Rate-adapted Dynamic-clamp of the Funny Current in Sinoatrial Pacemaker Cells

Chiara Bartolucci[1], Enrico Ravagli[1], Annalisa Bucchi[2], Mirko Baruscotti[2], Dario DiFrancesco[2]
Stefano Severi[1]

[1]Department of Electrical, Electronic and Information Engineering, University of Bologna, Italy
[2]Department of Biosciences, University of Milan, Italy

Abstract

A typical feature of sinoatrial node (SAN) pacemaker cells is the presence of an ionic current, named 'funny current', I_f, that activates upon hyperpolarization. We aimed to: 1) test the impact of different mathematical models of I_f (Maltsev-Lakatta and Severi-DiFrancesco) in real cells by using the Dynamic Clamp (DC) technique; 2) adapt the DC protocol to adequately simulate a shift of the I_f activation curve.

Two different protocols were implemented: i) the I_f was selectively blocked by Ivabradine and substituted with a "synthetic" I_f dynamically reconstructed from the experimentally recorded action potentials of a single isolated rabbit SAN cell according to the mathematical models; ii) virtual negative shift of the I_f activation curve by the injection of a compensatory current using DC.

The Severi-DiFrancesco model allowed the restoration of control pacemaking rate while the Maltsev-Lakatta model did not. The compensatory current used to reproduce the negative shift of the activation curve did not yield the expected results; we therefore adapted the Dynamic Clamp to the single-cell specific behavior by scaling the estimated If current based on the spontaneous firing rate. This adaptation protocol succeeded in reproducing the effects of a negative activation shift. The Ivabradine protocol was then repeated with this adjustment and the results were fully consistent with those previously obtained.

1. Introduction

The intrinsic pacemaker activity, or spontaneous electrical activity, of sinoatrial node cells (SAN) is based on the spontaneous diastolic depolarization (DD) which drives the membrane potential towards the action potential threshold. The DD is associated with a tiny net inward current across the cell membrane of no more than a few picoamps, which is the result of a complex interaction of multiple inward and outward ion currents. A particularly relevant role in this phase is played by a hyperpolarization-activated current known as "funny current", I_f.

In recent years, I_f has become a target for pharmacological reduction of heart rate. The specific I_f blocker ivabradine (66% current block and 16% pacemaking rate decrease at 3μM [1,2]) has become available for clinical use, and represents a new approach in selective heart rate reduction [3].

The contribution of If to pacemaking has been recently debated also based on computational analysis, through the development of mathematical models of SAN action potential (AP) with quantitatively different I_f membrane densities, such as the Severi-DiFrancesco (SDiF, [4]) and Maltsev-Lakatta (ML, [5]) models.

To investigate both models, we used the Dynamic Clamp (DC), an electrophysiological investigation technique based on a real-time recalculation of the current to inject into the cell under study [6]. This technique was recently used in cardiac electrophysiology to inject a pharmacological blocked current [7] or an additional current with respect to the native ones [8].

The aims of this study are: 1) the use of the classical DC approach for model validation to assess: which of two conflicting mathematical models of rabbit SA node pacemaker cells, with largely different I_f intensities (SDiF, ML), restore the basal condition when used to calculate the DC-based re-injection of I_f blocked by Ivabradine; 2) the implementation and testing of a new DC protocol able to simulate the effects of a negative shift of the activation curve.

2. Methods

2.1. Cell preparation

Isolated rabbit SAN cells were obtained by an enzymatic and mechanical procedure. Spontaneous

activity was recorded by perforated-patch. External (Tyrode) solution (mM): 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES NaOH, 10 D-glucose, pH 7.4. Pipette solution (mM): 130 K-Aspartate, 10 NaCl, 2 CaCl2, (pCa=7), 2 $MgCl_2$, 10 HEPES, 5 EGTA, 2 ATP(Na-salt), 0.1 GTP, 5 Creatine Phosphate, pH 7.2. Amphotericin B (100–250 µg/ml) was added to the pipette solution. T = 37 ± 0.5 °C.

2.2. Experimental Setup

The experimental setup consisted of classical electrophysiology instrumentation and additional hardware and software for the implementation of the DC protocols.

An Axopatch 200B Amplifier (Axon Instruments) was set in current clamp mode, to inject a stimulus current and record cellular activity in the form of membrane potentials. The External Command connector was used to pilot the stimulus current shape and intensity in real-time.

Both signals (V_m, V_{Cmd}) were acquired by a PC running PCLAMP software (Molecular Devices), for the visualization and recording of current and potential tracks. Action potentials (APs) were recorded at a sampling rate of 2 kHz and processed with custom software for rate evaluation.

Axon's V_m and V_{Cmd} channels were also connected to a data acquisition board (DAQ, National Instruments), outfitted with 16-bit resolution analog I/O channels. The board was integrated in a second PC with the RT-Linux Operative System installed. The RTXI (Real-Time eXperiment Interface, [9]) software platform was used to implement and run the DC protocols described in the following paragraphs. Before each test, membrane capacitance is measured to allow for the injection of the correct current density.

2.3. Protocols

Two different DC protocols were implemented using custom RTXI modules, based on the real-time acquisition of the cell's membrane potential, the recalculation of an injection current, and a fast closed-loop response. This feedback mechanism was used in conjunction with Ivabradine (IVA protocol) and to simulate a negative shift in the activation curve (Activation Shift Protocol).

2.3.1. IVA protocol

We run this protocol to identify which model (SDiF or ML) provides a better quantitative description of the I_f current amplitude. The protocol started with the acquisition of AP traces in control and in the presence of 3 µM Ivabradine. The selective block of the I_f current induced by ivabradine, caused a rate slowing [1, 2]. We then compensated the drug-induced current decrease by injecting a synthetic current (by the DC SW interface), whose value was computed real-time from the two models (Figure 1A). The current value was scaled to 66% to reproduce the amount of current blocked by ivabradine.

2.3.2. Activation shift protocol

The activation curve shift protocol, shown in Figure 1B, was developed to study the substitution of the real I_f with a synthetic I_f whose quantitative value was calculated after imposing a negative shift (-4.95 mV) of the voltage-dependence of the I_f current This shift was necessary to reproduce the physiological effect of 10 nM acetylcholine [4].

APs were recorded before, during and after the injection of the synthetic current. Our custom modules allowed to update in real-time the current equation and gating kinetics of both control and shifted I_f current. The difference in the computed control and shifted current (differential DELTA I_f value) was then injected.

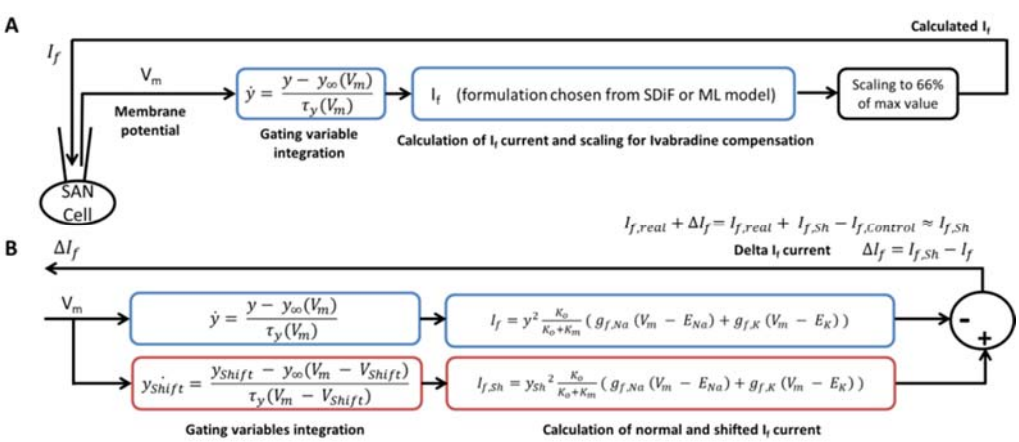

Figure 1A. IVA Protocol: injection of an artificial model-based If to substitute for the current blocked by Ivabradine. **1B**. Activation Shift Protocol: injection of a differential model-based current to simulate an activation shift.

2.4. Data processing and statistical analysis

AP recordings were under sampled from 2 k Hz to 500 Hz and exported in ASCII format on custom software for the evaluation of pacemaking rate evolution in time.

Current injection recordings were subject to smoothing to remove residual noise effects.

For each protocol, rate groups were subject to ANOVA test for repeated measures (F-Test Alpha = 0.05, Assumption Alpha = 0.1, Geisser-Greenhouse adjustment). After checking for ANOVA significance, rates were subject to Tukey-Kramer test for multiple comparisons, with p = 0.05.

3. Results

3.1. IVA protocol

Results obtained with the ivabradine protocol are shown in Figure 2 (top: superimposed AP traces; bottom: mean rate results).

The control pacemaking rate (2.77 ± 0.13 Hz) is significantly reduced after application of 3µM Ivabradine (2.20 ± 0.06 Hz, $p<0.05$), and the rate decrease (-20.6%) is comparable with values reported in literature (-23.8 ± 3.9 [10], -16.2 ± 1.5% [2]).

As already described in the Methods Section, the conductance scaling values for these tests (66%) were chosen in accordance with reported percentages of channel blockage by ivabradine (65.9 ± 2.4%, [2]). The injection of the $SDiF_{66}$ synthetic current increased the average pacemaking rate from the ivabradine level, restoring control value (2.70 ± 0.09, N.S. vs Control), while the injection of ML_{66} synthetic current was not sufficient for full recovery (2.39 ± 0.10, $p<0.05$ vs Control).

To check for the effectiveness of our investigation regarding the ML model, an additional test with 100% scaling value was carried out (ML_{100}), equivalent to the injection of the total calculated synthetic I_f current, which adds to the unblocked remaining current. The increase in current intensity in ML_{100} led to a rate value significantly different from the ivabradine level (2.35±0.08, $p<0.05$) but not high enough to reach the control value.

3.2. Adaptation of DC protocol

The injection of a current reproducing the effects of the negative shift of the activation curve led to an anomalous behaviour (Figure 3): in most of the cells beating with a frequency higher than 2.8 Hz, the injection did not induce the expected rate reduction; in cells with beating rates lower than 2.8 Hz the injection determined a deformation of the shape of the diastolic interval causing a large rate decrease (high rates -3 ± 1% vs low rates -71 ± 7 % $p<0.05$). This behaviour did not faithfully represent the real effect of cholinergic stimuli.

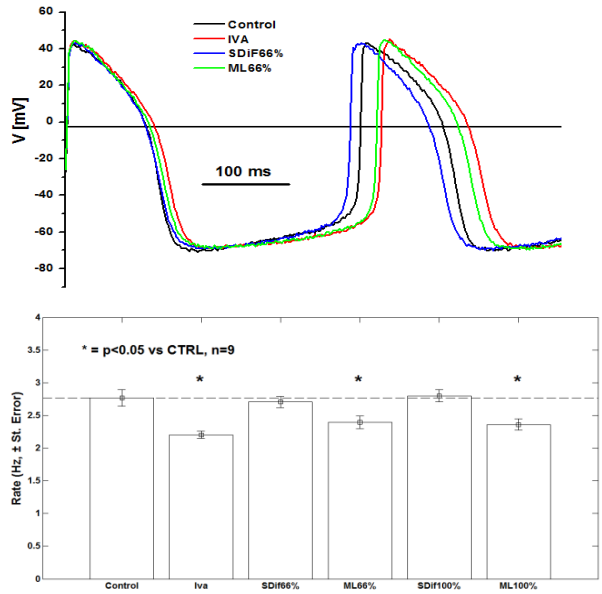

Figure 2. Example of superimposed AP traces recorded during the different stages of the IVA protocol (top). Mean rate values in control, in the presence of Iva (3µM) and after the injection of synthetic I_f calculated using the SDiF and ML models (bottom).

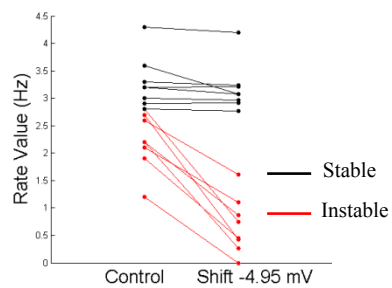

Figure 3.Rate values of sinoatrial cells obtained before (Control) and during the synthetic shifted current injection (shift -4.95 mV).

An additional scaling factor, $g_{Scaling}$ was defined for the output current; $g_{Scaling}$ was computed with a custom RTXI module that acquired control APs at the beginning of each test and returned the estimated scaling factor, used for the rest of the experiment. The estimation was based on the SDiF SAN cell model [3]: changing the maximum I_f conductance gF in the model by a scaling factor allowed (for a reasonable frequency range) to map the connection between $g_{Scaling}$ and Cycle Length:

$$g_{Scaling} = K_1 + K_2 \cdot e^{-\frac{Cycle\ Lengh}{\tau}}$$

To test the effectiveness of the new scaling protocol, negative shift experiments were repeated and rate

reductions similar to those expected were observed in all cells analyzed (Figure 4).

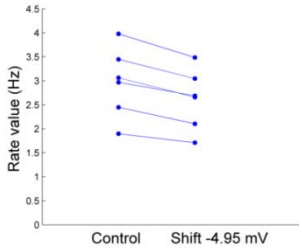

Figure 4. Rate values of sinoatrial cells obtained before (Control) and during the shifted current injection (shift -4.95 mV) after conductance scaling.

We then repeated the IVA protocol (n=6) with this modified scaling protocol using both the SDiF model and the ML models. While the $SDiF_{66}$ was able to effectively restore rate to control values, this was not the case for the ML model (Figure 5).

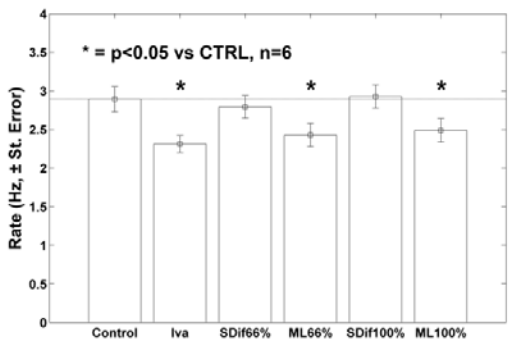

Figure 5. Mean rate values in control, in the presence of Iva (3μM) and after the injection of the synthetic I_f calculated using the SDiF and ML models modified with the scaling factor.

4. Discussion and Conclusion

Our results show that after the rate reduction induced by I_f inhibition with ivabradine only the injection of synthetic I_f calculated with the SDiF model was able to restore the beating frequency to control values. These data support the idea that the I_f current described in the SDiF model is quantitatively closer to the real current flowing in SAN cells than that calculated in the ML model.

Experiments carried out to evaluate how a negative shift of the activation curve of the I_f current affects spontaneous APs rate highlighted the importance of the adaptation of the computational model to the specific real single cell behavior. The introduction of a scaling factor which links the cycle length of the cells (and thus the real I_f current amplitude) of each cell to the injected current is mandatory to compensate for cell heterogeneity. In addition, the post-scaling IVA experiments confirmed the results obtained before the scaling.

References

[1] Bucchi A, Baruscotti M, DiFrancesco D. Current-dependent block of rabbit sino-atrial node I(f) channels by ivabradine. J Gen Physiol 2002 120:1-13.

[2] Bucchi A, Baruscotti M, Robinson RB, DiFrancesco D. Modulation of rate by autonomic agonists in SAN cells involves changes in diastolic depolarization and the pacemaker current. J Mol Cell Cardiol 2007;43:39-48.

[3] Roubille F, Tardif JC. New therapeutic targets in cardiology: heart failure and arrhythmia: HCN channels. Circulation 2013;127:1986-96

[4] Severi S, Fantini M, Charawi LA, DiFrancesco D. An updated computational model of rabbit sinoatrial action potential to investigate the mechanisms of heart rate modulation. J Physiol 2012;590:4483-99.

[5] Maltsev VA, Lakatta EG. Synergism of coupled subsarcolemmal Ca2+ clocks and sarcolemmal voltage clocks confers robust and flexible pacemaker function in a novel pacemaker cell model. Am J Physiol Heart Circ Physiol 2009;296:594-615.

[6] Wilders R. Dynamic clamp: a powerful tool in cardiac electrophysiology. J Physiol 2006;576:349-59.

[7] Bartolucci C, Altomare C, Bennati M, Furini S, Zaza A, Severi S. Combined action potential- and dynamic-clamp for accurate computational modelling of the cardiac IKr current. J Mol Cell Cardiol 2015;79:187-94.

[8] Sala L, Hegyi B, Bartolucci C, Altomare C, Rocchetti M, Mostacciuolo G, Severi S, Szentandrassy N, Nanasy PP, Zaza A Effects of species-dependent differences in action potential shape in setting β-adrenergic-stimulation induced current. Computing in Cardiology 2013, vol.40, pp 381-384.

[9] http://www.rtxi.org/.

[10] Thollon C, Cambarrat C, Vian J, Prost JF, Peglion JL, Vilaine JP. Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49. Br J Pharmacol 1994;112:37-42

Address for correspondence.

Stefano Severi
DEI, University of Bologna, Cesena,
Via Venezia 52, 47521, Cesena (FC), Italy
stefano.severi@unibo.it