Facilitation of the L-type calcium current in rabbit sino-atrial cells: effect on cardiac automaticity

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Abstract

Objective: The L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)) contributes to the generation and modulation of the pacemaker action potential (AP). We investigated facilitation of \(I_{\text{Ca,L}}\) in sino-atrial cells. Methods: Facilitation was studied in regularly-beating cells isolated enzymatically from young albino rabbits (0.8–1 kg). We used the whole-cell patch-clamp technique to vary the frequency of the test depolarizations evoked at \(-10\) mV or the conditioning diastolic membrane potential prior to the test pulse. Results: High frequencies (range 0.2–3.5 Hz) slowed the decay kinetics of \(I_{\text{Ca,L}}\) evoked from a holding potential (HP) of \(-80\) mV in 68% of cells resulting in a larger Ca influx during the test pulse. The amount of facilitation increased progressively between 0.2 and 3.0 Hz. When the frequency was changed from 0.1 to 1 Hz, the averaged increase in the time integral of \(I_{\text{Ca,L}}\) was 27±7% (\(n=22\)). Application of conditioning voltages between \(-80\) and \(-50\) mV induced similar facilitation of \(I_{\text{Ca,L}}\) in 73% of cells. The maximal increase of Ca entry occurred between \(-60\) and \(-50\) mV, and was on average 38±14% for conditioning prepulses of 5 s in duration (\(n=15\)). Numerical simulations of the pacemaker activity showed that facilitation of \(I_{\text{Ca,L}}\) promotes stability of sino-atrial rate by enhancing Ca\(^{2+}\) entry, thus establishing a negative feedback control against excessive heart rate slowing. Conclusion: Facilitation of \(I_{\text{Ca,L}}\) is present in rabbit sino-atrial cells. The underlying mechanism reflects modulation of \(I_{\text{Ca,L}}\) decay kinetics by diastolic membrane potential and frequency of depolarization. This phenomenon may provide an important regulatory mechanism of sino-atrial automaticity.

Keywords: Ca-channel; Impulse formation; Sinus node

1. Introduction

The spontaneous activity of sino-atrial ‘pacemaker’ myocytes underlies cardiac autrrhythmicity [1]. Several ionic currents, with complex reciprocal interaction, contribute to the sino-atrial automaticity. Their electrophysiological description has allowed the development of numerical models of the electrical activity of pacemaker cells [2,3]. Two types of Ca\(^{2+}\) current (\(I_{\text{Ca}}\)) have been identified in single sino-atrial myocytes. One is activated by moderate depolarization and is referred to as a low-voltage-activated T-type \(I_{\text{Ca}}\) (\(I_{\text{Ca,T}}\)) [4,5]. Stronger depolarization leads to the activation of a high-voltage-activated L-type \(I_{\text{Ca}}\) (\(I_{\text{Ca,L}}\)) [4–6]. \(I_{\text{Ca,L}}\) is highly sensitive to dihydropyridines (DHPs) and is upregulated by catecholamines and other neurotransmitters that increase intracellular cAMP [7,8]. Finally, sino-atrial cells are characterized by the presence of a sustained component (\(I_{\text{st}}\)) described recently [9]. \(I_{\text{st}}\) is activated early upon depolarization (about \(-70\) mV), and shows sensitivity to \(\beta\)-adrenergic agonists like \(I_{\text{Ca,L}}\). Together with the hyperpolarization-activated ‘pacemaker’ current \(I_{\text{P}}\), \(I_{\text{Ca,L}}\) and \(I_{\text{st}}\) account for most of the \(\beta\)-adrenergic stimulation of the mammalian heart rate [9,10].

\(I_{\text{Ca,L}}\) is widely expressed throughout the whole myocardium, where it plays a major role in the modulation of the...
AP duration, and in the excitation–contraction (E–C) coupling [7,8,11]. However, several of its properties are also consistent with an important role in the generation and modulation of the pacemaker AP [12]. For example, \( I_{\text{Ca,L}} \) is rapidly activated and inactivated upon depolarization and is modulated in opposite ways by \( \beta \)-adrenergic and muscarinic receptor stimulations (increase and decrease in current amplitude, respectively) [8,13]. These basic regulatory mechanisms are involved in the control of sino-atrial cells automaticity and, thus, in the beat-to-beat modulation of cardiac activity. Although voltage is its primary effector, \( I_{\text{Ca,L}} \) is modulated by intracellular messengers, such as cAMP, cGMP and \( Ca^{2+} \), that activate various protein kinases [7,8]. Direct modulation via a membrane-delimited pathway mediated by G-proteins has also been proposed [14]. However, another interesting basic property of \( I_{\text{Ca,L}} \) is its regulation by the frequency of depolarization, a phenomenon described first in frog atrium, and then in rat, guinea-pig, dog and human cardiomyocytes [11,15–23]. In mammalian cardiomyocytes, an increase in the frequency of depolarization slows the decay kinetics of \( I_{\text{Ca,L}} \). This mechanism induces larger \( Ca^{2+} \) influx during each depolarization. It may have critical effects on the AP amplitude and duration. Similar type of facilitation of \( I_{\text{Ca,L}} \) induced by a moderate depolarization of the diastolic membrane potential (in the range of −80 to −40 mV) has also been described in rat and human cardiomyocytes [24]. In the present work, we have addressed the question of whether facilitation of \( I_{\text{Ca,L}} \) is operant in spontaneously beating sino-atrial myocytes.

2. Methods

2.1. Rabbit sino-atrial cells isolation

Spontaneously-beating sino-atrial cells were isolated from young albino rabbits weighing 0.8–1 kg as described before [25,26]. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US national Institute of Health (NIH Publication No. 85-23, revised 1996). Briefly, beating hearts were removed under pentobarbital (3 ml/kg) and ketamine (1 ml/kg, Sanofi Veterinary) anesthesia. The sino-atrial region was excised in a normal Tyrode solution containing (mM): NaCl, 140; KCl, 5.4; CaCl₂, 1.8; MgCl₂, 1; Hepes–NaOH, 5; and d-glucose, 5.5; (pH 7.4). Strips of tissues were enzymatically digested in a low-\( Ca^{2+}, \) low- \( Mg^{2+} \) solution containing (mM): NaCl, 140; KCl, 5.4; MgCl₂, 0.5; CaCl₂, 0.2; KH₂PO₄, 1.2; taurine, 50; d-glucose, 5.5; Hepes–NaOH, 5; pH 6.9. Collagenase type II (224 U/ml, Worthington), elastase (1.9 U/ml, Worthington), and bovine serum albumin (BSA) 1 mg/ml were added. The digestion step was carried out for about 15 min under gentle mechanical agitation at 36°C. Tissue strips were then washed out, and transferred into a modified ‘Kraftbrühe’ (KB) medium [27] containing (mM): L-glutamic acid, 70; KCl, 20; KOH, 80; \( \beta \)-OH-butyric acid, 10; KH₂PO₄, 10; taurine, 10; BSA, 1 mg/ml; and Hepes–KOH, 10; pH 7.4. Single sino-atrial myocytes were manually dissociated in KB solution by employing a flame-forged glass pipette. Finally, cell automaticity was recovered by gradually increasing the extracellular \( Ca^{2+} \) concentration up to 1.3 mM [25]. The final storage solution contained (mM): NaCl, 100; KCl, 50; CaCl₂, 1.3; MgCl₂, 0.7; BSA 1 mg/ml; pH 7.4, and gentamycin (50 \( \mu \)g/ml). Cells were then stored at 4°C until use.

2.2. Electrophysiological recordings

For electrophysiological recordings, cells were harvested in 35-mm Petri dishes, and mounted on the stage of an inverted microscope (Nikon). The whole-cell patch-clamp technique [28] was employed to record \( I_{\text{Ca,L}} \) in spontaneously beating beating cells at room temperature (22°C). The voltage-clamp circuit was provided by an Axopatch 200A (Axon Instruments) patch-clamp amplifier. Recording pipettes were fabricated from borosilicate glass. Final electrode resistances were 3 MΩ. The intracellular recording solution contained (mM): Cesium(Cs)-aspartate, 120; CsCl, 10; MgCl₂, 2; ATP-Na⁺ salt, 2; GTP Na⁺ salt, 0.1: EGTA–CsOH, 10; Hepes–CsOH, 10; pCa=8, (pH 7.2) with CsOH. After seal formation the electrode capacitance was compensated electronically before patch rupture, and establishment of the whole-cell configuration. Seal resistances were in the range of 1–2 GΩ. Voltage errors resulting from the uncompensated series resistance were always \( \leq 3 \) mV and were not corrected. Cell input resistances were in the range of 0.2–2 GΩ. Whole-cell membrane capacitance was calculated by integrating the capacitive currents recorded during +10 nV voltage steps from a HP of −80 mV. The standard extracellular solution was chosen to block inward Na⁺, and outward K⁺ currents, and contained (mM): tetraethylammonium chloride, 130; CaCl₂, 2; MgCl₂, 1; 4-aminopyridine, 4; Hepes, 25; (pH 7.4 with TEAOH).

\( I_{\text{Ca,L}} \) was routinely recorded by applying brief (60–100 ms) step depolarizations to a test potential which activated maximal current amplitude (usually −10 mV). To measure the current–voltage relationship (I–V), depolarizations were applied in 10 mV increments between −70 and +60 mV from HPs set between −100 and −50 mV at 0.1 Hz. To study the frequency-dependent facilitation of \( I_{\text{Ca,L}} \), depolarizing steps were applied from an HP of −80 mV at variable frequency (0.1–5 Hz). Depolarization-dependent facilitation was assessed by applying conditioning voltage of variable duration (100 ms–5 s) and amplitude (−100 to −10 mV), prior to the test potential. For each type of facilitation, we measured \( I_{\text{Ca,L}} \) peak amplitude and the time integral between the zero current level after 40 ms depolarization. Although the zero current level does not correspond precisely to the zero \( I_{\text{Ca,L}} \) level (as measured
ideally after specific $I_{Ca,L}$ blockade) the offset is slight and was estimated to be less than 5% at $-10$ mV, by measuring the current $I$-V relation in the absence of extracellular Ca$^{2+}$. Experimental parameters, such as HPs, test potentials, and sampling intervals were controlled with an IBM PC connected to a Digidata 1200 interface (Axon). Current signals were filtered at 3–5 kHz prior to digitization and storage. Data acquisition and analysis were performed using the PCLAMP software package (Axon), and the ORIGIN 6 data analysis software (Microcal).

2.3. Fittings

$I_{Ca,L}$ current-to-voltage relation were fitted according to Eq. (1), which corresponds to the sum of two independent (2) and (3) Boltzmann equations

$$I(V) = f_1(V) + f_2(V)$$

$$f_1(V) = g_a(V - E_{rev})/1 + \exp [(V - h_1)/s_1]]$$

$$f_2(V) = g_b(V - E_{rev})/1 + \exp [(V - h_2)/s_2]]$$

where $I$ is the current, $f(V)$ are the two Boltzmann equations, $V$ is the membrane voltage, $g_a$ and $g_b$ are normalized conductances, $E_{rev}$ is the current reversal potential, $h_1$, and $h_2$ are half-activation factors, and $s_1$ and $s_2$ are the slope factors.

$I_{Ca,L}$ inactivation was best fitted by the sum of two exponential components according to the equation

$$I_{Ca,L}(t) = I_{Ca,L(fc)} \exp (-t/\tau_{fast}) + I_{Ca,L(sc)} \exp (-t/\tau_{slow})$$

where $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$ are the amplitudes, and $\tau_{fast}$ and $\tau_{slow}$ are the time constants of the fast and slow components of $I_{Ca,L}$, respectively. The zero time was set slightly before the peak of the current to determine $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$ and, in all cases, the sum of $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$ accounted for the peak current amplitude. Activation and inactivation time constants were also obtained from simultaneous multiexponential fitting of current activation, and inactivation. The time constants obtained from these two fitting methods were not significantly different.

$I_{Ca,L}$ time integrals were calculated according to the trapezoidal rule (ORIGIN 6 built-in function). The voltage-dependency of the ratio $I_{Ca,L(fc)}/I_{Ca,L(sc)}$ was fitted according to a two-parametric exponential function

$$Y = a \exp (bV)$$

where $Y$ is the ratio, $a$ is a numerical coefficient, $b$ is a quantity used to adjust the rate constants $k_i$ and $k_b$ in the model calculations and $V$ is the membrane voltage.

Voltage-dependent facilitation curves were also fitted according to the Boltzmann formulation

$$I_{norm}(V) = a_1(V) + b_1(V)$$

$$a_1(V) = A_2 + (A_1 - A_2)/1 + \exp [(V - V_{1/2})/s_1]]$$

$$b_1(V) = A_2 + (A_1 - A_2)/1 + \exp [(V - V_{1/2})/s_2]]$$

where $I_{norm}$ is the normalized current, $a_1(V)$ is the Boltzmann equation corresponding to current facilitation, $b_1(V)$ corresponds to current steady-state inactivation, $V$ is the membrane voltage, $A_1$ and $A_2$ are current values, $V_{1/2}$ and $V_{1/2s}$ is the voltage for half-facilitation and inactivation, respectively, $s_1$ and $s_2$ are the slope factors. All fittings were performed by numerical iteration, employing a Levenberg–Marquart based algorithm (ORIGIN 6 built-in). To fit the experimental data shown in each figure, the calculated parameters were used to build a simulation curve for each function.

2.4. Model simulations

For numerical simulation of $I_{Ca,L}$ kinetics, we adapted a model described previously [21]. This model is based on a state diagram which describes the time-dependent changes in the probability of a channel being in each of two proposed kinetic states, referred to as $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$ for the fast- and slow-inactivating component, respectively. The adaptation of this model to sino-atrial $I_{Ca,L}$, as well as the complete set of equations employed, is described in the Appendix section. $I_{Ca,L}$ voltage-clamp simulations were accomplished using the XPPAUT software [29] with the instantaneous probabilities over time obtained by numerical integration. For integrative calculations we have employed the CVODE numerical integration algorithm (software built-in). The integration step was 100 μs for simulations of the frequency-dependent facilitation, and 50 μs for depolarization-dependent facilitation. The calculated current waveforms were generated according to the equation

$$I_{Ca,L} = (O_t + O_s) g (V - E_{rev})$$

where $O_t$ and $O_s$ are the open state probabilities, for the channel being open in the fast- and slow-inactivating pathway, $g$ is the membrane conductance, $V$ is the membrane potential, and $E_{rev}$ is the apparent reversal potential for Ca$^{2+}$, which we have set to +50 mV. The voltage-dependent deactivation rate constants used in the numerical simulations were those used in our previous model [21]. The activation rate constants were measured after subtraction of the capacitative transient. obtained in the absence of extracellular Ca$^{2+}$. The inactivation rate constants were measured in the presence of extracellular Ca$^{2+}$, and Ba$^{2+}$. The various rate constants were subsequently adjusted until the model generated currents with time- and voltage-dependent properties consistent with experimental recordings in sino-atrial myocytes. Finally, the model equations were incorporated into the OXSOFT heart program [30]. The original equations for $I_{Ca}$ in the
Noble et al. [31] single cell model of sino-atrial automaticity were replaced by these equations to calculate change in cycle length, and AP duration. Intracellular Ca\(^{2+}\) buffering was calculated according to the equations in the Demir et al. model [3].

3. Results

3.1. Separation of T- and L-type Ca\(^{2+}\) currents

Only spontaneously-beating myocytes were used. The membrane capacitance of these cells was 28±2 pF (n = 25). Our recording conditions were optimized to isolate \(I_{Ca,L}\) from Na\(^+\) and K\(^+\) currents. In particular, we used intracellular Cs\(^+\) instead of K\(^+\) and extracellular TEA\(^+\) instead of Na\(^+\) in the presence of 4 mM 4-AP to block influx of TEA through Ca\(^{2+}\)-independent \(I_{Na}\) K\(^+\) channels [32]. Accordingly, a depolarizing ramp protocol applied to the cell evoked no inward current in the absence of extracellular Ca\(^{2+}\) (Fig. 1A). Step depolarizations from a HP of −100 mV led to the activation of two types of \(I_{Ca}\) exhibiting differential sensitivity to the membrane voltage. The first one, which was transient with fast decay kinetics, activated at a threshold of around −60 mV and reached maximal peak amplitude at −30 mV (Fig. 1Ba). This current, which was fully inactivated at a HP of −60 mV, corresponded to \(I_{Ca,T}\) described in pacemaker myocytes [4,5,33]. The other current had much slower decay kinetics, started to activate between −50 and −40 mV, and peaked at −10 mV (Fig. 1C). It was not inactivated at a HP of −50 mV (Fig. 1Bb). It corresponded to \(I_{Ca,L}\). Its averaged density was 18±3 pA/pF (n = 24). In contrast to \(I_{Ca,L}\), detected consistently (24/25 cells), \(I_{Ca,T}\) was recorded in only five cells and its amplitude was less than 20% of that of \(I_{Ca,L}\) (data not shown). Co-expression of \(I_{Ca,L}\) and \(I_{Ca,T}\) was observed in only one cell. However, to ensure that our recordings were representative of pure \(I_{Ca,L}\), we routinely employed the differential sensitivity of \(I_{Ca,T}\) and \(I_{Ca,L}\) to the HP as a test (Fig. 1B). This method appeared to be unequivocal, and did not require the use of pharmacological agents such as Ca\(^{2+}\) antagonists with use- and/or voltage-dependent effects that could alter the amplitude and kinetics of \(I_{Ca,L}\). We obtained a satisfactory mathematical fit of the current–voltage relationship of \(I_{Ca,L}\) (Fig. 1C), by employing the expression (1) detailed in the Methods section. Since \(I_{st}\) is inhibited in Na\(^+\)-free conditions [9], we did not need to separate \(I_{Ca,L}\) from \(I_{st}\).

3.2. Frequency-dependent facilitation of \(I_{Ca,L}\)

To study the modulation of \(I_{Ca,L}\) by the frequency of depolarization, we used test pulses activating maximal peak current (unless otherwise noted in Fig. 2). At the start of an experiment, the cells were stimulated for several min at 0.1 Hz to allow \(I_{Ca,L}\) to stabilize. At this rate, we observed no evidence for any change in terms of amplitude and kinetics. Then post-rest stimulations were applied at various rates. Rates lower than 0.2 Hz had no effect. Higher rates induced two types of effect: a slight augmentation of the peak current, and a slowing of the decay kinetics (Fig. 2Aa,b). These effects resulted into facilitation of \(I_{Ca,L}\) with steady-state achieved in four stimulations (Fig. 2Ba). Therefore, the trace corresponding to the fourth stimulation (\(t_f\)) was taken as the reference in all experiments. In addition, steady-state facilitation was reached independently from the frequency of depolarisation, and therefore length of diastolic interval, per se (Fig. 2Bb). This was highly consistent among cells. There was no further change with additional stimulations, even for several min after the start of an experiment. When changes eventually occurred, they involved only a decrease in peak current amplitude (with no change in decay kinetics) due to either a time-dependent accumulation of the channels in the inactivated state at the highest frequencies (>3.5 Hz) or, sometimes, an irreversible rundown process.

We routinely measured both the current peak, and the current time integral, to better evaluate the total change in Ca\(^{2+}\) entry. These parameters were measured at the first and at the fourth depolarization after the start of a train. The histogram in Fig. 2Ab illustrates the typical net augmentation of \(I_{Ca,L}\) peak, and time integral measured for the experimental data shown in Fig. 2Aa. At 1 Hz the averaged increase in \(I_{Ca,L}\) time integral was 27±7% (n = 22). Facilitation was observed for different levels of depolarization throughout the \(I–V\) relationship. For example, step depolarizations at voltages corresponding to \(I_{Ca,L}\) threshold (−40 mV, Fig. 2C), and at positive potentials (+10 mV, Fig. 2D) induced facilitation of \(I_{Ca,L}\). When the frequency of depolarization was >3.5 Hz, a negative effect on the peak current amplitude was observed which limited Ca\(^{2+}\) entry (Fig. 2Bb). This phenomenon reflected incomplete reactivation of Ca\(^{2+}\) channels (data not shown).

3.3. Depolarization-dependent facilitation of \(I_{Ca,L}\)

We investigated whether, as described in other types of cardiomyocytes [20,21,24], depolarization-induced facilitation of \(I_{Ca,L}\) is also present in sino-atrial cells. We applied various conditioning potentials from a HP of −80 mV, followed by 100-ms test pulses to −10 mV. The results of a representative experiment are shown in Fig. 3. Both short (100 ms in duration) and long (5 s) conditioning potentials were tested. When 100-ms duration prepulses were applied, both \(I_{Ca,L}\) peak amplitude and time integral increased gradually with increasing conditioning depolarizations between −80 and −50 mV (Fig. 3A,C and D). The resulting facilitation of \(I_{Ca,L}\) consisted of an augmentation of the current peak and a slowing of the inactivation kinetics (e.g. between −80 and −60 mV in Fig. 3A). At conditioning voltages positive to −50 mV, \(I_{Ca,L}\) started to decrease, due to the steady-state, voltage-dependent in-
Fig. 1. Voltage-dependent separation of $I_{Ca,L}$ and $I_{Ca,T}$ in rabbit sino-atrial myocytes. (A) Application of a voltage ramp between $-100$ and $+100$ mV did not elicit inward current in the absence of extracellular Ca$^{2+}$. Application of 2 mM Ca$^{2+}$ evoked inward Ca current. In (Ba), $I_{Ca,T}$ is observed upon depolarization to the indicated test potentials from an HP of $-100$ mV (left). $I_{Ca,T}$ is completely inactivated at HP $-60$ mV (right). (Bb) $I_{Ca,L}$ was insensitive to depolarizing the HP from $-100$ (left) to $-50$ mV (right). (C) $I_{Ca,L}$ current-to-voltage relation for traces shown in (Bb) at the HPs of $-100$ (filled circles), and $-50$ mV (open circles), respectively. The curve was fitted according to Eq. (1) in the Methods section.

activation process (Fig. 3C and D). For conditioning depolarizations lasting 5 s, facilitation was markedly enhanced and its voltage-dependence to reach the maximal effect was shifted to the left for both peak amplitude and Ca$^{2+}$ entry (with a peak of time integral at $-50$ mV instead of $-40$ mV; Fig. 3D). The part of the curve corresponding to steady-state inactivation (for depolarizations $>-50$ mV) was also shifted to the left as expected. Among 15 cells investigated, 11 showed facilitation. There was no change in current waveform for depolarisations between $-80$ and $-50$ mV in the four remaining cells. The averaged maximal increase of Ca$^{2+}$ entry when the conditioning potential was depolarized from $-80$ to $-60$ mV was $38\pm14\%$ ($n=15$). On average, peak amplitude varied by less than 1% ($0.99\pm0.06\%$ of control) suggesting that the main effect occurs on current decay
Fig. 2. Frequency-dependent facilitation of $I_{\text{Ca,L}}$. (A) Facilitation of $I_{\text{Ca,L}}$ induced with the protocol shown in inset. (Aa) $I_{\text{Ca,L}}$ was evoked by a train of stimulations at $-10$ mV from an HP of $-80$ mV. In (a) current facilitation is assessed by comparing the first trace at 0.1 Hz, with the fourth trace at 1 Hz. In (b) facilitation of $I_{\text{Ca,L}}$ as measured on peak, and time integral for trace 1 (open bars 100%, control value), and trace 4 (filled bars). (B) Equilibrium for steady-state frequency-dependent facilitation of $I_{\text{Ca,L}}$ is reached after four stimulations (Ba), and is independent from the frequency of stimulation (Bb). (C) Facilitation of $I_{\text{Ca,L}}$ evoked at its activation threshold ($-40$ mV) by a train of stimulations at 1 Hz. (D) Facilitation of $I_{\text{Ca,L}}$ evoked at the positive test potential of $+10$ mV by a train of stimulations at 3 Hz.

3.4. Analysis of decay kinetics of $I_{\text{Ca,L}}$

When extracellular $\text{Ba}^{2+}$ was used instead of $\text{Ca}^{2+}$ as the permeating ion, the decay of $I_{\text{Ca,L}}$ was dramatically slowed, indicating that the fast decay of the current is, in major part, related to $\text{Ca}^{2+}$-dependent inactivation (Fig. 4Aa). Since facilitation was related to slowing of inactivation, we analyzed more closely the kinetics of $I_{\text{Ca,L}}$. In extracellular $\text{Ca}^{2+}$, $I_{\text{Ca,L}}$ had a biexponential decay, best-fitted by two time constants (Fig. 4Aa,b). The fast rate kinetics. In pilot experiments, we determined that no facilitation occurs in a voltage range between $-100$ and $-80$ mV.
Fig. 3. Depolarization-dependent facilitation of $I_{\text{Ca,L}}$ in sino-atrial myocytes. Representative traces of $I_{\text{Ca,L}}$ recorded for a preconditioning pulse of 100 ms (A) and 5 s (B) at voltages ranging from $-80$ to $-10$ mV prior to the test pulse at $-10$ mV (the experimental protocols are shown in the upper part of the panels). (C) Normalized value of current peaks, and (D), the time integral of $I_{\text{Ca,L}}$. $I_{\text{Ca,L}}$ was measured upon applying a 5-s (open circles), and a 100-ms (filled circles) conditioning depolarization. Arrows indicate their respective fittings according to Eq. (6) detailed in the Methods section.

constant ($\tau_{\text{fast}}$) ranged between 2 and 5 ms, and showed no voltage dependence. The slow rate constant ($\tau_{\text{slow}}$) ranged between 10 and 40 ms. The inactivation rate constants measured in extracellular Ba$^{2+}$ for depolarizations of 100 ms in duration were similar to $\tau_{\text{slow}}$ (Fig. 4A) confirming that the fast decay of $I_{\text{Ca,L}}$ is due to Ca$^{2+}$-dependent inactivation. For each cell, therefore, we measured the amplitudes (in pAs) of the fast-inactivating component $I_{\text{Ca,L(fc)}}$, and the slow-inactivating component $I_{\text{Ca,L(sc)}}$. The current peak, and the relative amplitude of $I_{\text{Ca,L(fc)}}$, and $I_{\text{Ca,L(sc)}}$ at each voltage were used to construct their respective current-voltage relationships (Fig. 4B). The voltage-dependence of $I_{\text{Ca,L(sc)}}$ is 20 mV more positive than that of $I_{\text{Ca,L(fc)}}$. The voltage-dependence of the ratio between $I_{\text{Ca,L(fc)}}$ and $I_{\text{Ca,L(sc)}}$ throughout the $I_{\text{Ca,L}}$ current-voltage relation obeyed to an exponential relation (Fig. 4B). Accordingly, the time integral of $I_{\text{Ca,L}}$ had a voltage-dependence that matched that of $I_{\text{Ca,L(sc)}}$ more closely than that of $I_{\text{Ca,L(fc)}}$ (data not shown).

Since the sino-atrial $I_{\text{Ca,L}}$ is kinetically the sum of a fast and slow components, we investigated whether depolarization- and frequency-dependent facilitation enhanced the relative contribution of $I_{\text{Ca,L(sc)}}$ to the total $I_{\text{Ca,L}}$ (Fig. 5).

To this aim we used spindle-shaped cells, in which frequency- and depolarization-dependent facilitation are co-expressed on the same cell, and are directly comparable ($n=8$, Fig. 5A and B). Here, $I_{\text{Ca,L}}$ was up-regulated by rates of stimulation up to 3 Hz (Fig. 5A) which reflected a decrease in the ratio between $I_{\text{Ca,L(fc)}}$ and $I_{\text{Ca,L(sc)}}$ (Fig. 5B). Robust depolarization-induced facilitation was observed in the voltage window between $-70$, and $-50$ mV (Fig. 5B). In this case, facilitation of $I_{\text{Ca,L}}$ reflected an increase of $I_{\text{Ca,L(sc)}}$ (Fig. 5B).

3.5. Model for simulation of $I_{\text{Ca,L}}$

We attempted to evaluate the potential influence of modulation of the decay kinetics of $I_{\text{Ca,L}}$ by frequency of stimulation, and diastolic membrane potential on pacemaker activity. To do this, we performed numerical simulations of both $I_{\text{Ca,L}}$ and AP waveforms. First, we used a mathematical model to simulate the voltage-, time- and Ca$^{2+}$-dependent behavior of macroscopic $I_{\text{Ca,L}}$. This model takes into account the predicted fraction of Ca$^{2+}$ channels inactivated by Ca$^{2+}$, and the fraction inactivated independently of Ca$^{2+}$ at any time and any voltage. This approach
Fig. 4. Kinetics of $I_{\text{Ca,L}}$ in sino-atrial myocytes. (Aa) Kinetics of $I_{\text{Ca,L}}$ decay, recorded in extracellular 2 mM Ca$^{2+}$ and Ba$^{2+}$, at the peak of their respective current-to-voltage relation. (Ab) Voltage-dependence of $t_{\text{fast}}$, and $t_{\text{slow}}$, in extracellular 2 mM Ca$^{2+}$ and Ba$^{2+}$. (Ba) Current-to-voltage relations for $I_{\text{Ca,L(fc)}}$ (open circles), $I_{\text{Ca,L(sc)}}$ (filled squares), and current peak (filled circles). (Bb) Voltage dependence of the ratio $I_{\text{Ca,L(fc)}}/I_{\text{Ca,L(sc)}}$ for the current-to-voltage relation shown in (Ba). The curve was fitted according to Eq. (6) detailed in the Methods section. All the data are from the same cell.

has the advantage of being independent from the exact mechanism of Ca$^{2+}$-dependent inactivation, as well as to allow to calculate $I_{\text{Ca,L}}$ behaviour in terms of a voltage-dependent evolution of the ratio $I_{\text{Ca,L(fc)}}/I_{\text{Ca,L(sc)}}$ which follows an exponential relationship as experimentally observed (see Fig. 4Bb). This voltage-dependence is also observed for conditioning potentials applied prior to the test depolarisation in a range of voltages that are presumed too weak to activate significant macroscopic $I_{\text{Ca,L}}$ (Fig. 3; Fig. 5Ba,b). The voltage- and time-dependent equilibrium model correctly predicted the negative effect on current peak, as expected when channel voltage-dependent reaction becomes limiting. Numerical simulations also reproduced the effect of changing the voltage from which the step depolarization is evoked (Fig. 6B). Consistent with our experimental observations (Fig. 3), depolarization-induced facilitation was dependent upon the duration of the preconditioning pulse and was associated with a decrease of the ratio between $I_{\text{Ca,L(fc)}}$ and $I_{\text{Ca,L(sc)}}$ as expected from the time-dependent change in the distribution of $I_{\text{Ca,L(fc)}}$ and $I_{\text{Ca,L(sc)}}$ (Fig. 6B).
tion was maximal when the conditioning potential was changed from $-80$ to $-50$ mV, for 2 s, as experimentally verified.

### 3.6. Model simulation of AP automaticity

We thus integrated our equations in the DiFrancesco–Noble model of sino-atrial automaticity (Fig. 7A). For consistency, we used the same parameters values as in voltage-clamp simulations, except for $I_{Ca,L}$ conductance which was scaled to give consistent $I_{Ca,L}$ current density (Fig. 7B and C). This simulation reproduced AP waveform, and diastolic depolarization (Fig. 7A). The Na$^+$–Ca$^{2+}$ exchanger time-dependent kinetics (Fig. 7D), and the intracellular Ca$^{2+}$ transients amplitude and kinetics (data not shown), were similar to those calculated in Demir et al. model. Three voltage-dependent currents were present in the diastolic depolarization: a decaying outward delayed rectifier current component (Fig. 7E), the hyperpolarization-activated $I_t$ current (Fig. 7G), and $I_{Ca,T}$ (data not shown). Furthermore, two voltage-independent current components also contributed to the diastolic depolarization: the time-independent background conductances (Fig. 7F), and the Na$^+$–Ca$^{2+}$ exchanger current. Finally, $I_{Ca,L}$ was also present as an inward current component in the vicinity of the AP threshold.

Our first goal was to evaluate whether and how the complex kinetics of $I_{Ca,L}$, that had never been taken into account before in numerical simulations, would influence sino-atrial automaticity. Fig. 8 shows a comparison between the DiFrancesco–Noble model, which exhibits only mono-exponential decay of $I_{Ca,L}$, and our variant model. Simulation showed that inclusion of the biphasic decay of $I_{Ca,L}$ and its modulation by rate of stimulation and diastolic membrane potential slowed the basal rhythm, and hyperpolarized the maximum diastolic potential. Both effects
are due to enhanced contribution of $I_{\text{Ca,L}(sc)}$ to the AP plateau (Fig. 8Ba). Since $I_{\text{Ca,L}(fc)}$ and $I_{\text{Ca,L}(sc)}$ have differential dependence upon frequency of stimulation, and diastolic membrane potential, it was interesting to test the relative contribution of the two current components to automaticity at different rhythms. Fig. 9A shows that a 30% reduction in the amplitude of $I_{\text{Ca,L}}$ decreased the APs frequency from 4 to 2.8 Hz (Fig. 9A). The ratio $I_{\text{Ca,L}(fc)}/I_{\text{Ca,L}(sc)}$ was increased, because $I_{\text{Ca,L}(sc)}$ was diminished (Fig. 9B and C). The greater contribution of $I_{\text{Ca,L}(fc)}$ at low APs frequencies was confirmed by comparing the influence of suppressing $I_{\text{Ca,L}(fc)}$ on automaticity, at each of the two frequencies tested (Fig. 9D and E, respectively). These simulations suggested that the Ca$^{2+}$-inactivating component of $I_{\text{Ca,L}}$ contributes significantly to regulation of the basal rhythm but its role is, however, not determinant in supporting automaticity. In contrast, suppression of the Ca$^{2+}$-independent component $I_{\text{Ca,L}(sc)}$ arrested automaticity (Fig. 9F) suggesting that $I_{\text{Ca,L}(sc)}$ has a major contribution. Our last attempt was to test how sino-atrial frequency, and diastolic depolarization would influence $I_{\text{Ca,L}}$ kinetics, and thus Ca$^{2+}$ entry, during pacemaker cycle in physiologically

Fig. 6. Numerical simulations of facilitation of $I_{\text{Ca,L}}$. (A) Numerical simulations of frequency-dependent facilitation, and relative amplitudes of $I_{\text{Ca,L}(Aa)}$, $I_{\text{Ca,L}(Ab)}$, and $I_{\text{Ca,L}(Ac)}$, observed between 1 and 5 Hz. Current waveforms were calculated by setting the HP to $-80$ mV, and applying a 100 ms long depolarizing step to $-10$ mV. In each panel, the dotted trace represents reference at 1 Hz. (B) Time-dependency of the depolarization-dependent facilitation. Currents were calculated by applying a 20 (Ba), 200 (Bb), and 2-s (Bc) long preconditioning voltages. In each panel, the dotted lines represent $I_{\text{Ca,L}(c)}$ and $I_{\text{Ca,L}(sc)}$. Traces evoked upon preconditioning at $-80$ mV (reference), and $-50$ mV are indicated by arrows.
relevant conditions (Fig. 10A). A spectrum of steady-state frequencies ranging from 4.6 to 3.2 Hz was generated by progressive activation of background $I_{K(\text{ATP})}$, current [34]. Slow rates resulted in a prolongation of the diastolic depolarization, together with a shift of the maximum diastolic potential (Fig. 10Aa). At 3.2 Hz, the simulation resulted into facilitation of $I_{\text{Ca,L}}$, as observed on current waveforms during APs (Fig. 10Ab), and instantaneous $I–V$ plots (Fig. 10Ac). The time integral ratio of $I_{\text{Ca,L}}$ was augmented with prolongation of the diastolic interval (Fig. 10B) which, despite the slowing of APs frequency, was the predominant factor. During the diastole, intracellular $\text{Ca}^{2+}$ was also increased which resulted in turn in an enhancement of the $\text{Na}^{+}–\text{Ca}^{2+}$ exchange current at 3.2 Hz (data not shown).

4. Discussion

This study describes for the first time the regulation of the decay kinetics of $I_{\text{Ca,L}}$ by frequency of depolarisation, and diastolic membrane potential in rabbit sino-atrial
pacemaker cells. To summarize our results: (i) $I_{\text{Ca,L}}$ has a biexponential decay best-fitted by two time constants with the fast one being Ca$^{2+}$-dependent and the slow one Ca$^{2+}$ independent; (ii) both higher frequencies of stimulation and depolarized diastolic membrane potential between $-70$ and $-50$ mV prevent fast inactivation of $I_{\text{Ca,L}}$; (iii) the resulting facilitation of $I_{\text{Ca,L}}$ promotes larger Ca$^{2+}$ influx; (iv) numerical simulations suggest that the Ca$^{2+}$-independent slow-inactivating component of $I_{\text{Ca,L}}$($I_{\text{Ca,L,(sc)}}$) supports pacemaker activity whereas the Ca$^{2+}$-dependent fast-inactivating component ($I_{\text{Ca,L,(fc)}}$) is a secondary regulator and (v) the depolarisation-induced facilitation, which develops during prolongation of the diastolic interval, is expected to oppose excessive rhythm slowing.

The decay kinetics of $I_{\text{Ca,L}}$ are under the control of both the rate of stimulation and the diastolic membrane potential in rabbit sino-atrial cells. This observation is new and worth considering when studying Ca$^{2+}$ channels regulation in cardiac pacemaker cells. This modulation of Ca$^{2+}$ channel activity underlies the mechanism widely referred to as facilitation. Indeed, spindle-shaped sino-atrial cells present robust frequency- and depolarization-dependent facilitation of Ca$^{2+}$ channel-mediated Ca$^{2+}$ entry which may provide them with an important regulation of automaticity for several reasons. First, facilitation is detected in the majority of cells. Second, the overall net increase of Ca$^{2+}$ entry can be substantial. Third, the amount of facilitation is graded, being function of increasing frequencies, or depolarization of the diastolic potential (range between $-70$ and $-50$ mV) from which the Ca$^{2+}$ channels are activated, suggesting that Ca$^{2+}$ channel activity is finely modulated in a wide spectrum of pacemaker rates and diastolic membrane potentials. Fourth, frequency-dependent facilitation reaches equilibrium within three to four stimulations, which is consistent with a beat-to-beat regulation of cell automaticity.

In cardiac cells, there is a joint dependence of the inactivation of $I_{\text{Ca,L}}$ on both voltage and intracellular Ca$^{2+}$ [8,20,35,36]. The early fast decay of $I_{\text{Ca,L}}$ (and its related component $I_{\text{Ca,L,(fc)}}$) is determined by a Ca$^{2+}$ release-induced inactivation in a microdomain hardly accessible to EGTA or even BAPTA [37–40]. A local Ca$^{2+}$ signalling has been demonstrated, for example, by depleting the SR Ca$^{2+}$ content using ryanodine, thapsigargin or yet using phospholamban deficient mice which resulted into slowed inactivation of $I_{\text{Ca,L}}$ [19,24,38–42]. The Ca$^{2+}$-dependent inactivation can be totally inhibited using Ba$^{2+}$ as the charge carrier or, alternatively, using high BAPTA concentration allowing to isolate slow voltage-dependent inactivation [8,20,35–37; present results]. The slowing of

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**Fig. 8.** Modulation of $I_{\text{Ca,L}}$ inactivation kinetics by facilitation regulates the AP duration, and slows the basal rhythm. (Aa). Time course of a monophasic $I_{\text{Ca,L}}$ which sustains spontaneous activity shown in (Ab). Facilitation enhanced Ca$^{2+}$ entry (Ba), and promoted activation of $I_{\text{k}}$ (data not shown) which, in turn, hyperpolarized the maximum diastolic potential. $I_{\text{Ca,L}}$ facilitation also prolonged the AP duration.
Fig. 9. Differential contribution of $I_{Ca,L}$ and $I_{Ca,L}^{(sc)}$ during sino-atrial automaticity in our model. (A) Changing $I_{Ca,L}$ amplitudes generates two independent rhythms of 2.8 Hz (dotted line), and 4 Hz (solid line). Note that the hyperpolarization of the maximum diastolic potential is due to reduced $I_{Na,Ca}$ activity (not shown). (B) Instantaneous $I-V$ plots of the ratio $I_{Ca,L}^{(fc)}$ and $I_{Ca,L}^{(sc)}$ at 2.8 Hz (dotted line), and 4 Hz (solid line). (C) Corresponding instantaneous $I-V$ plots for the two $I_{Ca,L}$ components. In (B) and (C) note the increment of $I_{Ca,L}^{(sc)}$ contribution at lower frequency. (D) Computed change in sino-atrial rhythm at 4 Hz (solid line) when $I_{Ca,L}^{(fc)}$ was set to zero (dotted line). (E) $I_{Ca,L}^{(fc)}$ has been set to zero at 2.8 Hz. (F) $I_{Ca,L}^{(fc)}$ is essential to sustain sino-atrial automaticity: the spontaneous activity ceased when the slow-inactivating component was set to zero.

$I_{Ca,L}$ decay kinetics involved in facilitation is likely to reflect reduced Ca$^{2+}$-dependent inactivation and of its fast-inactivating component ($I_{Ca,L}^{(fc)}$). This occurs with a concomitant increase in the Ca$^{2+}$-independent component $I_{Ca,L}^{(sc)}$, because the fraction of channels that are sensitive to Ca$^{2+}$ then inactivate slowly. The precise mechanism may involve reduced SR-Ca$^{2+}$ release inactivation of $I_{Ca,L}$ as reported recently in myocardial cells [19,24,42]. However, this mechanism, which seems likely for repetitive activation of $I_{Ca,L}$ at high frequencies, is less evident for the depolarisation-induced facilitation. For example, it seems unrelated to Ca$^{2+}$ channel opening during conditioning depolarizations that are considered too negative (range −70 to −50 mV) to generate a significant macroscopic $I_{Ca,L}$. In addition, the amount of facilitation is proportional to the prepulse duration which is reminiscent of a voltage- and time-dependent mechanism. Therefore, two mechanisms could account for this intriguing phenomenon: a voltage-driven Ca$^{2+}$ release from the SR as proposed in cardiomyocytes [43] or, alternatively, Ca$^{2+}$ entry via very few activatable Ca$^{2+}$ channels. Since such current is hardly detectable at the macroscopic level, it should be postulated that this latter mechanism generates localized Ca$^{2+}$ influx with the gain coupling Ca$^{2+}$ influx and Ca$^{2+}$ release high enough to deplete the SR during the conditioning depolarisation. The high driving force for Ca$^{2+}$ at hyperpolarized membrane voltages may play a crucial role. Finally, another possibility could be that, in
addition to decreasing the driving force for Ca\(^{2+}\), high voltages render the Ca\(^{2+}\)-binding site hardly accessible to Ca\(^{2+}\) ions and thus Ca\(^{2+}\)-induced inactivation becomes less important with larger depolarizations. Indeed, when evoked from hyperpolarized holding potentials, currents in the left branch of the \(I-V\) curve (test pulses less than 0 mV), where the fast inactivating component is predominant, are much faster than the currents in the right branch (>0 mV).

The depolarization-dependent facilitation of \(I_{Ca,L}\) may be involved in different physiological situations associated with modulation of the diastolic membrane potential. In both cases, the increase in transmembrane Ca\(^{2+}\) entry generates an additional depolarizing current, which is expected to influence the AP waveform. Since sinoatrial cells generate an electrical oscillation, we expected the decay kinetics of \(I_{Ca,L}\) to regulate the pacemaker activity and, in turn, to be regulated by the AP cycle length and/or diastolic membrane potential. Since both the pacemaker rate and the diastolic membrane potential contribute
to shape the waveform of $I_{Ca,L}$, and are interdependent, it was difficult to predict their relative influence on the pacemaker activity based on an experimental approach. For example, one limitation of AP-clamp experiments is the need of a pharmacological subtraction of currents in order to define their time-course during the AP. Manipulation of pharmacological agents with potential use- and voltage-dependent effects (such as Ca$^{2+}$ channel antagonists) is also critical. Here, we used simplified experimental conditions to allow precise measurements of $I_{Ca,L}$ kinetics and to have access to the mechanistic details. We isolated $I_{Ca,L}$ from the other major contaminating currents ($I_{Na,L}$, $I_K$) and used intracellular EGTA (to protect cells from Ca$^{2+}$ paradox during recordings) to study facilitation in good experimental conditions. However, the disadvantage of our approach was that was the impossibility to get an insight into the precise mechanism of facilitation by itself, thus rendering difficult to extrapolate directly the role of facilitation to in vivo conditions. For example, it was difficult to establish the contribution (or interference) of the various mechanisms regulating Ca$^{2+}$ homeostasis (Na$^+$–Ca$^{2+}$ exchange, SR Ca$^{2+}$) to facilitation.

To overcome some of these problems, the development of a DiFrancesco–Noble-based numerical model of sinoatrial electrical activity [31] helped us to evaluate the possible consequences of $I_{Ca,L}$ facilitation on automaticity. Our simulations showed that enhanced Ca$^{2+}$ entry due to facilitation prolongs the AP duration, hyperpolarizes the maximum diastolic potential, and slows the basal pacemaker rate (Fig. 8) which is consistent with some of the observations made previously in a numerical model of the toad sinus venosus [44]. Our simulations also showed that the two kinetically distinct current components of $I_{Ca,L}$ have differential contribution in the regulation of cell automaticity. Indeed, the slow-inactivating Ca$^{2+}$-independent component $I_{Ca,L(sc)}$ is mandatory to maintain automaticity (Fig. 9). The properties of $I_{Ca,L(sc)}$ suggest that it could play a role in the latter phase of diastolic depolarization [45], and in the regulation of the AP plateau [46], as proposed in the Demir et al. model [3] or by the biphasic waveform of the nifedipine-sensitive current recorded during AP-clamp experiments [47]. In contrast, the Ca$^{2+}$-dependent fast-inactivating component $I_{Ca,L(fc)}$ is not crucial to maintain automaticity but it regulates the basal rhythm, in particular by having a greater contribution at low APs frequencies.

Pacemaker rate and diastolic membrane potential provide a way to modulate $I_{Ca,L}$ indirectly. This may occur in conditions where autonomous neurotransmitters and/or intracellular second messengers have no direct effect on $I_{Ca,L}$ per se. For example, pacemaker slowing at low parasympathetic tone involves muscarinic inhibition of $I_f$ at agonist concentrations that have no effect on $I_{Ca,L}$ [48]. Since $I_{Ca,L}$ regulates the pacemaker rate, the time-dependent facilitation of voltage-gated Ca$^{2+}$ entry induced by slow depolarization of the diastolic membrane potential could thus represent a protection mechanism against excessive sino-atrial frequency slowing (see for example, Fig. 10). Prolonged sino-atrial pauses are expected to enhance Ca$^{2+}$ channel activity and, depending on the membrane diastolic potential during the pause, favor the recovery of the pace-maker cycle. Recovery from sino-atrial pauses often depends on reactivation by atria, a phenomenon which implies a role for cell depolarization [49]. Since $I_{Ca,L}$ is up-regulated even at its threshold for activation (Fig. 2C), facilitation could enhance the contribution of $I_{Ca,L}$ to the late diastolic depolarisation at low pacemaker rates. Interestingly, increased Ca$^{2+}$ entry due to facilitation is also expected to activate Ca$^{2+}$ sensitive ionic currents such as, for example, the Ca$^{2+}$-dependent K+ conductance ($I_{K Ca,L}$) [50] and/or $I_K$ via a yet unidentified intracellular signaling cascade [51,52].

In summary, pacemaker cells display facilitation of $I_{Ca,L}$. Facilitation is based on regulation of the inactivation rate of $I_{Ca,L}$ by the pacemaker frequency and the diastolic membrane potential. Numerical simulation demonstrate that, in turn, modulation of $I_{Ca,L}$ decay kinetics is a potentially important physiological mechanism for normalizing and regulating cardiac automaticity. This mechanism may be involved in the neurohormonal modulation of pacemaker rate.

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Appendix A

Standards units used in the following set of equations

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane potential</td>
<td>V</td>
</tr>
<tr>
<td>Membrane current</td>
<td>nA</td>
</tr>
<tr>
<td>Membrane conductance</td>
<td>nS</td>
</tr>
<tr>
<td>Time constant</td>
<td>s</td>
</tr>
<tr>
<td>Rate constant</td>
<td>s$^{-1}$</td>
</tr>
</tbody>
</table>

$I_{Ca,L}$ model equations

Our equations for solving $I_{Ca,L}$ correspond to the parallel channel gating model state diagram [21] shown below. This is based on eight closed states named $C_{11}$–$C_{14}$ for the channel in the fast mode of inactivation ($C_f$), and $C_{21}$–$C_{24}$
for the channel in the slow mode of inactivation ($C_s$), respectively. Two open states, $O_1$ and $O_2$ correspond to the channel open in the fast ($O_1$) or slow ($O_2$) mode, respectively. Finally, two inactivated states $I_1$ and $I_2$ correspond to the channel inactivated in the fast ($I_1$) or slow ($I_2$) mode.

\[ I_{Ca,L(fc)} = O_1 \ g_1 \ (V - E_{rev}) \]  
\[ I_{Ca,L(sc)} = O_2 \ g_2 \ (V - E_{rev}) \]

where $g_1$ and $g_2$ are the membrane conductances for $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$, respectively. Given the total $I_{Ca,L}$ being the sum of the fast- and slow-inactivating current component, and in the hypothesis that $I_{Ca,L(fc)}$ and $I_{Ca,L(sc)}$ have the same conductance we can write

\[ I_{Ca,L} = (O_1 + O_2) \ g \ (V - E_{rev}) \]  

Parameter values

\[ k_{14}, k_{24} = 1000 \ \text{s}^{-1} \]
\[ k_{-14}, k_{-24} = 300 \ \text{e}^{-(-5 \ \text{V})} \]
\[ k_{11-13,21-23} = 5000 \ \text{e}^{(40 \ \text{V})} \]
\[ k_{-11-13,21-23} = 1000 \ \text{e}^{(-30 \ \text{V})} \]
\[ k_{15} = 260 \ \text{s}^{-1} \]
\[ k_{25} = 35 \ \text{s}^{-1} \]
\[ k_{-16,-26} = 0 \]
\[ k_{16} = 0.4 \ \text{e}^{(-54 \ \text{V})} \]
\[ k_{26} = 0.8054 \ \text{e}^{(-26.5 \ \text{V})} \]
\[ k_1 = 30 \ \text{e}^{(70 \ \text{V})} \]
\[ k_2 = 0.1 \ \text{e}^{(-20 \ \text{V})} \]

Initial values

\[ O_1 = 8.8734993 \ \text{e}^{-7} \]
\[ O_2 = 1.9716894 \ \text{e}^{-7} \]
\[ C_{11} = 0.8576811 \]
\[ C_{12} = 0.12937438 \]
\[ C_{13} = 0.0078748195 \]
\[ C_{12} = 0.0011878541 \]
\[ C_{13} = 7.2287337e^{-5} \]
\[ C_{14} = 1.090584e^{-5} \]
\[ C_{14} = 6.4820296e^{-7} \]
\[ C_{24} = 9.9668384e^{-6} \]
\[ I_1 = 4.4703534e^{-6} \]
\[ I_2 = 7.9400161e^{-7} \]

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