**Materials and Methods**

**The numerical model**

This model is based on the published models of Lindblad et al. [1], Cortassa et al. [2], Nguyen et al. [3] and Yaniv et al. [4]. The model describes the atrial membrane potential, ionic currents, Ca2+ cycling, energetics and force generation.

**Action potential, membrane currents and intracellular Ca2+**

This part of our model aims to model AP and the intracellular mechanisms of the rabbit atrial cell.

It is based on the model of Lindblad et al. [1] and consists of two components: an equivalent

electrical circuit corresponding to the sarcolemmal and ionic channels, pumps and exchangers

(Figure 1A) and a model of the intracellular space molecules and SR (Figure 1B). The sarcolemma

is modeled as a capacitor (Cm [pF]), powered by various ionic currents, pumps and exchangers,

which have been previously shown to exist in the rabbit atrial cell membrane[5]. We assumed

that the transmembranal potential (V) is spatially uniform and time varying, and that the total net

ionic current (Inet) is linearly related to the time derivative of V:

This model utilizes Hodgkin-Huxley (HH) based equations in order to characterize each ionic

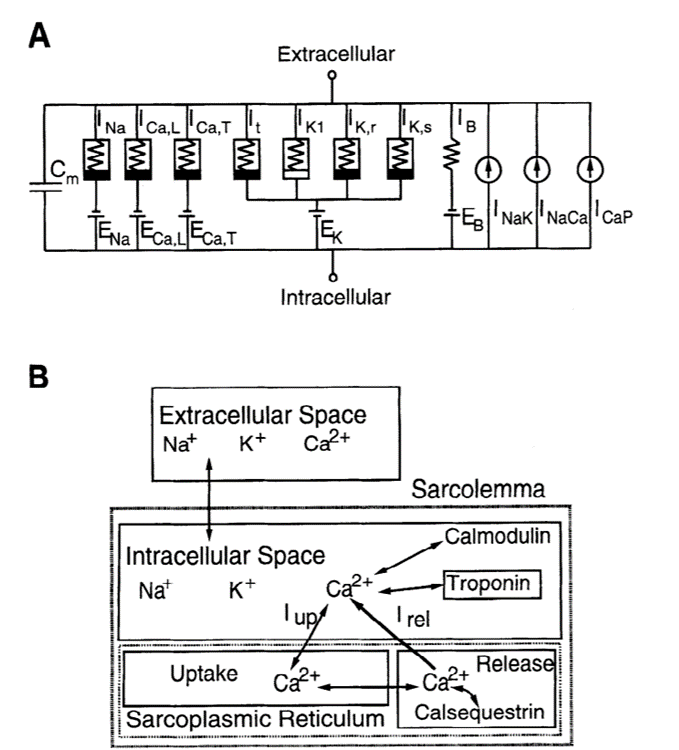
current through the following general equation form:

Where *E*i is the Nernst potential for the *i*th ionic species, is the maximal whole cell conductance for the *i*th current, and the terms are HH-type gating varibales with the exponents for the *i*th ionic current characteristics.

The gating variables, through HH equation characteristics, demonstrate first-order kinetics through the general equation:

Where represents the steady-state value of *y* as a function of V and is the voltage-dependent time constant of *y*.

To summarize, this part our model assumes that: (i) the extracellular ionic concentrations are constant, (ii) the model is considered a single component model for the distribution of Na+, K+ and Ca2+, (iii) the model equations are based on HH formalism, and (iv) the atrial cell possesses a uniform cylindrical geometry.



**A**

**B**

**Figure 1:** The basis of the atrial electrophysiological model: (A) A membrane equivalent circuit. The boxes around the resistors indicate voltage-dependent conductances. The filled portion of boxes demonstrate the time-varying conductances. (B) A fluid compartments model. The intracellular Ca2+ concentration is buffered by calmodulin and troponin in the cytosol and by calsequestrin in the SR release compartment. Adapted from [1].

The reversal (“Nernst”) potentials of the main ions are calculated by:

Where are the ion concentrations in the cytoplasm and in the intracellular space accordingly. R is the ideal gas constant; T is the temperature and F is Faraday’s constant.

The membrane potential is calculated by:

Where is the total membranal current, calculated in eq. (8[6]), is a stimulus current, if present, and is the membrane capacitance.

Fast delayed rectifier current

Where, is the maximal conductance for the fast-delayed rectifying channels, is the membrane potential calculated in eq. (7) and is the Nernst potential of potassium calculated in eq. (4). \*The left option is the actual implementation used in the simulation in Aslanidi and colleagues[7] , the right is the equation stated in both the article [7] and Lindblad’s model [1] In our implementation we have both options.

Slow delayed rectifier current

Where, is the maximal conductance for the slow delayed rectifying channels.

Inward rectifier channel

Where, is the maximal conductance of the Inward rectifier channels and is the equilibrium binding constant for [K+] dependence of the channels.

Transient outward channel

\*The left option is the actual implementation used in the simulation in Aslandi[7], the right is the equation stated in both the article and Lindblad’s model[1]. In our implementation we have both options.

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Where, is the maximal conductance of the transient outward channels.

Sustained outward current

- ATPase

Where, is the maximal current of the Na-K pumps, is the equilibrium binding constant of potassium to the pump and is the equilibrium binding constant of sodium.

exchanger (NCX)

Where, is the position of Eyring rate theory energy barrier controlling voltage dependence of the NCX and is a constant fitted empirically.

Fast channels

1. *(in Lindblad’s paper 0.00035)*
2. *(in Lindblad’s paper 0.00295)*

Membranal pump

Where, is the maximal current of the membranal pumps.

L-type channels

Where is the maximal conductance of the L-type calcium channels and is the reversal potential for the L-type calcium channels. Note that it doesn’t necessary match the Nernst potential of calcium in the cell.

T-Type calcium channels

Where is the maximal conductance of the T-type calcium channels and is the reversal potential for the T-type calcium channels. Note that it doesn’t necessary match the Nernst potential of calcium in the cell.

Background currents

Where, and are the leakage conductance for sodium and calcium.

Total membranal current

Sodium and potassium concentration in cytoplasm

Where, is the intracellular volume and is the cytoplasmic volume.

**Sarcoplasmic Reticulum (SR) and Calcium Handling** [1], [7]

The modeling of calcium concentration in the cell is based on division of the cell to several inner compartments, each with different capabilities of calcium buffering. In this section we model the buffering of all compartments and the intracellular calcium currents between compartments. The inner compartments modeled are the cytoplasm (annotated by “in”) and two SR compartments, an uptake compartment and a release compartment.

Uptake current of to the SR

Where, is the maximal calcium uptake current by the SR, is the equilibrium binding concentration on cytosol side, is the equilibrium binding concentration on uptake compartment of the SR side, is a translocation constant and is the calcium concentration in the uptake compartment of the SR.

Transfer current between the uptake and release compartments of SR

Where, is the calcium concentration in the release compartment of the SR, is the volume of the uptake compartment of the SR and is a transfer time constant.

Release current of calcium from the SR

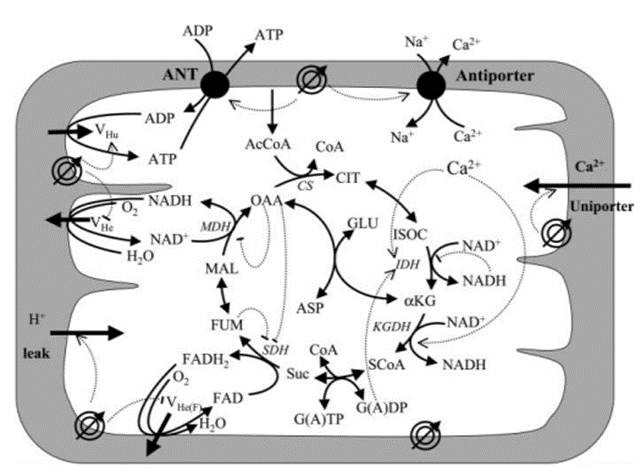
Where, is the rate constant for calcium release, is the volume of the release compartment of the SR, is the rate constant for recovery of the calcium dependent calcium release channels from inactivated state and is the Equilibrium binding constant of SR release gate for .

\*This constant takes different values in different places. This version is the one stated in Aslanidi’s article[7], in their implementation they used 240 and the original value in Lindblad’s article[1] was 203.8.

Where, is the intracellular magnesium concentration and is the intracellular volume for .

**Mitochondrial energy metabolism, Ca2+ dynamics and oxygen consumption**

This part of the model describes the control of atrial mitochondrial metabolism, Ca2+ dynamics and oxygen consumption. The model incorporates the main mitochondrial electrophysiological and metabolic processes and their interactions (Figure 3). This model describes the tricarboxylic acid (TCA) cycle and its regulating enzymes and oxidative phosphorylation. In this model we assume that the cell is within its energy production capabilities, and thus the ATP concentration is constant. The consumption calculated is the consumption of the main dynamic processes that are influenced solely from APs. Other energy demanding processes which are governed by other stimuli such as neuronal or hormonal activations, are not modeled.



**Figure 3:** The mitochondrial component describes the production (F1, F0ATPase) and transport (ANT) of ATP, Ca2+ transport, and Ca2+ activation of the tricarboxylic acid (TCA) cycle dehydrogenases. Adapted from [2].

Mitochondrial energetics and EC coupling

EC coupling and mitochondrial energetics are linked through ATP, adenosine diphosphate (ADP), creatine, creatine phosphate and mitochondrial and cytoplasmic Ca2+ concentrations. Two separate ATP pools are defined within the cytoplasm: ATPi (EC coupling linked) and ATPic­ ­(associated with constitutive cytoplasmic ATPases). In each pool the total concentration of adenine nucleotides is constant and the total concentration of adenine nucleotide CA is the same. Therefore, the following equations apply:

The TCA cycle

In our model, the TCA cycle is split into two pathways: the tricarboxylate (from oxaloacetate

(OAA), to α-ketoglutarate (αKG) via citrate (CIT) and dicarboxylate (from αKG to OAA via

succinate (Suc)). In the tricarboxylate pathway, acetyl CoA (AcCoA) and OAA react to produce

αKG, reduced nicotinamide adenine dinucleotide (NADH) and carbon dioxide (CO2). In turn, αKG

serves as a substrate of the dicarboxylate pathway producing OAA that will resupply the first

pathway. Alternatively, a shunt may directly lead from OAA into αKG through the aspartate amino

transferase (AAT) catalyzed reaction.

1. Citrate synthase (CS)

Where [AcCoA] is acetyl CoA concentration, is the catalytic constant of CS, is CS concentration, is the Michaelis constant of AcCoA, and KMOAA is the Michaelis constant of OAA.

1. Aconitase (ACO)

Where is the forward rate constant of ACO and is the equilibrium constant of ACO.

1. Isocitrate dehydrogenase (IDH)

Where is the activation constant by ADP, is the activation constant for Ca2+, is the inhibition constant by NADH, is the IDH rate constant, is IDH concentration, is the Michaelis constant for isocitrate, is the Michaelis constant for NAD+, is the isocitrate cooperativity, is matrix proton concentration, and and are the ionization constants of IDH.

1. Alpha-ketoglutarate dehydrogenase (KGDH)

When is the activation constant for Mg2+, is the activation constant for Ca2+, is the KGDH concentration, is the KGDH rate constant, is the Michaelis constant for NAD, is the Michaelis constant for αKG, is the Hill coefficient of KGDH for αKG and is the Mg2+ concentration in the mitochondria.

1. Succinyl CoA lyase (SL)

Where is the SL forward rate constant, is the SL reaction equilibrium constant and is the CoA concentration.

1. Succinate dehydrogenase (SDH)

Where is the SDH rate constant, is the SDH enzyme concentration, is the succinate Michaelis constant, is the fumarate inhibition constant, and is the OAA inhibition constant.

1. Fumarate hydratase (FH)

Where is the FH forward rate constant and if the FH equilibrium constant.

1. Malate dehydrogenase (MDH)

Where , , and are MDH ionization constants, is a pH independent term in the pH activation factor of MDH, is the MDH rate constant, is the total MDH enzyme concentration, is the malate Michaelis constant, is the OAA inhibition constant, and is the NAD+ Michaelis constant.

1. Aspartate amino transferase (AAT)

Where is glutamate concentration, is the AAT forward rate constant, is the AAT equilibrium constant and is the rate constant of aspartate.

In summary, because the TCA cycle and the AAT shunt are closed from the point of view of carbon intermediates, a conservation equation relating all TCA metabolites is shown here. Thus, the level of CIT is the result of the balance of all other intermediates in the cycle as follows:

Where is the sum of TCA cycle intermediates' concentration.

Oxidative phosphorylation

1. The respiration-driven proton pump

The main reaction steps in this section are the O2 consumption flux (VO2) and its linked proton efflux, VHe. The rate of these two processes depends on Δ*µ*H and the redox potential as driving forces. Oxygen consumption rate is studied as a function of NADH and ΔΨm. NADH exerts its influence on VO2 through the redox potential Ares.

Where , , ,, , and are the sum of products of rate constants, is the concentration of electron carriers of respiratory complexes I-III-IV, and is the equilibrium constant of respiration.

In a modification of the original formulation, it is also considered that the complex II electrons input by Suc through FADH2 to the respiratory chain:

The flux of protons driven by FADH2 oxidation (VHe(F)) has the same form as VHe except for the adjustment of the redox potential and the H+ stoichiometry. A previous analysis reveals that the sensitivity of the overall O2 consumption with complex II is very low, which leads us to consider FADH2 as a parameter is this model. Mitochondrial NAD+ is assumed to be conserved according to the following relation:

Where is the concentration of electron carriers (respiratory complexes II-III-IV), is the phase boundary potential, is the voltage correction factor, is the equilibrium constant of FADH2 oxidation, is reduced FAD concentration, is oxidized FAD concentration, and is the total sum of mitochondrial pyridine nucleotides.

1. The F1F0-ATPase

According to the concept of respiratory control, mitochondrial function is governed by the availability of ADP and Pi. The chemiosmotic hypothesis dictates that ΔΨm is lowered by an H+ influx, which drives the production of ATP by the F1Fo-ATPase.

Where , , ,, , and are the sum of products of rate constants, is the F1F0-ATPase concentration, is the equilibrium constant of ATP hydrolysis, is the inorganic phosphate concentration and is the total sum of mitochondrial adenine nucleotides.

1. Adenine nucleotide translocator (ANT) and proton leak

In order to complete the description of the major membrane oxidative phosphorylation associated processes, the exchange of adenine nucleotides across the mitochondrial membrane as well as the proton leak, are considered. The ANT model is modeled according to a sequential mechanism of the carrier. VANT, the flux of ANT-mediated exchange between cytosolic ADP and matrix ATP is considered to be electrogenic and dependent on the gradients of both ATP and ADP across the inner mitochondrial membrane as follows:

The proton leak is considered to be a linear function of the Δ*µ*H through a proportionally constant given by the H+ conductance gh:

Where is the maximal ANT rate, if the fraction of , is the ionic conductance of the inner membrane, and is the pH gradient across the inner membrane.

Mitochondrial Ca2+ handling processes

This model assumes that mitochondrial Ca2+ dynamics is controlled by three processes [3]: Ca2+  influx through the Ca2+ uniporter, Ca2+ efflux through the NCX and Ca2+ buffering.

1. The Ca2+ uniporter

The mitochondrial Ca2+ uniporter depends on the electrochemical driving force for Ca2+. Thus, Ψm and extracellular Ca2+ concentration are the primary determinants of the uniporter flux. The uniporter is assumed to be an ion channel permeable only to Ca2+.

The uniporter flux (*J*uni) can be described by:

Where is Ca2+ valence, is the Ca2+ uniporter permeability, is mitochondrial Ca2+ activity coefficient and is the extra-mitochondrial Ca2+ activity coefficient.

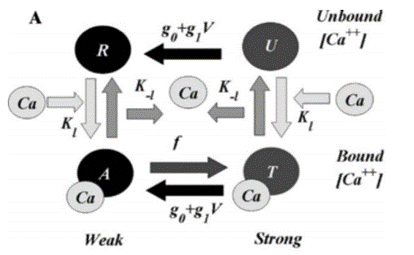
1. The Na+/Ca2+ exchanger

The NCX flux (*J*NC) can be described by:

Where is the Na+/Ca2+ exchanger maximal velocity, is the Na+/Ca2+ exchanger Ca2+ affinity and is the Na+/Ca2+ exchanger Na+ affinity.

**Force generation and energy consumption**[6]

In the implementation we present here, we assume isometric contraction, which is equivalent to assumption of an infinite load placed on a surface. The effect of sarcomere length on the atrial cells’ force generation is modeled as well.



**Figure 4:** The force generation component describes that Ca2+ kinetics and XB cycling control the transitions between the four states of the regulatory units. Adapted from [4].

Where, is the minimal length of the sarcomere, is the reciprocal of the cross section of the tissue, is the cross-bridge independent coefficient of calcium affinity, is the cooperativity coefficient. Describes the dependence of calcium affinity on the number of strong cross-bridges, is Hill coefficient, is the half-maximal cross-bridge Ca2+ affinity, is the rate constant of calcium binding to troponin low-affinity sites, is the cross-bridge turnover rate from the weak to the strong conformation, is the cross-bridge weakening rate at isometric regime, is the mechanical-feedback coefficient (describes the dependence of the XB weakening rate on the shortening velocity), and are constants representing the dependence of force generation on the energy state of the cell and is the unitary force per cross-bridge at isometric regime.

Where, is the maximal ATP consumption by the sarcomeres.

**Single atrial cell isolation and electrical stimulation**

Animals were treated in accordance with the Technion Ethics Committee. The experimental protocols were approved by the Animal Care and Use Committee of the Technion (Ethics number: IL-118-10-13). Single, spindle shaped atrial cells were isolated from rabbit hearts as previously described [8], and placed in HEPES solution  containing (in mM): NaCl 140, KCl 5.4, HEPES 5, Glucose 10, MgCl2 2, CaCl2 1 (pH 7.4 with NaOH( at 36°C. Oxygen consumption was measured using a pair of platinum electrodes as previously described in [8].

**Oxygen consumption**

Oxygen consumption was measured in cell suspensions of atrial cells, either quiescent or stimulated to contract at 1,2 and 3 Hz, using Clark-type electrodes (MT200, Strathkelvin Instruments Ltd.). Atrial cell suspensions were centrifuged at 1000 RPM for 10 min, and the supernatant was removed. The atrial cells were incubated in fresh HEPES solution (as above). The cell suspension was divided equally into 2 aliquots: the first aliquot was designated to be electrically stimulated and the second was used as a control. The atrial cell suspensions were stirred gently under quiescent conditions in 36ºC in HEPES buffer for 2 min, then electrically stimulated at 1,2 and 3 Hz for 1 min, respectively and again under quiescent conditions for 1 min. To measure oxygen consumption in electrically stimulated atrial cells, we designed a custom sealed plunger for the oxygen chamber that included platinum wires. Following measurements of oxygen consumption, total protein concentration (BCATM Protein Assay) and the number of viable cells were determined in the cell suspension. The oxygen consumption of the atrial cells was normalized to the protein concentration.

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