states of the charged drug form.

As a result, the fraction of channels blocked by the charged form (\(b_C\)) is described in Equation (2).
In this case, the association and dissociation rate constants are \( k_C \) and \( l_C \), respectively.

The exponential function modifying the unbinding rate in the above expression is derived from the work required to move a charged molecule of the drug across the membrane potential. The complete model is represented by the kinetic diagram shown in Figure 1.

In order to introduce the effects of lidocaine on \( I_{Na} \), as formulated in Equation (3) by Luo and Rudy (34), we have introduced the factor \( (1-b) \) in the \( I_{Na} \) equation, as shown in Equation (4).

\[
\frac{db_C}{dt} = \left[ m^2 h j \cdot k_C \right] [D_C] \cdot (1 - b_N - b_C) - \\
\left[ m^2 h j \cdot e^{-\frac{\nu_{Na}}{RT} \cdot I_C} \right] \cdot b_C - I_p \cdot b_C + k_p [H^+] \cdot b_N
\] (2)

\( I_{Na} = \overline{g}_{Na} \cdot m^3 h j \cdot (V_m - E_{Na}) \) (3)

\( I_{Na} = \overline{g}_{Na} \cdot (1 - b) \cdot m^3 h j \cdot (V_m - E_{Na}) \) (4)

\( Na \) is the maximum conductance; \( m, h \) and \( j \) are the channel gates; \( V_m \) is the membrane potential; and \( E_{Na} \) is the reversal potential; \( b \) stands for the total fraction of channels blocked by the neutral and charged forms of lidocaine.

**Action potential model**

In this work, the Luo-Rudy (LRd) model of the AP (17; 34) was used to simulate the guinea pig ventricular electrical activity. This model includes the formulation of membrane ionic channel currents following the Hodgking-Huxley formalism, ionic pumps, and exchangers that regulate ionic concentration changes; as well as dynamic changes of intracellular calcium. Intracellular processes represented in the model include Ca\(^{2+}\) uptake and Ca\(^{2+}\) release by the sarcoplasmic reticulum (SR); and Ca\(^{2+}\) buffering by calmodulin and troponin (in the myoplasm) and calsequestrin (in the SR).

To simulate AP propagation in one-dimensional and bi-dimensional tissues, we used the reaction-diffusion equation described in Equation (5).
\[
\frac{1}{S_v} \left( \frac{1}{\rho_x} \frac{\partial^2 V_m}{\partial x^2} + \frac{1}{\rho_y} \frac{\partial^2 V_m}{\partial y^2} \right) = C_m \frac{\partial V_m}{\partial t} + \sum I_{ion} + I_{st}
\]  

where \(C_m\) is the membrane capacitance (1\(\mu\)F/cm\(^2\)), \(I_{ion}\) stands for the sum of ionic current densities, \(I_{st}\) is the stimulus current density, \(S_v\) is the surface-to-volume ratio, and \(\rho_x\) and \(\rho_y\) are the cellular resistivities in the longitudinal (x) and transversal (y) directions, respectively.

Equation (5) was solved using the operator-splitting method. The LRd equation was solved using an explicit Euler method with a lookup table for the voltage-dependent parameters. The diffusion equation was solved using the Cranck-Nicholson method. This method was also used in our previous publications (21; 40; 41; 55; 56). ‘No-flux’ boundary conditions were applied. A time-step of 24 \(\mu\)s was chosen in order to satisfy the von Neumann linear stability criterion.

Longitudinal CV in normal conditions was 0.3 m/s. Different values for longitudinal and transverse resistance were used so as to achieve anisotropy in the bi-dimensional tissue with a 3.5:1 longitudinal-transverse CV ratio in normal tissue.

Model of regional acute ischemia

To simulate regional acute ischemia we used a model described in detail in our previous publications (22; 55; 56). In this model, the heterogeneity introduced by acute ischemia was simulated by three main components: hyperkalemia, hypoxia, and acidosis. Hypoxia was taken into account by partial activation of ATP-sensitive K\(^+\) current (\(I_{K(\text{ATP})}\)) (21), hyperkalemia was considered by the elevation of the extracellular K\(^+\) concentration ([K\(^+\)]\(_o\)); \(I_{Na}\) and the calcium (Ca\(^{2+}\)) current through the L-type channels (\(I_{CaL}\)) were reduced to mimic acidosis.
These components were altered in the central ischemic zone (CZ) and a border ischemic zone (BZ) was also considered where the parameters varied linearly from the normal values (present in the normal zone (NZ)) to the ischemic values. Different degrees of severity of acute ischemia (i.e. at different minutes after the onset) were also simulated by increasing the severity of the altered parameters in the CZ (see Trenor et al. 2007 (56) for details).

We assumed that lidocaine did not reach the CZ because of restricted blood flow, and that the diffusion is linear in the BZ. The concentrations of the drug considered in the simulations were 20, 50, and 100 µmol/L.

The effects of the drug under ischemic conditions were analyzed initially in a regionally ischemic one-dimensional fiber composed of 370 nodes (100 µm); and later in a regionally ischemic bi-dimensional tissue composed of 550 x 550 nodes (100 µm × 100 µm) (see Trenor et al. 2007 (56)).

Protocols and Parameters

In all the simulations conducted in the present study, we first reached the steady-state for the lidocaine block. For this purpose, the cells or tissue were paced continuously with a basic train of 10 pulses, 1.5 times the diastolic threshold in amplitude, and using basic cycle lengths (BCLs) of 200, 500, and 1000 ms in various simulations.

To evaluate the action of lidocaine on AP characteristics, dV/dt_{max} and CV were measured in the central nodes of the 370 fiber nodes to avoid border effects.

Protocol S1-S2 was used to measure the window of block (WB) in the one-dimensional fiber and the vulnerable window (VW) in the bi-dimensional tissue. The basic stimulation (S1) consisted of 10 rectangular current pulses of 2 ms in duration and 1.5 times the diastolic threshold in amplitude. Subsequently, an extraestimuli (S2) was delivered at different coupling intervals (CIs). The WB was defined as the interval of CIs for which the
delivery of $S_2$ led to propagation block. Finally, the VW was defined as the interval of CIs for $S_2$ delivery giving rise to a reentrant circuit.

RESULTS

Drug-ion channel interaction

To model the drug-ion channel interaction, we first determined the numeric values of the rate constants present in the formulation of the lidocaine model described in the methods section. For this purpose, we used the genetic algorithm method described by Houck et al. (27). Using this algorithm, we fitted Equations (1), (2), and (4) to the experimental data obtained by Schwarz et al. (42). The equations represent the kinetics of the drug when it binds and unbinds the receptor of the channel in both forms of lidocaine (neutral and charged). The experimental data from Schwarz et al. represent the fraction of unblocked Na$^+$ channels by 0.2 mmol/L lidocaine for different pH values (pH 6 and 7.2). The BCLs used were 200 ms, 400 ms, and 800 ms and the use-dependent effect was observed. It is worth noting that such observations and experimental data are scarce in the scientific literature. Table 1 shows the fitted values for the association and dissociation rate constants obtained. The goodness of fit is shown in Figures 2A and 2B where the maximum error obtained was less than 10%.

Effects of lidocaine on sodium current

To characterize the action of lidocaine on the sodium current, we measured the inhibition of peak Na$^+$ current for different pH values, BCLs, and concentrations of the drug when the steady-state was achieved. We conducted voltage clamp simulations applying a test pulse of -20 mV from a holding potential of -140 mV. Peak $I_{Na}$ was then measured and normalized to the control value. Figure 3 represents the dose-response curve showing the decrease of the normalized peak $I_{Na}$ for increasing lidocaine concentrations for various BCL and pH values. We also obtained the concentration of the drug that provoked
a 50% inhibition ratio (IC$_{50}$) on the Na$^+$ current, which yielded 52, 68, and 185 µmol/L for BCL values of 300, 500, and 1000 ms, respectively in the case of 6.4 pH (see panel A Figure 3). In the case of a pH of 7.4 (see panel B Figure 3), the value of IC$_{50}$ increased to 92, 107, and 161 µmol/L, respectively for the same BCL values. Our results highlight the pH- and use-dependence of IC$_{50}$ values for the first time in experimental studies.

An important advantage of the proposed mathematical model of lidocaine is the opportunity to characterize by simulation the specific interaction of the two forms of the drug with the Na$^+$ channel, i.e. how the pH modulates the action of the charged and neutral forms of lidocaine. For this study, we applied a train of pulses (BCL of 500 ms) in the presence of 100 µmol/L of lidocaine for pH values of 6.4 and 7.4, and observed the blockade produced by both forms of the drug, as represented in Figure 4. Our results show that the inhibition of peak I$_{Na}$ was higher for pH 6.4 than for pH 7.4 (see panels A and B in Figure 4). The recovery from block was slowed for the lower pH value. Panels C and D in Figure 4 depict the total and partial blockade of the charged (b$_C$) and neutral (b$_N$) forms of lidocaine for both pH values. For a pH of 6.4, b$_C$ contributed the most to the total blockade (b) due to the increase in the fraction of the charged form. However, if extracellular pH increases, the fraction of neutral lidocaine is enhanced and becomes the main responsible for the total blockade.

**Effects of lidocaine on action potential**

We incorporated the model of lidocaine into the LRd AP model (34) to simulate the effects of lidocaine on AP features. Specifically, we measured the reduction of the maximum AP upstroke and the CV for different stimulation frequencies and pH values when increasing concentrations of the drug.

Firstly, we validated our model by comparing simulated dV/dt$_{max}$ for different concentrations of lidocaine and BCLs against experimental recordings obtained by Nawada
and Enhring et al. (16; 37), as depicted in Figure 5 panels A and B, respectively. This comparison reveals the consistency of the model in reproducing experimental observations. The maximum relative errors with respect to experimental data (dots) were 6.4% and 12% in panels A and B.

Secondly, we explored the influence of pH and stimulation frequency on the CV decrease provoked by lidocaine and the results are shown in Figure 5 panels C and D. Under normal conditions, the CV was 0.3 m/s, whereas in the presence of 100 µmol/L lidocaine for a BCL of 200 ms the CV was 0.22 m/s (75% of control value) and 0.27 m/s (92% of control value) for pHs 6.4 and 7.4, respectively. The simulation results showed again the pH and use-dependent effects of lidocaine on CV.

Effects of lidocaine under ischemic conditions

We also explored the action of lidocaine under ischemic conditions, since several experimental studies have suggested that antiarrhythmic drugs modify their action when acidosis is present, i.e. under pathological situations, such as ischemia (7; 30; 62). Firstly, we analyzed the effects of lidocaine on I_{Na} on CV and on conduction block in an acute regional ischemic fiber. Secondly, the study was extended to bi-dimensional regionally ischemic tissue.

We first measured peak I_{Na} in control and in the presence of 100 µmol/L lidocaine for different zones (NZ, BZ, and CZ) of a 370 node fiber (see Figure 6B, where only values up to node #264 are shown). Peak I_{Na} in the presence of the drug decreased to 42%, 64%, and 99% of the control value in NZ (node #54), BZ (node #94) and BZ (node #174), respectively. Note that peak I_{Na} was unaffected in the CZ, as we assumed that lidocaine did not have access to the central ischemic zone due to restricted blood flow.

Regarding the lidocaine effects on CV, Figure 6C highlights the higher reduction of CV in the NZ than in the BZ. Whereas the decrease was similar in all nodes of the NZ, the
262 reduction in the BZ was inhomogeneous, being more accentuated in the proximal BZ and less pronounced in the distal BZ. As expected, CV was not altered in the CZ. The simulations also reproduced supernormal conduction in the BZ regardless of lidocaine concentration, as observed experimentally in regionally ischemic tissues (29; 45). Longitudinal conduction velocity showed a biphasic behavior across the different zones of the tissue. In the NZ, CV remained constant, while in the proximal BZ, supernormal conduction was observed due to mild hyperkalemia (44). As the degree of hyperkalemia increased in the distal BZ, where acidosis was also present, CV was reduced. Note that lidocaine enhanced supernormal conduction. Indeed, in the absence of the drug, CV increased by 6.2% from the normal to border zone; while in the presence of different concentrations of lidocaine (20, 50, and 100 µmol/L) the increase was 8.7%, 9%, and 12%, respectively. This effect is related to the linear diffusion of the drug in the BZ modeling a restricted blood flow. In the NZ, the reduction of I_{Na} was higher than in the proximal BZ, where mild hyperkalemia and a lesser reduction of I_{Na} accentuated CV regional increases.

276 The alteration of I_{Na} and CV provoked by lidocaine led to changes in propagation patterns in the regionally ischemic fiber, as depicted in the right panels of Figure 6. In order to analyze the changes in propagation patterns with lidocaine administration, simulations were conducted using an S_{1}-S_{2} protocol and the WB was subsequently evaluated. Figures 6E, 6F, and 6G depict the three patterns of propagation obtained for different CIs. A short CI did not allow AP propagation (Figure 6E) because of refactoriness, intermediate CIs led to a propagation block (Figure 6F), and later CIs allowed propagation throughout the fiber. These simulations were systematically carried out for different concentrations of the drug and different ischemic conditions and several minutes of acute regional ischemia. Table 2 shows the different WBs obtained under the different conditions. For minutes 6 and 7 after coronary occlusion, the WB yielded 8 and 51 ms in the absence of the drug,
respectively. Lidocaine decreased these values to 0 and 27 for minutes 6 and 7, respectively. Indeed, the reduction of I_{Na} by lidocaine led to safer conduction (43) and decreased the likelihood of conduction block. In conclusion, we observed that the WB decreased for all concentrations of lidocaine, suggesting that the drug could exert an antifibrillatory effect by hampering wavefront breaks.

The final step taken to evaluate lidocaine effects under ischemic conditions was to simulate the effects of the drug on a regionally ischemic bidimensional tissue prone to reentrant circuits, where an NZ, a BZ, and a CZ were defined (see Figure 7, panel A). Simulations were also carried out using an S_{1}-S_{2} protocol applied to the top edge of the tissue; and the VW for reentry was calculated for various degrees of severity of ischemia and various concentrations of the drug (20, 50, and 100 µmol/L) as shown in Figure 7A.

Figure 7D shows the existence of different patterns of activation obtained after the delivery of S_{2} applied at different CIs. These patterns were no propagation (NP), reentry, bidirectional block (BDB), and complete propagation or collision. In the first case, the delivery of S_{2} at early CIs produced no propagation because of refractoriness. When S_{2} was delivered slightly later, two patterns were observed: reentry or BDB. When S_{2} was delivered, a conduction block around the CZ took place, so that the wavefront travelled around the CZ and invaded retrogradely the distal CZ after its recovery from refractoriness, and gave rise to a reentrant circuit. Figure 7B shows the establishment of a complete figure-of-eight reentry in the absence of lidocaine (upper snapshots) and in the presence of 100 µmol/L lidocaine (lower snapshots). By comparing both reentrant patterns, it is notable that although the reentry pattern did not show dramatic changes, the propagation was considerably slowed in the presence of lidocaine. In the case of BDB (patterns not shown), S_{2} was blocked in the proximal CZ and as the two alternative pathways surrounded the ischemic zone tried to reenter retrogradely, a second block was observed in the distal CZ.
For later CIs, $S_2$ propagation could be achieved throughout the tissue or collision of the various wavefronts occurred. The VWs for reentry are shown in Figure 7C, and follow a unimodal behavior with ischemia time course peaking (35 ms) 6 minutes after the onset of ischemia. If the concentration of lidocaine was increased, the VW would become wider, indicating a higher vulnerability to reentry in the presence of the drug. This effect can be due in part to the heterogeneous action of the drug in the diverse zones of the tissue, contributing to a dispersion of CV and setting the stage for reentry.

Finally, to throw some light on the mechanism of the action of lidocaine, an analysis of the upper and lower limits of the VW shown in Figure 7D was carried out. In all cases studied, the upper limit of the VW was shifted to higher CIs. For CIs above this limit, collisions occurred and the upper limit was higher for increasing concentrations of lidocaine. Indeed, the drug decreased CV so that the collisions between antegrade and retrograde wavefronts took place for later CIs. The lower limit of vulnerability decreased with higher lidocaine concentrations at minute 7 after the onset of ischemia, so widening the VW (see Figure 7C). This phenomenon can be explained by the effect previously described in one-dimensional propagation in the regional ischemic fiber. Lidocaine decreased CV and led to safer conduction, while increasing the time for recovery from refractoriness of the ischemic tissue. As a result, propagation is possible when the retrograde wavefront reaches the distal ischemic zone and a reentrant circuit is established.

For the same CI in the absence of the drug, BDB would take place as propagation goes faster and the CZ is not ready to develop AP.

In conclusion, our simulations demonstrate that the slow conduction provoked by lidocaine could facilitate the onset of reentries in regionally ischemic tissues by widening
the VW; but at the same time the drug can hamper wavefront breaks by slowing CV and exerting antifibrillatory effects.

**DISCUSSION**

**Model of lidocaine**

Several models have been proposed to explain how lidocaine blocks cardiac sodium channels (12; 25; 36). However, no model has been successful in reproducing several important drug effects, especially pH-dependent effects. Irvine (32) and Comtois et al. (12) formulated a model of lidocaine based on the modulated receptor hypothesis. Moreover, Furukawa et al. (23) formulated lidocaine interaction using the GRT. These models effectively reproduced the concentration- and use-dependence block. However, they did not take into account the action of lidocaine when pH was modulated, although ample experimental studies provide evidence that acidosis potentiates Na⁺ channel blockade by lidocaine. Finally, Starmer et al. (50) proposed a model of the effects of a tertiary drug on sodium channels that took into account pH modulation. Nevertheless, the model could not quantify the kinetic parameters representing the interaction of both forms of lidocaine with Na⁺ channels. In the present work, the incorporation of both hydrophilic and hydrophobic paths described by Hille (26) in the model of lidocaine improves on previously formulated models of the drug. The formulated model takes into account the different interactions with Na⁺ channels according to the form of lidocaine. As hypothesized by Clarkson et al. (11) under normal pH, lidocaine associates and dissociates with the receptor in the activated and inactivated states. Accordingly, in our model, the neutral form binds and unbinds the receptor of the channel mainly in the activated and inactivated states. However, the interaction with the resting state was also considered, as binding of the drug also takes place at holding potential (3; 23). It should also be noted the association rate constant in the resting state (k_R) yielded a much smaller value than in the inactivated state (k_I), or in the
activated state \((k_A)\) in the model. With respect to the charged forms, there is ample experimental evidence suggesting that the block takes place when the channel is open, and then the drug is trapped in the closed or inactivated states (36; 58; 61). These observations were also taken into account in our model.

With reference to the parameter fitting, our results demonstrated that the GA is a useful tool to determine the rate constants in a binding and unbinding process of the drugs. GAs are currently used by many authors to fit parameters describing the biological behavior of ionic currents, drugs, and pathologies (15; 20; 38; 47; 52). In the present work, a good fit to experimental data was obtained using GA and our model was validated against experimental data from various authors.

The formulated model reproduced experimental observation of IC\(_{50}\) values. Under normal pH conditions, the IC\(_{50}\) value predicted by our model was 166 µmol/L, very close to the value of 134 µmol/L that was experimentally observed (28). Furthermore, the lidocaine potency increased (IC\(_{50}\) value decreased) for lower pH values, as experimentally evidenced (53). An additional and relevant aspect of the present work is the finding that IC\(_{50}\) was reduced when a high rate of stimulation was applied and in the presence of acidosis. Indeed, in the scientific literature, there is no experimental or simulation data describing this observation, which is an important contribution of the present study. The variation of peak I\(_{Na}\) block by lidocaine with pH and stimulation rates obtained in our simulations was also in agreement with the scarce experimental data extracted from the scientific literature (11; 23).

Additionally, the dose- and frequency-dependent effects of lidocaine on CV, which are determinant in assessing the role of the drug in arrhythmogenesis, was also reflected in our model, together with experimental results. Indeed, we obtained a reduction of 14% in CV
for a BCL of 200 ms and 20 µmol/L lidocaine, similar to the reduction obtained by Anderson et al. (1)(Figure 2) and Quinteiro et al. (39) (Table 1) under the same conditions.

**Effects of lidocaine under ischemic conditions**

The action of lidocaine under regional ischemic conditions has been analyzed in both one dimensional and bi-dimensional tissues. The substantial fall in external pH during myocardial ischemia changes the kinetics of drug-binding to Na\(^+\) channels and gives rise to the controversial actions of the drug. These actions have been mechanistically approached in the present simulation study.

An important result obtained in our simulations of the regional ischemic fiber was that lidocaine reduced the heterogeneously peak for I\(_{\text{Na}}\) and CV while decreasing the WB, thus improving AP propagation in the ischemic fiber. Other computational and theoretical studies have pointed out how under conditions of depressed excitability and conduction velocity, AP propagation can become safer (40; 45; 56). Shaw and Rudy suggested that when I\(_{\text{Na}}\) is partially blocked or depressed, I\(_{\text{CaL}}\) becomes more important in terms of supporting conduction, leading to slower but safer conduction (45). Furthermore, the decrease in CV provoked by lidocaine delays activation time, so that cells have more time to recover their excitability before the arrival of the premature stimulus. Indeed, the recovery from refractoriness is determinant to assuring conduction; and several studies suggest that the availability of sodium channels (as an indicator of the recovery of refractoriness) must reach a certain threshold to assure wavefront propagation (4; 21). However, Starmer et al. (51) explained how a complex balance of CV, recovery time from block, and balance of background ionic currents could be responsible for the antiarrhythmic effect of lidocaine. In agreement with this reasoning, we observed how lidocaine reduced CV and favored a safer conduction, so tending to narrow the WB.
In this way, the presence of lidocaine under ischemic conditions can prevent propagation block and wavefront fragmentation; and may exert antifibrillatory effects. The effectiveness of lidocaine against fibrillation was also experimentally observed by Cardinal et al. (8) during coronary occlusion, preventing the fractionation of wavefronts into multiple wavelets and micro-reentrant circuits.

It must be noted that several factors, such as the tissue condition or the applied concentration of the drug, are responsible for the controversial pro or antiarrhythmic effects of lidocaine (19; 30) and that simulation studies provide a powerful tool for gaining a greater understanding of the underlying mechanisms.

In the present study, lidocaine exerts proarrrhythmic effects in terms of increasing the probability for reentry generation. Vulnerability to reentry was quantified by the width of the VW, which increased with lidocaine concentration. Similar results have been obtained by other authors (10; 49). For instance, Cimponeriu et al. carried out a simulation work, using a simple formulation of the block of Na\(^+\) channels and quantifying the VW for reentry under ischemic conditions. In their study, the VW was also widened when I\(_{Na}\) was partially blocked. In a different experimental study undertaken by Starmer et al. (49) the block of sodium channels also exerted proarrrhythmic effects and increasing the VW, although these experiments were carried out in rabbit atrium.

The increase in the vulnerability to reentry is associated with dispersion of the electrophysiological characteristics of the tissue (5; 17; 22). In our simulations, lidocaine affected conduction heterogeneously in regionally ischemic tissue and enhanced the already present heterogeneities. Indeed, the drug enhanced the supernormal conduction effect in ischemic BZ, accentuating CV dispersion with respect to control conditions. Regional differences in CV can lead to a situation in which the propagation of premature stimuli is faster in one myocardial region and slower in another, so that repolarization time
will also be heterogeneous across the myocardium. This suggests that regional slowing of
CV causes regional changes in repolarization time and refractoriness, and hence spatial
dispersion. Thus, spatial dispersion of CV may also cause a spatial dispersion in
refractoriness and could facilitate the formation of reentrant waves. Several studies have
extensively demonstrated this effect under ischemic conditions (19; 31; 46; 62).

There are several experimental and theoretical studies of the time course of vulnerability
to arrhythmias after coronary occlusion that follow unimodal behaviors (13; 40; 56; 59).
Our simulations support this observation and as ischemia progressed in the absence of the
drug, the VW increased – peaking for minute 6 and then decreasing again for more severe
ischemic.

Furthermore, it has been experimentally observed that when the degree of myocardial
injury increased, lidocaine facilitated the generation of reentrant circuits (6; 9). In
examining this effect, we observed an important decrease in the lower limit of the VW. In
the absence of the drug, and for low CIs, bidirectional block occurred; whereas reentry and
thus unidirectional block occurred in the presence of the drug for the same CIs. In this way,
lidocaine increased the probability of unidirectional block and prevents bidirectional block.
This observation is in accordance with our one-dimensional results, as lidocaine favors
conduction, and prevents the retrograde block in the distal zone, and thereby gives rise to
the reentrant circuit. The reduction in CV also allows the distal zone of the tissue to recover
from refractoriness when reached by the retrograde wavefront.

**Clinical implications**

Lidocaine is classified as a class Ib antiarrhythmic agent used to suppress arrhythmias.
However, classification is a difficult task since many of the antiarrhythmic agents have
multiple modes of action. The use of lidocaine is generally accepted in antiarrhythmic
therapies during the acute phase of myocardial infarction. However, there is ample evidence that this drug exerts proarrhythmic effects by slowing conduction, so facilitating the formation of reentries under ischemic conditions. This observation has been supported by experimental and clinical studies undertaken by several authors (9; 31; 43; 46; 62) and also by the present work. The possible effectiveness of lidocaine under ischemic conditions is related to the termination of wavefront fragmentation into multiple wavelets and micro-reentrant circuits, exerting antifibrillatory effects. This effect has been proven by Yin (62) and by our results using 1D fibers. To analyze the effects of lidocaine under such situations further theoretical investigations should be undertaken in 2D or 3D tissues prone to fibrillation.

Other authors have investigated the action on lidocaine after 10-15 minutes of coronary occlusion (2; 18). In such studies, an important finding was that lidocaine mainly prevented Na$^+$ loading, thus preventing Ca$^{2+}$ overload and so reducing cardiac damage. In this way, lidocaine exerts antiarrhythmic effects under ischemic conditions.

**Limitations of the study**

Some difficulties were encountered regarding the model of lidocaine. Specifically, data reflecting the use- and pH-dependence effects was scarce in the experimental scientific literature. Data published in 1977 by Schwarz were used to fit the model parameters. However, the experimentally observed effects of lidocaine were reproduced by our model with a high degree of fidelity.

In this work, we have used experimental data from frog because guinea-pig data for pH- and use-dependent block by lidocaine were not available. Yet, the model was validated against data from guinea-pig and accurately reproduced the action of the drug under normal and pathologic conditions.
The most relevant electrophysiological changes have been in the model of ischemia. However, it is well documented that other mechanisms are altered, such as the depression of the sodium potassium pump or other alterations in ionic currents. Furthermore, although regional heterogeneity in the tissue has been considered, the contribution of transmural heterogeneity in the tissues, which was not considered in our case, may have an important role in the genesis of reentries and in arrhythmogenesis when lidocaine is applied. Despite these limitations, our simulations rigorously reproduced the action of lidocaine under normal and ischemic conditions. The similarities between the results obtained from our simulations and experimental observations support the robustness of our model. Our model can be regarded as a new tool to study the mechanism of the action of lidocaine under different pathologies.

Conclusions

We have proposed a model to characterize the behavior of both neutral and charged forms of lidocaine in the cellular electrical activity of ventricular guinea pig ventricles. The model is based on experimental results and takes into account the experimental evidence that suggests an interaction with the channel in a pH-, concentration- and use-dependent manner. Simulations of the effects of lidocaine were conducted and a higher reduction of $I_{Na}$, $dV/dt_{\text{max}}$, and CV was obtained at lower pH and BCL values. Under ischemic conditions, the effect of lidocaine on the conduction block was investigated. In a one-dimensional fiber, our simulations showed a decrease in the window of the block for the different concentrations of lidocaine as a result of the reduction of $I_{Na}$ and CV; with $I_{CaL}$ supporting conduction and so leading to slower but safer conduction. Thus, lidocaine could hamper wavefront fragmentation, and exert antifibrillatory effects. In a bi-dimensional tissue, lidocaine exerts a proarrhythmic effect by increasing the vulnerability to reentries as a consequence of slow conduction and the enhancement of conduction dispersion.
ACKNOWLEDGEMENTS

This work was partially supported by the European Commission preDiCT grant (DG-510 INFSO-224381) through the National Plan for Scientific Research, Development, and Technological Innovation of the Spanish Ministry of Science and Innovation (TEC2008-512 02090, TIN2004-03602), by the Research and Development Support Plan (PAID-06-09-513 2843) of the Universidad Politécnica de Valencia, and by the Directorate-General for Scientific Policy of the Valencian regional government (GV/2010/078) and by the Generalitat Valenciana (BEST/2010/102). The work of K. Cardona is fully supported by the Spanish Ministry of Science and Education (TIC2001-2686).
Reference List


6. **Campbell TJ and Hemsworth PD.** Selective depression of maximum rate of depolarization of guinea-pig ventricular action potentials by amiodarone and


30. Li GR and Ferrier GR. Effects of lidocaine on reperfusion arrhythmias and
electrophysiological properties in an isolated ventricular muscle model of

31. Lin JW, Garber L, Qi YR, Chang MG, Cysyk J and Tung L. Region
[corrected] of slowed conduction acts as core for spiral wave reentry in cardiac

32. Lisa A. Irvine. *Models of the Cardiac Sodium Channel and the Action of

and lidocaine block of heart Na+ channels: evidence from experiments with

34. Luo CH and Rudy Y. A dynamic model of the cardiac ventricular action
potential. I. Simulations of ionic currents and concentration changes. *Circ Res*

35. Moorman JR, Yee R, Bjornsson T, Starmer CF, Grant AO and Strauss HC.
pKa does not predict pH potentiation of sodium channel blockade by lidocaine
and W6211 in guinea pig ventricular myocardium. *J Pharmacol Exp Ther* 238:
159-166, 1986.

36. Moorman JR, Yee R, Bjornsson T, Starmer CF, Grant AO and Strauss HC.
pKa does not predict pH potentiation of sodium channel blockade by lidocaine


FIGURE LEGENDS

Figure 1: Lidocaine interaction with Na$^+$ channels in both forms of the drug: neutral (b$_N$) and charged (b$_C$).

Figure 2: Comparison between experimental data (dots) obtained by Schwarz et al. and simulation data (lines). The simulations were carried out with the model of lidocaine interaction and the same protocol used by Schwarz et al. (42) at pH 6 (A) and 7.2 (B). All currents are normalized (Nor) to the peak current during the first pulse of a train.

Figure 3: Dose response curves for lidocaine at pH 6.4 (A) and 7.4 (B) for different BCLs (300, 500, and 1000 ms). I$_{Na}$ amplitude was normalized to the amplitude determined in the absence of lidocaine.

Figure 4: Panels A and B represent the reduction of the peak I$_{Na}$ when a train of pulses of BCL 500 ms was applied, in the presence of 100 µmol/L lidocaine at pH values of 6.4 and 7.4. Panels C and D show the total blockade (b) produced by lidocaine; and the partial blockade of the charged (b$_C$); and neutral (b$_N$) forms of lidocaine at pH values of 6.4 and 7.4.

Figure 5: Panels A and B show the validation of our model (dashed lines) with experimental data (dots) obtained by different authors: Nawada et al. (37) and Ehring et al. (16). Panels C and D show the effects of lidocaine on CV at pH values of 6.4 and 7.4 for different BCLs. The CV was measured once steady-state was achieved and normalized (Nor) with respect to CV in the absence of the drug.

Figure 6: Effects of 100 µmol/L lidocaine in acute ischemic tissue (six minutes after occlusion). Panel A represents the one-dimensional fiber used for the simulation. The fiber was composed of 370 nodes (100 µm each). Panel B shows I$_{Na}$ in normal conditions (dashed lines) and in the presence of 100 µmol/L lidocaine (solid lines) along the fiber. Panel C shows CV for different concentrations of lidocaine (20, 50, and 100 µmol/L) along
the fiber. Panel D shows the protocol used (S₁-S₂ protocol) to obtain the different patterns of propagation in the presence of lidocaine: no propagation (panel E), propagation block (panel F), and complete propagation (panel G).

Figure 7: Panel A shows regional acute ischemia bi-dimensional tissue including a NZ, BZ, and CZ. S₁-S₂ protocol was applied to the top edge of the tissue. Panel B shows reentry patterns in control conditions and in the presence of 100 µmol/L lidocaine. Each panel contains eight gray-coded voltage snapshots of the virtual tissue after S₂ stimulation. Panel C shows the width of the VW in ms for different degrees of ischemia (5, 6, and 7 minutes after occlusion) and concentrations of lidocaine (20, 50, and 100 µmol/L). Panel D shows bar diagrams of the VW, showing the upper and lower CIs limits of each VW. Various patterns of activation are presented: no propagation (light gray), reentry (gray), BDB (black), and complete propagation or collision (white).

TABLE LEGENDS

Table 1: Values of association and dissociation rate constants for lidocaine.

Table 2: Window of block for different concentrations lidocaine at minutes 6 and 7 after coronary occlusion.
<table>
<thead>
<tr>
<th>Association constants</th>
<th>Dissociation constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_A$ (M$^{-1}$ms$^{-1}$)</td>
<td>5173</td>
</tr>
<tr>
<td>$k_I$ (M$^{-1}$ms$^{-1}$)</td>
<td>4998.4</td>
</tr>
<tr>
<td>$k_R$ (M$^{-1}$ms$^{-1}$)</td>
<td>0.000196</td>
</tr>
<tr>
<td>$k_C$ (M$^{-1}$ms$^{-1}$)</td>
<td>1288</td>
</tr>
<tr>
<td>$k_P$ (M$^{-1}$ms$^{-1}$)</td>
<td>5000</td>
</tr>
</tbody>
</table>
Table 2.

<table>
<thead>
<tr>
<th>Lidocaine (µmol/L)</th>
<th>Minute 6</th>
<th>Minute 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>51</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
<td>27</td>
</tr>
</tbody>
</table>