A Computational Model of Cytosolic and Mitochondrial $[Ca^{2+}]$ in Paced Rat Ventricular Myocytes

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We carried out a series of experiment demonstrating the role of mitochondria in the cytosolic and mitochondrial Ca^{2+} transients and compared the results with those from computer simulation. In rat ventricular myocytes, increasing the rate of stimulation (1~3 Hz) made both the diastolic and systolic $[Ca^{2+}]$ bigger in mitochondria as well as in cytosol. As L-type Ca^{2+} channel has key influence on the amplitude of Ca^{2+} -induced Ca^{2+} release, the relation between stimulus frequency and the amplitude of Ca^{2+} transients was examined under the low density (1/10 of control) of L-type Ca^{2+} channel in model simulation, where the relation was reversed. In experiment, block of Ca^{2+} uniporter on mitochondrial inner membrane significantly reduced the amplitude of mitochondrial Ca^{2+} transients, while it failed to affect the cytosolic Ca^{2+} transients. In computer simulation, the amplitude of cytosolic Ca^{2+} transients was not affected by removal of Ca^{2+} uniporter. The application of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) known as a protonophore on mitochondrial membrane to rat ventricular myocytes gradually increased the diastolic $[Ca^{2+}]$ in cytosol and eventually abolished the Ca^{2+} transients, which was similarly reproduced in computer simulation. The model study suggests that the relative contribution of L-type Ca^{2+} channel to total transsarcolemmal Ca^{2+} flux could determine whether the cytosolic Ca^{2+} transients become bigger or smaller with higher stimulus frequency. The present study also suggests that cytosolic Ca^{2+} affects mitochondrial Ca^{2+} in a beat-to-beat manner, however, removal of Ca^{2+} influx mechanism into mitochondria does not affect the amplitude of cytosolic Ca^{2+} transients.

Key Words: Mitochondria, Ca^{2+} transient, Rat ventricular myocytes, Computational model

INTRODUCTION

The major role of ventricular myocardium is to contract regularly in response to electrical excitation in order to pump oxygenated blood out of ventricle. Two key elements are directly involved in the normal cycle of contractile activity in ventricular myocytes. One is intracellular Ca^{2+} -cycling and the other is ATP synthesis by mitochondria. Upon release from sarcoplasmic reticulum (SR), Ca^{2+} binds to troponin C to move the tropomyosin away exposing the myosin-binding site of actin filament. If ATP and Ca^{2+} are high enough near the contractile apparatus, the cross bridge cycle continues and muscle would contract. In the end of contraction, majority of Ca^{2+} is pumped back into the Ca^{2+} store by SR Ca^{2+} pump then muscle relaxes.

Therefore, disruption of either Ca^{2+} -cycling or ATP synthesis can disturb normal cycle of ventricular contraction. ATP synthesis by mitochondria can directly or indirectly affect the Ca^{2+} -cycling by regulating the ATP-driven SR Ca^{2+} pump [1,2] and sarcolemmal Na^+/K^+ pump [1]. Metabolic inhibitors are supposed to suppress the normal cycle of contraction via this mechanism. There has been evidence that Ca^{2+} -cycling affects the ATP synthesis in mitochondria. Increased mitochondrial Ca^{2+} influx across the inner mitochondrial membrane has been known to stimulate F_0F_1 -ATPase activity [3,4]. Increase in mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) has also been known to stimulate tricarboxylic acid (TCA) cycle dehydrogenases [5,6]. The resultant increase in NADH could affect the ATP synthesis of mitochondria. This is supposed to be the control mechanism that matches energy demand for bigger contraction induced by higher cytosolic (or intracellular) $[Ca^{2+}]$ ($[Ca^{2+}]_i$) [7]. There is a controversial issue on the possibility that $[Ca^{2+}]_m$ changes in a beat-to-beat manner. Beat-to-beat mitochondrial Ca^{2+} transients have been identified on adult

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ABBREVIATIONS: FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; SR, sarcoplasmic reticulum; TCA, tricarboxylic acid; AP, action potential.

rabbit cardiac myocytes [8] and on rat cardiac myocytes [9]. In contrast, beat-to-beat change in $[Ca^{2+}]_m$ was not identified on cat ventricular myocytes [10]. Further complicating thing is that the ATP depletion can affect action potential (AP) waveform by stimulating ATP sensitive K^+ channel. Reduced action potential duration (APD) by activation of ATP sensitive K^+ channel can reduce the energy demand during contraction. All above observations demonstrate how complex the interaction among Ca^{2+} -cycling, ATP production, and electrical excitation could be. If all those elements are properly modeled, an integrated mathematical model could be developed and utilized to investigate those complex interactions.

Recently, cardiac models integrating electrophysiology, Ca^{2+} cycling, and energy metabolism have emerged based on experimental results from guinea pig ventricular myocytes [11,12]. Although they were able to couple mitochondrial energetics to excitation-contraction coupling, the description of mitochondrial ion transport is limited and the modeling of pH_m and pH_i is lacking which is a key element in ischaemia-related pathophysiology. We present an advanced model which incorporates an expanded set of mitochondrial ion transport including Na^+ , K^+ , Ca^{2+} , and H^+ based on mitochondrial physiology [13]. Our model is also based on the experimental results on rat ventricular myocytes. The waveform of AP from rat ventricular myocytes is relatively short and 'triangular', whereas those from guinea pig and rabbit show longer and 'spike and a dome'-like configuration. The different waveform of AP can be a factor determining contribution of Ca^{2+} influx across the plasma membrane to the Ca^{2+} -cycling. The effect of metabolic inhibition on the waveform of AP could be different between them. Although there is a model reproducing the electrophysiology and Ca^{2+} cycling of rat ventricular myocytes, it lacks mitochondrial energetics and ion transport [14]. In order to develop and assess our model, we carried out a series of experiment investigating the relation between mitochondrial and cytosolic Ca^{2+} transients under various rates of stimulation. The developed model was able to reproduce main features of rat ventricular myocytes related with stimulus frequency and metabolic inhibition.

METHODS

Cell isolation and Ca^{2+} measurement

All experimental procedures were reviewed and approved by the Institutional Review Board (IRB) of Animal, Inje University College of Medicine, and the procedure was carried out in accordance with the guidelines of the IRB on the ethical use of animals. The methods of isolation of single rat ventricular myocytes and measurement of mitochondrial Ca^{2+} signal are the same as described by Kang et al [15]. In order to measure $[Ca^{2+}]_i$, Fura-2 fluorescence ratio was taken. The isolated myocytes were incubated in normal Tyrode with 0.5 mM Ca^{2+} and the cell-permeant acetoxymethyl ester of Fura-2 (5 μ M) for 10 min at room temperature (24°C). Cells were then perfused with normal Tyrode and excited alternatively at 340 and 380 nm by ultraviolet (UV) light in a DeltaRam fluorescence measurement system (Photon Technologies International). The emission signal at 510 nm was collected at a rate of 100 Hz and the ratio during excitation at 340 and 380 nm was used to record changes in $[Ca^{2+}]_i$. All the background fluorescence

was subtracted from the recorded signals before calculation of the ratio. Ca^{2+} transients were induced by field stimulation. Pulses for field stimulation were generated by an external pulse generator (S-95, Medical Systems Corp) and stimulus isolator (A385, WPI) and were given to cells in a superfusion chamber via two built-in platinum electrodes. The stimuli were 30~50 V in amplitude and 2~5 ms in duration. Temperatures during recording were between 22 and 25°C.

Electrophysiology

Recording of AP was carried out using an Axon interface and Axopatch 1C amplifier (Axon Instruments, Union, CA). All experimental parameters, such as pulse generation and data acquisition, were controlled using our own software (PatchPro). In order to generate APs, square electrical pulses of 1~2 nA and 5 ms duration were applied to the ventricular myocytes through the patch pipette. Patch pipettes were pulled from thin-walled borosilicate capillaries (Clark Electromedical Instruments, Pangbourne, UK) using a PP-83 vertical puller (Narishige, Tokyo, Japan). The voltage and current signals were filtered at 1.0~5.0 kHz, 4-pole Bessel-type low-pass filter and were sampled at a rate of 1~3 kHz. Electrode resistance before sealing was 3~4 M Ω with K^+ -rich recording solution, and it was around 5 G Ω after sealing. The liquid-junction potentials between normal Tyrode and pipette solution, which were calculated based on ionic mobilities, were <5 mV. Temperatures were 22~25°C during recording.

Solutions and drugs

Normal Tyrode solution was used for the perfusion of myocytes. It contained (in mM): 143 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5.5 glucose, and 5 HEPES (N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]) (pH 7.4). The perforated patch pipette solution contained (in mM): 115 K-aspartate, 22 KCl, 8 NaCl, 1 $MgCl_2$, 0.1 EGTA (ethylene-glycol-bis (β -aminoethyl ether)-N,N,N',N'-tetraacetic acid), and 10 HEPES (pH 7.2 with KOH). Nystatin was dissolved in dimethyl sulfoxide (60 mg/ml) and added to the pipette solution to make a final concentration of 200 μ g/ml. Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Mathematical model

General approach

This model aims to reproduce ion currents, membrane potential, ion concentrations and energy metabolites in each of the subcellular compartments and contractile force during excitation-contraction cycle of single rat ventricular myocyte. The extramitochondrial part generally follows the same approach as described by Matsuoka et al [11] while the mitochondrial part follows the approach by Cortassa et al [12]. As the two models are based on the data of guinea pig ventricular myocytes, lots of modifications were made to fit those of rat ventricular myocytes by exploiting the electrophysiological data from rat. The numerical integration was carried out by a fourth-order Runge-Kutta method in most cases. For the two-state or three-state gating model, the alternative method was used to prevent large errors accumulated during repetitive calculation as previously described by Youm et al [16].

Myocyte ultrastructure

The value of cell capacitance was chosen to be 140 pF which is averaged value from 143 adult (2~4 months old) rat ventricular myocytes [17]. The accessible cell volume was estimated from the geometry [18] and ultrastructural analysis [19] of adult rat ventricular myocytes. A representative myocyte was 116 μm in length, 23 μm in width, and 14 μm in depth. The cell shape was assumed to be a rectangular parallelepiped. The estimation gives the total myocardial cell volume to be 37.3 pL. The accessible volume was then calculated to be 13.4 pL based on the assumption that 36% of total myocardial cell volume is accessible to ions [19]. The volume of mitochondrial matrix was calculated to be 6.35 pL applying the ratio (0.17) of mitochondrial matrix to total myocardial cell volume [19]. The volumes of release and uptake site of SR were also calculated based on the ratio of each of them to total myocardial cell volume (Table 1).

Part I. Extramitochondrial space

Most of modeling in extramitochondrial space is based on the Kyoto Model developed by Matsuoka et al [11]. Main components specific to rat ventricular myocytes are described in following section. Complete list and corresponding equations including parameters are presented in part I of Appendix (Eqs. 1~227).

Ca^{2+} -independent transient outward K^+ current (I_{to})

The transient outward K^+ current (I_{to}) is responsible for the initial fast repolarization phase of AP. Our formulation of I_{to} is based on the experimental finding that it is composed of two distinct populations (fast and slow component) of channel with kinetically different recovery from inactivation [20]. The voltage dependence of steady-state activation and inactivation was formulated based on the experimental data by Nie and Meng [21]. The voltage dependence of steady-state inactivation in slow component of I_{to} was supposed to be the same with that of fast component. The voltage dependence of activation time constants was formulated based on the experimental data obtained between -30 and +60 mV [22]. The voltage dependence of time constants of inactivation and recovery from inactivation in fast component of I_{to} was determined from experimental data of rat ventricular myocytes [23].

The slow component of inactivation in I_{to} is revealed on its recovery from inactivation during repolarization after depolarizing voltage steps. Time constants of slow component from the experimental data of Shimoni et al [20] were utilized to formulate its voltage dependence.

The relative contribution of fast and slow component to total I_{to} and its amplitude were determined from the experimental data by Volk et al [24].

Steady-state outward K^+ current (I_{ss})

The steady-state outward K^+ current (I_{ss}) has been known to take part in the slower phase of AP repolarization back to the resting membrane potential in adult rat ventricular myocytes [22]. The formulation of I_{ss} is the same as used by Pandit et al [14].

Table 1. General parameters

Symbol	Definition	Value	Unit
V_i	Accessible cell volume	13,400	fL
V_{rel}	Volume of SR release site	130.6	fL
V_{up}	Volume of SR uptake site	1,175	fL
V_{mito}	Volume of mitochondrial matrix	6,350	fL
C_m	Membrane capacitance	140	pF
C_{mito}	Inner membrane capacitance of mitochondria	$1.812 \cdot 10^{-3}$	$\text{mM} \cdot \text{mV}^{-1}$
V	Membrane potential	-80.173	mV
F	Faraday constant	96.4867	$\text{C} \cdot \text{mmol}^{-1}$
R	Universal gas constant	8.3143	$\text{C} \cdot \text{mV} \cdot \text{K}^{-1} \cdot \text{mmol}^{-1}$
T	Absolute temperature	297.15	K

Delayed rectifier K^+ current, rapid component (I_{Kr})

The formulation of I_{Kr} is the same as that in Kyoto Model by Matsuoka et al [11]. The maximum conductance was adjusted to reproduce the same current density (0.36 $\text{pA} \cdot \text{pF}^{-1}$) from rat ventricular myocytes with the same voltage-clamp protocol [25].

Inward rectifier K^+ current (I_{K1})

The inward rectifier K^+ current (I_{K1}) contributes most to the formation of resting membrane potential and takes part in the later phase of AP repolarization. Conductance of I_{K1} decreases with depolarization while it increases with repolarization. The formulation of I_{K1} generally follows the approach by Pandit et al [14]. The conductance was modified to be proportional to $[\text{K}^+]_e$ following the experimental data describing the dependence of single channel conductance on $[\text{K}^+]_e$ [26]. The conductance was also adjusted to reproduce the same current density as that of experimental data from rat ventricular myocytes [27].

ATP-sensitive K^+ current ($I_{K_{ATP}}$)

The formulation for $I_{K_{ATP}}$ was adapted from the work of Nichols and Lederer [28] where rat ventricular myocytes were used to incorporate the ATP sensitive K^+ channel into AP model.

Na^+ current (I_{Na})

The formulation of I_{Na} is almost same as used by Luo and Rudy [29]. The maximum conductance was adjusted to reproduce the same current density from rat ventricular myocytes [30].

L-type Ca^{2+} current (I_{CaL})

The formulation of I_{CaL} is mostly based on the work by Pandit et al [14] with following adjustments. The voltage-dependence of steady-state activation and inactivation was formulated on the basis of experimental data from Katsube et al [17]. The maximum conductance was adjusted

to reproduce the same current density ($6.9 \text{ pA} \cdot \text{pF}^{-1}$) of rat ventricular myocytes [31] with a voltage clamp step from -45 to -5 mV . The inactivation of I_{CaL} is also dependent on the Ca^{2+} -calmodulin complex [32]. Detailed equations and parameters governing Ca^{2+} -calmodulin complex-mediated inhibition were adopted from Sun et al [33].

Non-selective cation channel (I_{bNSC})

Non-selective cation channels are characterized to be non-selective to cations such as Na^+ , K^+ , and Ca^{2+} . It potentially contributes to slight depolarization of myocytes against the equilibrium potential of K^+ (E_{K}). The formulation of non-selective cation channel is based on the model of rat heart [34] which incorporated the selective permeability of channels to various cations. The maximum conductance was adjusted to reproduce the appropriate AP shape of rat ventricular myocytes.

Background Ca^{2+} leak current (I_{Cab})

Ca^{2+} leak or background Ca^{2+} channel has been reported in adult rat ventricular myocytes [35,36]. This channel showed a significant permeability to divalent cations but not to monovalent cations such as K^+ and Na^+ ions [35]. This channel was suggested to play a role in the Ca^{2+} overload induced by metabolic inhibition or free radical exposure [36] and is the candidate channel which partly contributes to the cardiac muscle activation during postrest recovery [37]. The current density was adjusted to reproduce the experimental observation of postrest recovery from rat ventricular myocytes by Bers [37].

Ca^{2+} dynamics

The uptake of cytosolic Ca^{2+} into SR by SR Ca^{2+} pump and release of Ca^{2+} by ryanodine receptor are based on the Kyoto Model by Matsuoka et al [11]. The buffering of Ca^{2+} by calmodulin, troponin C, and calsequestrin are also based on the model. The L-type Ca^{2+} channel-dependent activation of ryanodine receptor was adjusted to fit the amplitude of Ca^{2+} transients in rat ventricular myocytes [38] (Table 2).

Na^+/K^+ pump and $\text{Na}^+/\text{Ca}^{2+}$ exchanger

The lumped two-state model for Na^+/K^+ pump and $\text{Na}^+/\text{Ca}^{2+}$

Table 2. Ca^{2+} -binding proteins

Symbol	Definition	Value	Unit
$[\text{T}]_{\text{t}}$	Total concentration of troponin C	0.07	mM
K_{mT}	Michaelis constant of troponin C for Ca^{2+}	$7.7 \cdot 10^{-4}$	mM
$[\text{CMDN}]_{\text{t}}$	Total concentration of calmodulin	0.05	mM
K_{mCMDN}	Michaelis constant of calmodulin for Ca^{2+}	$2.38 \cdot 10^{-3}$	mM
$[\text{CSQN}]_{\text{t}}$	Total concentration of calsequestrin	10	mM
K_{mCSQN}	Michaelis constant of calsequestrin for Ca^{2+}	$8 \cdot 10^{-1}$	mM

Ca^{2+} exchanger developed by Matsuoka et al [11] was adopted with modifications to fit the $[\text{Na}^+]_{\text{i}}$ and $[\text{Ca}^{2+}]_{\text{i}}$ in rat ventricular myocytes [39]. The cooperative constant for ATP_i -dependency of Na^+/K^+ pump was modified to fit the steep rise in $[\text{Na}^+]_{\text{i}}$ by inhibition of Na^+/K^+ pump using strophanthidin. The activation constant of Na^+/K^+ pump by extracellular K^+ was also modified to fit the $[\text{Na}^+]_{\text{i}}$ under resting and paced state. The activation constants of $\text{Na}^+/\text{Ca}^{2+}$ exchanger for Na^+_{i} and $\text{Ca}^{2+}_{\text{i}}$ were modified to fit diastolic and systolic level of Ca^{2+} transients [38].

Na^+/H^+ exchanger and pH_i

Change in $[\text{H}^+]_{\text{i}}$ for a given amount of H^+ flux across the inner membrane of mitochondria was calculated by dividing the flux by intrinsic buffering power [40]. The H^+ flux was simulated by integration of flux by Na^+/H^+ ex-

Table 3. Initial values of parameters related with state variables

Category	Parameters	Value	Unit
I_{Na}	I_{Na}	$-2.014 \cdot 10^{-2}$	pA
	m	$3.320 \cdot 10^{-3}$	
	h	$9.582 \cdot 10^{-1}$	
	j	$9.726 \cdot 10^{-1}$	
I_{CaL}	I_{CaL}	$-1.769 \cdot 10^{-1}$	pA
	d	$5.360 \cdot 10^{-5}$	
	f_{11}	$9.998 \cdot 10^{-1}$	
	f_{12}	$9.998 \cdot 10^{-1}$	
	Ca_{inact}	$9.673 \cdot 10^{-1}$	
I_{K1}	I_{K1}	$3.393 \cdot 10^1$	
I_{Kr}	I_{Kr}	$8.311 \cdot 10^{-3}$	pA
	y1	$1.678 \cdot 10^{-3}$	
	y2	$1.695 \cdot 10^{-3}$	
	y3	$9.555 \cdot 10^{-1}$	
I_{to}	I_{to}	1.892	pA
	y1	$4.496 \cdot 10^{-2}$	
	y2	$8.532 \cdot 10^{-1}$	
	y3	$8.118 \cdot 10^{-1}$	pA
I_{ss}	y1	$2.643 \cdot 10^{-2}$	
	y2	$2.938 \cdot 10^{-3}$	
	y3	$3.327 \cdot 10^{-1}$	
I_{NaK}	I_{NaK}	$2.084 \cdot 10^1$	pA
	Y	$4.493 \cdot 10^{-1}$	
	V_{NHE}	$3.939 \cdot 10^{-9}$	$\text{mM} \cdot \text{ms}^{-1}$
I_{NaCa}	I_{NaCa}	-4.306	pA
	Y	$9.806 \cdot 10^{-1}$	
	I_{bNSC}	$-4.527 \cdot 10^1$	pA
I_{Cab}	I_{Cab}	-7.548	pA
I_{KATP}	I_{KATP}	$6.249 \cdot 10^{-1}$	pA
I_{RyR}	I_{RyR}	$2.628 \cdot 10^2$	pA
	Close	$4.229 \cdot 10^{-1}$	
	Open	$2.700 \cdot 10^{-3}$	
	Unavailable	1·Close·Open	
$I_{\text{SR T}}$	$I_{\text{SR T}}$	$2.626 \cdot 10^2$	pA
$I_{\text{SR L}}$	$I_{\text{SR L}}$	$1.033 \cdot 10^3$	pA
$I_{\text{SR U}}$	$I_{\text{SR U}}$	$1.294 \cdot 10^3$	pA
	Y	$4.033 \cdot 10^{-1}$	
I_{PMCA}	I_{PMCA}	1.067	pA
contraction	$[\text{TCa}]$	0.0014	mM
	$[\text{TCa}^*]$	$8.599 \cdot 10^{-5}$	mM
	$[\text{T}^*]$	$1.759 \cdot 10^{-5}$	mM
	$[\text{T}]$	0.0684	mM

Table 4. Initial concentrations of ions and energy metabolites in extramitochondrial space

Symbol	Definition	Value	Unit
$[\text{Na}^+]_i$	Cytosolic (intracellular) Na^+ concentration	9.008	mM
$[\text{Na}^+]_e$	Extracellular Na^+ concentration	140	mM
$[\text{K}^+]_i$	Cytosolic K^+ concentration	139.6	mM
$[\text{K}^+]_e$	Extracellular K^+ concentration	5.4	mM
$[\text{H}^+]_i$	Cytosolic H^+ concentration	$7.054 \cdot 10^{-5}$	mM
$[\text{Ca}]_i$	Total cytosolic calcium concentration	$3.546 \cdot 10^{-4}$	mM
$[\text{Ca}^{2+}]_i$	Cytosolic Ca^{2+} concentration	$1.622 \cdot 10^{-5}$	mM
$[\text{Ca}^{2+}]_e$	Extracellular Ca^{2+} concentration	2.0	mM
$[\text{Ca}]_{\text{rel}}$	Total calcium concentration in SR release site	8.195	mM
$[\text{Ca}^{2+}]_{\text{rel}}$	Ca^{2+} concentration in SR release site	1.570	mM
$[\text{Ca}^{2+}]_{\text{up}}$	Ca^{2+} concentration in SR uptake site	2.251	mM
$[\text{ATP}]_i$	EC coupling linked ATP concentration	7.580	mM
$[\text{ATP}]_{\text{ic}}$	Cytosolic ATP concentration not linked to EC coupling	7.507	mM
$[\text{ADP}]_i$	EC coupling linked ADP concentration	$4.247 \cdot 10^{-1}$	mM
$[\text{ADP}]_{\text{ic}}$	Cytosolic ADP concentration not linked to EC coupling	$4.933 \cdot 10^{-1}$	mM
$[\text{CrP}]_i$	Mitochondrial linked creatine phosphate concentration	14.788	mM
$[\text{CrP}]_{\text{ic}}$	Cytosolic creatine phosphate concentration	14.783	mM
$[\text{Cr}]_i$	Mitochondrial linked creatine concentration	10.211	mM
$[\text{Cr}]_{\text{ic}}$	Cytosolic creatine concentration	10.217	mM

changer on sarcolemma and flux between cytosol and mitochondria. Calculated $[\text{H}^+]_i$ was converted into pH_i.

Cell contraction

The contraction model developed by Negroni and Lascano [41] reproduces the sarcomere cross-bridge dynamics in relation with calcium kinetics. Almost all equations and parameters were adopted from the model integrating the consumption of ATP and other energy intermediates by cell contraction with synthesis of ATP by mitochondria (Eqs. 195~227) (Table 3).

Energy intermediates linking the mitochondria and cell contraction

The mitochondrial energetics and the cell contraction are linked by various energy intermediates such as ATP, ADP, creatine, and creatine phosphates. Creatine kinase provides the cell with energy buffer by catalyzing the transfer of phosphates from creatine phosphate to ADP to generate ATP. Modeling of this reaction was adopted from the model by Cortassa et al [12], which was then integrated into the cell contraction.

Ionic linkage between mitochondria and cytosol

Flux of ions and energy intermediates including Na^+ , K^+ , Ca^{2+} , ATP, and ADP from mitochondria was integrated to the calculation of cytosolic concentrations of them by considering the ratio of volume (V_{mito}/V_i) (Table 4, 5).

Part II. Mitochondria

The modeling in mitochondrial space integrates TCA cycle, oxidative phosphorylation, ATP/ADP exchange, mitochondrial electrophysiology, and handling of ions including Na^+ , K^+ , Ca^{2+} , and H^+ (pH). Coupling of extra-mitochondrial space and mitochondria is accomplished by modeling the transport of ions between mitochondria and

extramitochondrial space. Main components of modeling in mitochondria are briefly described in this section and the detailed equations governing all the components are presented in the part II of Appendix (Eqs. 228~362).

Physical dimension

Physical dimension of mitochondria was estimated from the ultrastructural analysis [19] of adult rat ventricular myocytes and the volume of mitochondrial matrix was presented in Table 1. The capacitance of inner membrane ($\text{mM} \cdot \text{mV}^{-1}$) was adopted from Cortassa et al [12] and was converted into the unit of pF, when the mitochondrial volume and current amplitude rather than flux are utilized to calculate ionic concentrations (Table 1).

The TCA cycle

TCA cycle provides electron transport chain with NADH and FADH_2 by oxidizing the AcCoA into CO_2 . TCA cycle is regulated by Ca^{2+} which can increase the activity of dehydrogenases such as citrate synthase (CS), isocitrate dehydrogenase (IDH), α -ketoglutarate dehydrogenase (KGDH), and malate dehydrogenase (MDH). The model of TCA cycle is mostly based on that of Cortassa et al [12]. Concentration of acetyl CoA (AcCoA) feeding the TCA cycle is set to a variable in order to see the effect of substrate supply to the TCA cycle. Detailed equations and initial values are presented in Appendix (Eqs. 228~300) and Table 5, respectively.

Oxidative phosphorylation

Electron transport chain or respiratory chain on the mitochondrial inner membrane begins the oxidative phosphorylation with the input of NADH and FADH_2 from TCA cycle. Complex I~IV oxidize them and provide the inner membrane with the electrochemical gradient by pumping the proton (H^+) out of mitochondrial matrix. Complex V (F_1F_0 -ATPase) utilizes the electrochemical gradient for pro-

Table 5. Definition and initial values of parameters in mitochondrial space

Symbol	Definition	Value	Unit
[AcCoA]	Acetyl CoA concentration	$6.352 \cdot 10^{-7}$	mM
[OAA]	Oxaloacetate concentration	$7.748 \cdot 10^{-1}$	mM
[CIT]	Citrate concentration	$9.984 \cdot 10^{-3}$	mM
[ISOC]	Isocitrate concentration	$2.199 \cdot 10^{-2}$	mM
[α KG]	α -ketoglutarate concentration	$6.461 \cdot 10^{-5}$	mM
[GLU]	Glutamate concentration	10	mM
[SCoA]	Succinyl CoA concentration	$8.392 \cdot 10^{-3}$	mM
[Suc]	Succinate concentration	$1.967 \cdot 10^{-4}$	mM
[CoA]	CoA concentration	$2 \cdot 10^{-2}$	mM
[FUM]	Fumarate concentration	$9.237 \cdot 10^{-2}$	mM
[MAL]	Malate concentration	$9.190 \cdot 10^{-2}$	mM
[ATP] _m	ATP concentration	1.145	mM
[ADP] _m	ADP concentration	$3.547 \cdot 10^{-1}$	mM
Pi	Inorganic phosphate concentration	2	mM
[NADH]	NADH concentration (reduced)	$2.496 \cdot 10^{-5}$	mM
[NAD ⁺]	NAD ⁺ concentration (oxidized)	9.999	mM
[FADH ₂]	FADH ₂ concentration (reduced)	1.240	mM
[FAD]	FAD concentration (oxidized)	$1 \cdot 10^{-2}$	mM
ρ^{res}	Concentration of electron carriers (respiratory complexes I-III-IV)	$3.0 \cdot 10^{-3}$	mM
$\rho^{\text{res(F)}}$	Concentration of electron carriers (respiratory complexes II-III-IV)	$3.75 \cdot 10^{-4}$	mM
ρ^{F1}	Concentration of F ₁ F ₀ -ATPase	1.5	mM
C_A	Total concentration of mitochondrial adenine nucleotides	1.5	mM
C_{PN}	Total concentration of mitochondrial pyridine nucleotides	10	mM
[Ca ²⁺] _m	Ca ²⁺ concentration	$6.604 \cdot 10^{-6}$	mM
[K ⁺] _m	K ⁺ concentration	44.93	mM
[Na ⁺] _m	Na ⁺ concentration	2.902	mM
[H ⁺] _m	H ⁺ concentration	$2.032 \cdot 10^{-5}$	mM
[Mg ²⁺] _m	Mg ²⁺ concentration	0.4	mM
$\Delta\Psi_m$	Negative value of mitochondrial membrane potential	142.62	mV

ton to phosphorylate matrix ADP into ATP. The adenine nucleotide translocator exchanges the matrix ATP with extramitochondrial ADP. The model of oxidative phosphorylation is mostly based on that of Magnus and Keizer [42]. Most of detailed parameters were adopted from the model of Cortassa et al [12]. Different from that of Cortassa et al [12], the proton gradient across the inner membrane (Δ pH) is regarded as a variable rather than a constant to calculate the proton motive force ($\Delta \mu$ H). Equations governing oxidative phosphorylation and initial values are presented in the section of oxidative phosphorylation reaction rates of Appendix (Eqs. 301~333) and Table 5.

Ionic homeostasis in mitochondria

Concentrations of Na⁺, K⁺, H⁺, and Ca²⁺ inside mitochondria were determined based on the flux of each ion across the inner membrane of mitochondria. Fluxes of Na⁺/Ca²⁺ exchanger (V_{NCX}) and Na⁺/H⁺ exchanger (V_{NHE}) were used to calculate [Na⁺]_m. Equations governing the fluxes are based on the model from Nguyen et al [43] with minor modification to fit the value of [Na⁺]_m obtained from experiment [44]. In order to simulate [K⁺]_m, fluxes of ATP-sensitive potassium channel (V_{KATP}) and K⁺/H⁺ exchanger (V_{KH}) were used. Modeling of V_{KATP} is based on the plasmalemmal ATP sensitive K⁺ channel with modifications for mitochondria. Modeling of V_{KH} is based on that of Nguyen et al [43]. Fluxes of Ca²⁺ uniporter (V_{uni}) and Na⁺/Ca²⁺ exchanger (V_{NCX}) were used to calculate [Ca²⁺]_m based on the modeling by Nguyen et al [43]. Change in [Ca²⁺]_m was calculated dividing the flux by Ca²⁺-buffering power in mitochondria [45]. [H⁺]_m or pH_m was calculated from the fluxes through electron transport chain (V_{Hu} , V_{Hleak} , V_{He} , and V_{HeF}) and transporters (V_{KH} , V_{NHE} , and V_{ANT}). Change in [H⁺]_m for a given amount of H⁺ flux across the inner membrane of mitochondria was calculated by dividing the flux by intrinsic buffering power assuming it is similar to the value of extramitochondrial space. Calculation of intrinsic buffering power is based on the method developed by Niederer and Smith [40]. All the detailed equations are presented in Appendix (Eqs. 334~362).

RESULTS

Reconstructed APs

The integrated computer model of rat ventricular myocytes was examined whether it could reproduce the waveform of experimentally generated APs. Single rat ventricular myocyte was whole-cell clamped in a perforated patch configuration and was stimulated in a current clamp mode. The amplitude and duration of single stimulus current were 1~2 nA and 5 ms, respectively. Smaller or shorter stimulus often failed to elicit the APs. Stimulus was applied at least 200 sec for the cell to reach steady-state condition.

The representative recording of elicited AP is shown in Fig. 1A. The plateau is hardly noticeable and the APD is relatively short compared with those of guinea-pig ventricular myocytes [46]. The resting membrane potential (−76.3 mV) was slightly positive to the calculated Nernst equilibrium potential of K⁺ at 24°C ($E_{\text{K}} = -83.0$ mV). The model-generated AP is demonstrated in Fig. 1B. The stimulus current was 1.5 nA in amplitude and 5 ms in duration. The waveform is similar to that of experimentally generated AP. The APD and resting membrane potential of model are also comparable to those of experimentally generated APs although the resting membrane potential of experimentally-generated AP is a little more depolarized (−76 versus −80.2 mV).

Stimulus frequency and cytosolic Ca²⁺ transients

The rate of stimulation has been known to increase the twitch amplitude of mammalian heart [47]. In case of rat ventricular myocytes, the relationship is controversial. A negative staircase has been reported within the range between 0 and 1 Hz in rat ventricular myocardium or myocytes, where increasing the rate of stimulation up to 1 Hz

Fig. 1. Comparison of APs generated by experiment (A) and model simulation (B). (A) Myocytes were stimulated at a rate of 1 Hz and a representative trace of AP was obtained after 200 sec of stabilization period. Resting membrane potential was at -76.3 ± 3.7 mV ($n=21$). APD_{50} and APD_{90} of the representative recording were 21.1 ms and 42.9 ms, respectively. Recordings were obtained at room temperature ($22 \sim 25^\circ\text{C}$). (B) Model-generated AP. Resting membrane potential was at -80.2 mV. APD_{50} and APD_{90} were 22.2 ms and 43.4 ms, respectively. Temperature was set at 24°C (Table 1).

Fig. 2. Stimulus frequency-dependent change in cytosolic Ca^{2+} transients recorded by experiment (A) and model (B). (A) Myocytes were field stimulated at a rate of 0.2 Hz for 200 sec and the rate was sequentially changed from 0.2 to 1 Hz, 2 Hz, and 3 Hz. Fura-2 fluorescence ratio (F_{340}/F_{380}) was recorded to see change in cytosolic Ca^{2+} transients. Expanded trace of fluorescence ratio during 2 Hz stimulation was demonstrated on the right panel. The expanded trace was chosen from our other experimental data. (B) Model-generated cytosolic Ca^{2+} transients. Expanded trace during 2 Hz stimulation was also displayed on the right panel. The downward arrow indicates the point where the expanded trace was extracted.

reduced the amplitude of twitch and Ca^{2+} transients [47-49]. Above the rate of 1 Hz stimulation, the rat ventricular myocardium or myocytes showed the positive staircase as well. Frampton et al [50], however, demonstrated the positive staircase along the whole range of stimulation rate between 0.1 and 2 Hz in rat ventricular myocytes. It

was supposed that the positive staircase is mediated by increased Ca^{2+} influx through L-type Ca^{2+} channel with increasing frequency of AP while negative staircase is related to the frequency-dependent decrease in restitution of SR Ca^{2+} [50]. Therefore, examining the stimulus frequency-dependent change in $[\text{Ca}^{2+}]_i$ would be appropriate to assess

Fig. 3. Negative staircase in the model with reduced I_{CaL} . (A) Model simulation with control. The rate of stimulation was switched between 0.2 and 0.8 Hz. (B) Model simulation with reduced I_{CaL} . Channel density of L-type Ca^{2+} channel was reduced to 1/10 of control. The stimulation protocol is the same as that one used in A. (C) Model simulation with increased I_{to} . Channel density of Ca^{2+} -independent transient outward K^+ current (I_{to}) was increased to 10-times of control.

the relative contribution of Ca^{2+} influx through L-type Ca^{2+} channel and Ca^{2+} release from SR to the cytosolic Ca^{2+} increase.

Fig. 2A shows the recording of Fura-2 fluorescence demonstrating the effect of changing stimulation rate on $[Ca^{2+}]_i$. Increasing stimulation rate elevated both the diastolic and systolic Ca^{2+} along the whole range of our stimulation protocol (0.2~3 Hz) implying that the contribution by L-type Ca^{2+} currents dominates the relationship between stimulus frequency and amplitude of Ca^{2+} transients of rat ventricular myocytes at least in our experimental condition. Model simulation was able to reproduce this relationship in agreement with our experimental results (Fig. 2B).

Borzak et al [49] found that the verapamil, an L-type Ca^{2+} channel blocker, makes the twitch amplitude smaller with increasing rate of stimulation in the whole range between 0.2 and 6 Hz suggesting that the L-type Ca^{2+} channel contributes to the mechanism of positive staircase. Since the verapamil makes the signal of Fura-2 fluorescence almost undetectable, we tested the hypothesis on model simulation.

Fig. 3 compares the effect of changing stimulus frequency

in the model of reduced I_{CaL} with that of control. The amplitude of Ca^{2+} transients with reduced I_{CaL} was much smaller than those of control during 0.2 Hz stimulation. Switching the rate of stimulation from 0.2 to 0.8 Hz made the amplitude even smaller revealing the negative staircase (Fig. 3B). In order to test whether changing the AP waveform itself can affect the frequency-dependent change in the amplitude of Ca^{2+} transients, the effect of raising channel density of I_{to} was simulated. 10-fold increase in I_{to} resulted in shortening of AP and revealed the negative staircase (Fig. 3C) whereas 5-fold increase still showed the positive staircase (data not shown). The APD_{90} with 10-fold increase in I_{to} , however, was less than 10 ms, which is extremely short for rat ventricular myocytes. The APD_{90} with reduced density of I_{CaL} was about 36 ms, which is in similar range to that of epicardial ventricular myocytes (for details see Fig. 1 of Clark et al [51]).

Stimulus frequency and mitochondrial Ca^{2+} transients

The ratio of mitochondrial matrix to total myocardial cell volume is estimated to be 0.17 and the accessible volume of cytosolic compartment is estimated to be 0.36 of total myocardial cell volume [18]. Therefore, the ratio of mitochondrial matrix to accessible volume could be estimated as 0.47. If there are channels or transporters for ions on the mitochondrial membrane with rapid kinetics, beat-to-beat change in $[Ca^{2+}]_i$ is supposed to induce beat-to-beat change in $[Ca^{2+}]_m$. Ca^{2+} uniporter (mCU) has been suggested to transport cytosolic Ca^{2+} into mitochondria utilizing the large electrochemical gradient for Ca^{2+} across the inner mitochondrial membrane [52,53]. A rapid mode of Ca^{2+} uptake (RaM) [54] and mitochondrial ryanodine receptors [55] are also suggested to transport Ca^{2+} into mitochondrial matrix. The mitochondrial Na^+/Ca^{2+} exchanger has been suggested to pump out $[Ca^{2+}]_m$ in exchange of $[Na^+]_i$ [56].

Fig. 4A shows the beat-to-beat change in $[Ca^{2+}]_m$ during field stimulation. Both diastolic and systolic level was frequency-dependent in a reversible manner. Similar to cytosolic Ca^{2+} , there were an immediate rise and a delayed rise in $[Ca^{2+}]_m$ by increasing the rate of stimulation. Switching the rate to the control frequency (0.2 Hz) slowly restored the $[Ca^{2+}]_m$ to its control level. The model was tested whether it could reproduce the frequency-dependence of $[Ca^{2+}]_m$ obtained from experiment (Fig. 4B). The diastolic and systolic level of $[Ca^{2+}]_m$ during 0.2 Hz stimulation were about 6.6 nM and 30 nM, respectively. Switching the stimulation rate from 0.2 Hz to 1 Hz increased both diastolic and systolic level of $[Ca^{2+}]_m$ (9.1 nM and 54.4 nM). The biphasic time course (immediate and delayed) was also evident.

The effect of ruthenium red on $[Ca^{2+}]_m$ and $[Ca^{2+}]_i$

In order to exclude the possibility that the Rhod-2 intensity represents the cytosolic Ca^{2+} rather than mitochondrial Ca^{2+} , a specific blocker of mitochondrial Ca^{2+} uniporter (ruthenium red) was applied to the myocyte during 0.2 Hz stimulation. Since ruthenium red exerts its inhibitory effect after it crosses the plasma membrane, cells were preincubated with it for 5 min before recording.

As shown in Fig. 5A, continuous perfusion of ruthenium red (10 μM) gradually reduced both diastolic and systolic level of Rhod-2 intensity. The difference between the two levels was also inhibited as high as 60% during 5 min recording. Ruthenium red, however, failed to reduce the

Fig. 4. Stimulus frequency-dependent change in mitochondrial Ca^{2+} transients by experiment (A) and model simulation (B). (A) Myocytes were field stimulated and the rate was switched between 0.2 Hz and test frequency (1 Hz, 2 Hz, and 3 Hz). Rhod-2 intensity was recorded to monitor change in mitochondrial Ca^{2+} transients. Output intensity was normalized to diastolic level at control in order to get a pseudo ratio unit (arbitrary unit). Expanded trace of normalized Rhod-2 intensity during 2 Hz stimulation was presented on the right panel. The downward arrow indicates the point where the expanded trace was extracted. (B) Model-generated mitochondrial Ca^{2+} transients. Expanded trace during 2 Hz stimulation was also shown on the right panel.

Fig. 5. The effect of ruthenium red ($10 \mu\text{M}$) on mitochondrial and cytosolic Ca^{2+} transients. Stimulation rate is 0.2 Hz. (A) Mitochondrial Ca^{2+} transients during perfusion of ruthenium red. Cells were preincubated with ruthenium red for 5 min before recording. (B) Cytosolic Ca^{2+} transients during perfusion of ruthenium red.

Fura-2 fluorescence ratio during 5 min of recording implying that it does not affect Ca^{2+} movement across the plasma membrane (Fig. 5B). If the Rhod-2 intensity represents the cytosolic Ca^{2+} , Rhod-2 intensity and Fura-2 fluorescence

should be affected in the same manner by ruthenium red. The selective inhibition of Rhod-2 intensity over Fura-2 fluorescence ratio by ruthenium red indicates the Rhod-2 intensity represents the mitochondrial Ca^{2+} rather than cyto-

Fig. 6. The effect of Ca^{2+} uniporter removal on mitochondrial and cytosolic Ca^{2+} transients in model simulation. Stimulation rate is 0.2 Hz. Mitochondrial Ca^{2+} transients with (A) or without (C) Ca^{2+} uniporter activity. Cytosolic Ca^{2+} transients with (B) or without (D) Ca^{2+} uniporter activity.

Fig. 7. The effect of mitochondrial protonophore on cytosolic Ca^{2+} transients. Stimulation rate is 0.2 Hz. 1 μM FCCP eventually abolished Ca^{2+} transients. Cells became round-up after 5 min perfusion of FCCP. Arrow (A) indicates the point where the expanded trace before FCCP was extracted while arrow (B) indicates the point where expanded trace after FCCP was extracted.

solic Ca^{2+} .

Although the block of Ca^{2+} uniporter did not significantly affect the cytosolic Ca^{2+} transients in experiment, there is still a possibility that removal of Ca^{2+} uniporter activity can affect the cytosolic Ca^{2+} transients. If the Ca^{2+} influx into mitochondria across the mitochondrial inner membrane is blocked, it will probably affect the Ca^{2+} balance in cytosolic space as well. We examined the possibility uti-

lizing the model simulation as shown in Fig. 6.

The removal of Ca^{2+} uniporter activity increased the amplitude of cytosolic Ca^{2+} transients as little as 0.5%. This small degree of change may be practically undetectable in the experiment considering that the signal to noise ratio in the experiment is no more than 10 in amplitude. Slow time course of inhibition by ruthenium red makes it further difficult to see the change clearly in a limited duration of

Fig. 8. Simulation of the effects of mitochondrial protonophore on cytosolic Ca^{2+} transients and ATP content. Stimulation rate is 0.2 Hz. 10^6 -fold increase in H^+ permeability through inner mitochondrial membrane was tested on the model simulation. (A) Change in cytosolic Ca^{2+} transients during stimulation. (B) Change in cytosolic ATP content during stimulation.

recording.

The effect of mitochondrial protonophore on $[\text{Ca}^{2+}]_i$ and energetics

Rat ventricular myocytes exposed to metabolic blockers show decline in twitch amplitude and eventually develops a 'rigor' contracture which is mediated by depletion of ATP content [28]. Mitochondrial protonophores can act as a metabolic blocker because the increase in H^+ permeability across the inner mitochondrial membrane by protonophores can disrupt mitochondrial membrane potential which is important in maintaining ATP synthesis [57]. It is then supposed that the protonophores can affect SR Ca^{2+} content by reducing the activity of ATP-driven SR Ca^{2+} pump. The depletion of ATP content can also affect the Na^+ balance by limiting the activity of Na^+/K^+ pump. Resultant increase in $[\text{Na}^+]_i$ can shift the balance of $\text{Na}^+/\text{Ca}^{2+}$ exchanger toward its reverse mode, which can increase the diastolic level of $[\text{Ca}^{2+}]_i$.

Fig. 7 demonstrates the effect of FCCP, a protonophore, on the cytosolic Ca^{2+} transients. FCCP gradually abolished the Ca^{2+} transients and induced an irreversible rise of diastolic Ca^{2+} . We examined whether the model simulation is able to reproduce those effects of FCCP by applying 10^6 -fold increase in the H^+ permeability on inner mitochondrial membrane. As shown in Fig. 8A, the model simulation was able to reproduce the similar response as those of experiment. The model simulation also shows the predicted accompanying change in ATP content by 10^6 -fold increase in the H^+ permeability (Fig. 8B). It can be explained that the protonophore disrupts the SR Ca^{2+} dynamics by re-

ducing ATP content.

DISCUSSION

Present study aimed to develop an integrated model of rat ventricular myocytes which could reproduce electrical excitability, intracellular Ca^{2+} -dynamics, muscle contraction, ATP production and consumption, and mitochondrial ion movement. The model was able to reproduce the stimulus frequency-dependent change in cytosolic and mitochondrial Ca^{2+} transients. It was also able to reproduce the effects of metabolic inhibition on Ca^{2+} transients and contractility. Mitochondrial ion movement and its effect on cytosolic ion concentration were also addressed. Since this model can simulate pH_i and pH_m and its interaction with other components, the ischaemia-related cellular events are expected to be elucidated by utilizing it.

Stimulation frequency and cytosolic Ca^{2+} transients

Stimulation frequency has been known to alter the force of contraction in cardiac muscle [47], by which the heart could meet the demand of work required. The force-frequency relationship could be manifested as either positive or negative depending on the species and experimental circumstances. In our experiment, the force-frequency relationship was positive in accordance with results from Frampton et al [50]. However, there are several studies that present the negative force-frequency relationship especially in rat ventricular myocytes [47,49,58]. Frampton et al [50] proposed that the negative force-frequency relationship is the result of Ca^{2+} loading caused by damage during preparation of tissue or isolated myocytes. This idea is based on the results by Capogrossi et al [48], where cells with Ca^{2+} overload had a negative force-frequency relationship.

Our study suggests that the density of I_{CaL} determines the force-frequency relationship (Fig. 3). The balance between transsarcolemmal Ca^{2+} influx through L-type Ca^{2+} channel and efflux through Ca^{2+} removing mechanism during AP seems to determine whether a myocyte shows the positive or negative force-frequency relationship. If the net Ca^{2+} movement is influx during AP, increasing stimulation rate probably accumulates diastolic Ca^{2+} , whereby increases Ca^{2+} uptake and release from SR. Increasing the rate of stimulation, however, reduces the time period for restitution of SR opposing the effects of Ca^{2+} accumulation during AP. Role of SR in force-frequency relationship has been investigated by using ryanodine which inhibits restitution of SR [49,59]. The net result of raising stimulation rate will be dependent on the net amount of Ca^{2+} influx during AP. If the net Ca^{2+} movement is efflux or very small influx during AP, increasing stimulation rate is supposed to further reduce the diastolic and systolic Ca^{2+} because opposing effect to SR Ca^{2+} release by reduced restitution of SR prevails over the net influx of Ca^{2+} during AP. Most important component determining the net Ca^{2+} influx during AP should be L-type Ca^{2+} channel since it predominantly opens during AP rather than resting period. Since the change in Ca^{2+} removing mechanism equally affects the Ca^{2+} movement during AP and resting period, it may not affect force-frequency relationship. Our simulation results confirmed this idea (data not shown). Change in background Ca^{2+} leak current (I_{Cab}) is also supposed to affect

the AP and resting period in a similar way as change in Ca^{2+} removing mechanism. Supporting this idea, it did not affect the positive force-frequency relationship in the simulation (data not shown). The contribution of L-type Ca^{2+} channel to the positive force-frequency relationship has been suggested because the use of verapamil, a specific blocker of L-type Ca^{2+} channel, converted the positive force-frequency relationship into the negative one in rat ventricular myocytes [49]. Although reproducing the same relation with Ca^{2+} transients rather than contractile force is almost impossible to get because of extremely small signal after application of blocker, simulation results could be an alternative to explain the role of L-type Ca^{2+} channel in positive staircase (Fig. 3). Role of repolarizing K^+ channel (I_{to}) in positive staircase was also addressed by model simulation (Fig. 3C). Increasing the K^+ channel conductance is supposed to shift the balance between transsarcolemmal Ca^{2+} influx and SR restitution towards the latter because Ca^{2+} influx would be terminated earlier with reduced APD. Negative staircase, however, was revealed under nonphysiological level of APD (<10 ms) implying that variation in I_{to} among species cannot explain the conflicting force-frequency relationship. Contradictory results among experiments in rat ventricular myocytes may arise from different level of I_{CaL} depending on the experimental circumstances or individual variation among animals or myocytes. The relation between stimulation frequency and force or $[\text{Ca}^{2+}]_{\text{i}}$ was also investigated by Shim et al [60] utilizing a new multi-scale simulation model. They proposed that $[\text{Ca}^{2+}]_{\text{i}}$ has a positive relation with pacing frequency whereas left ventricular (LV) pressure has a negative one. They explained that the negative relation in LV pressure arises from the decreased LV volume due to diminished filling time with higher pacing frequency. In case of force, their model showed a positive relation up to a frequency of 2.5 Hz and then it was abruptly reversed. Their results are basically similar to ours, however, the increase in $[\text{Ca}^{2+}]_{\text{i}}$ looks smaller with higher pacing frequency (Fig. 9 in Shim et al [60]).

Beat-to-beat change in $[\text{Ca}^{2+}]_{\text{m}}$

There has been a controversial issue whether the $[\text{Ca}^{2+}]_{\text{m}}$ changes in a beat-to-beat manner following cytosolic Ca^{2+} transients. In our study, $[\text{Ca}^{2+}]_{\text{m}}$ changed in a beat-to-beat manner with very similar time course to $[\text{Ca}^{2+}]_{\text{i}}$. Furthermore, $[\text{Ca}^{2+}]_{\text{m}}$ also showed the same positive staircase as $[\text{Ca}^{2+}]_{\text{i}}$. One may argue that Rhod-2 actually reflects $[\text{Ca}^{2+}]_{\text{i}}$ as well as $[\text{Ca}^{2+}]_{\text{m}}$. However, the response to inhibitor of Ca^{2+} uniporter clearly indicates that Rhod-2 intensity indeed arose from the mitochondria (Fig. 5). Similar results have been reported in rabbit cardiac myocytes [8], rat heart trabeculae [7], and guinea pig cardiac myocytes [61]. In order to resolve technical limitations related with selective localization of dyes into mitochondria, Robert et al [62] used targeted aequorins and ratiometric-pericams which are genetically encoded Ca^{2+} probes. Those probes are designed to selectively localize to the mitochondrial matrix and they found that $[\text{Ca}^{2+}]_{\text{m}}$ indeed oscillates rapidly and in synchrony with $[\text{Ca}^{2+}]_{\text{i}}$. In the study of Griffiths et al [63], $[\text{Ca}^{2+}]_{\text{m}}$ in rat ventricular myocytes were 59 nM at 0.25 Hz stimulation and 517 nM at 3 Hz stimulation, respectively when the cells are exposed to 50 nM norepinephrine. In rat heart trabeculae [7], diastolic $[\text{Ca}^{2+}]_{\text{m}}$ was estimated to be 440 nM at 0.25 Hz stimulation and

582 or 676 nM at 2 Hz stimulation, respectively. Single SR Ca^{2+} release was quantitatively measured by Andrienko et al [9] with typical values of 2–4 nM when diastolic $[\text{Ca}^{2+}]_{\text{m}}$ is around 30 nM. In our simulation, diastolic $[\text{Ca}^{2+}]_{\text{m}}$ was around 6.6 nM at 0.2 Hz stimulation and 26.3 nM at 3 Hz stimulation. Single SR Ca^{2+} release at 2 Hz stimulation was around 55 nM when diastolic $[\text{Ca}^{2+}]_{\text{m}}$ is around 15 nM. Considerable variations in results among groups may arise from the different technique in measurement and calibration of dye signal and species specific nature. There is also a possibility that the diastolic $[\text{Ca}^{2+}]_{\text{m}}$ might have been overestimated because of lysosomal/endosomal accumulation of ester-loaded fluorophores [8]. It could even obscure the beat-to-beat change in $[\text{Ca}^{2+}]_{\text{m}}$, which might be a major reason that some groups failed to get the beat-to-beat mitochondrial Ca^{2+} transients. Measurement by three-dimensionally resolved confocal microscopy has clearly advantage over microfluorometry in that respect [8]. There remains a question why cardiac myocytes need the beat-to-beat change in $[\text{Ca}^{2+}]_{\text{m}}$ in synchrony with $[\text{Ca}^{2+}]_{\text{i}}$. One of possible explanation is that energy demand of heart should decode amplitude and frequency of Ca^{2+} transients as well as diastolic $[\text{Ca}^{2+}]_{\text{i}}$. $[\text{Ca}^{2+}]_{\text{m}}$ is well known to regulate the activity of dehydrogenases in TCA cycle to cope with the energy demand. If $[\text{Ca}^{2+}]_{\text{m}}$ is tonic, mitochondria could help but match the energy demand for the averaged value of $[\text{Ca}^{2+}]_{\text{i}}$ rather than the amplitude and/or frequency of Ca^{2+} transients. It cannot cope with every circumstance related with cardiac work load. For example, if the diastolic level of $[\text{Ca}^{2+}]_{\text{i}}$ is elevated but the Ca^{2+} transients disappear, mitochondria with tonic change in $[\text{Ca}^{2+}]_{\text{m}}$ would simply increase the energy production, which is not plausible considering the reduced work demand of heart. In that respect, $[\text{Ca}^{2+}]_{\text{m}}$ needs to be in synchrony with $[\text{Ca}^{2+}]_{\text{i}}$ enabling beat-to-beat match of energy demand.

Contribution of mitochondria to the $[\text{Ca}^{2+}]_{\text{i}}$

The present study suggests that mitochondria have little contribution to the $[\text{Ca}^{2+}]_{\text{i}}$ under normal cyclic contraction based on the finding that the blocker of Ca^{2+} uniporter on the inner membrane of mitochondria does not significantly affect the $[\text{Ca}^{2+}]_{\text{i}}$ (Fig. 5). Simulation study also supports the experimental finding since removal of Ca^{2+} uniporter activity increased the amplitude of Ca^{2+} transients as little as 0.5% (Fig. 6). There have been studies which tried to calculate the contribution of mitochondria and SR Ca^{2+} pump to Ca^{2+} cycling, where it was estimated to be only 1–2% in rabbit, rat, and ferret ventricular myocytes [64–67]. The contribution of mitochondria alone to Ca^{2+} cycling should be even lower. These findings still cannot exclude the possibility that Ca^{2+} overload in cytosol is buffered by mitochondria. Contribution of mitochondria to Ca^{2+} cycling under saturating caffeine was estimated to be as high as 26% [68]. A possible explanation for these findings and idea is that the amount of Ca^{2+} influx through Ca^{2+} uniporter is very small in ordinary condition but Ca^{2+} uniporter could simply increase the amount of flux enough to buffer an excessive or pathologic increase in $[\text{Ca}^{2+}]_{\text{i}}$ in response to change in electrochemical gradient of Ca^{2+} . At present, we have no evidence that the mitochondria could act as a Ca^{2+} buffer in ordinary circumstances. As for the reason why removal of Ca^{2+} uniporter does not affect the amplitude of Ca^{2+} transients in our simulation study, it could be speculated that the flux is not so fast and large

enough to significantly attenuate the much faster and bigger Ca^{2+} -induced Ca^{2+} release. In our simulation, change in $[\text{Ca}^{2+}]_m$ during single beat is about 1/20 of that in $[\text{Ca}^{2+}]_i$. There is also a significant delay between the peaks of Ca^{2+} -induced Ca^{2+} release (6 ms after stimulation) and of mitochondrial Ca^{2+} transients (75 ms after stimulation). In fact, as we set the control permeability of Ca^{2+} uniporter more than 10-times bigger, removal of the transporter revealed significant increase (around 5%) in the amplitude of cytosolic Ca^{2+} transients. It is supposed that the flux through Ca^{2+} uniporter is just large and fast enough to decode beat-to-beat $[\text{Ca}^{2+}]_i$ change. Our experimental results (Fig. 5) are in accordance with the simulation results under the control permeability of Ca^{2+} uniporter (Fig. 6).

Disruption of mitochondrial function and $[\text{Ca}^{2+}]_i$

The FCCP is well known to uncouple the oxidative phosphorylation by transporting H^+ through the inner membrane of mitochondria [69]. It also depolarizes $\Delta\Psi_m$ by dissipating H^+ gradient across the inner membrane of mitochondria, which could affect ionic homeostasis in mitochondria [70]. Major consequences of uncoupling oxidative phosphorylation result from block or inhibition of ATP synthesis [71-73]. Our study demonstrates that disruption of mitochondrial function using FCCP elevates diastolic $[\text{Ca}^{2+}]_i$ and abolishes Ca^{2+} transients. The depletion of ATP by FCCP is considered to affect all the energy-dependent intracellular processes and carrier proteins such as plasmalemmal Ca^{2+} pump, SR Ca^{2+} -ATPase (SR Ca^{2+} pump), ATP-sensitive potassium channel, phospholamban, and ryanodine receptors [74]. Inhibition of Ca^{2+} pump on the plasmalemma and SR is supposed to increase diastolic $[\text{Ca}^{2+}]_i$ and decrease SR content and subsequent SR Ca^{2+} release, which is in agreement with our results. There is evidence that energy from glycolysis is preferentially utilized by membrane-bound transporters and ion channels, whereas energy from oxidative phosphorylation is preferentially utilized by contractile proteins [75-78]. The energy from oxidative phosphorylation, however, accounts for more than 95% of total ATP synthesis in non-ischaemic mammalian heart [79]. Approximately 65% of ATP hydrolysis is utilized by contractile proteins, and the remaining 35% is used to operate ion pumps including SR Ca^{2+} pump [80,81]. Therefore, inhibition of oxidative phosphorylation alone may be enough to inhibit all the compartmental energy utilization. Indeed, incubation of cells with $1\ \mu\text{M}$ FCCP for 20 min reduced the $[\text{ATP}]_i$ to submillimolar level (unpublished results from our laboratory). Depolarization of $\Delta\Psi_m$ itself can affect the ion movement across the inner membrane of mitochondria. Saotome et al [70] found that FCCP decreased $[\text{Ca}^{2+}]_m$ in a dose-dependent manner. They also suggested that dissipation of $\Delta\Psi_m$ opens the mitochondrial permeability transition pore (mPTP). In our simulation study, the $[\text{Ca}^{2+}]_m$ showed a transient increase after FCCP, but eventually decreased below its control level in agreement with experimental findings. Although time course of $[\text{Ca}^{2+}]_i$ with FCCP in simulation (Fig. 8A) is in similar scale with that in animal experiment (Fig. 7), depletion of $[\text{ATP}]_i$ (Fig. 8B) was too rapid compared with that obtained from rat heart using ^{31}P NMR technique where hearts were perfused through Langendorff apparatus [82]. Perfusion of whole heart with drugs might take much longer to take effect compared with perfusion of isolated myocytes in a small chamber. The concentration of FCCP (100 nM) was

also lower than that we used in animal experiment ($1\ \mu\text{M}$). A possible source of error from model simulation is the inappropriate modeling of ANT. As we set the maximal rate of ANT to lower value in simulation, the depletion rate of $[\text{ATP}]_i$ was significantly attenuated. Measurement of $[\text{ATP}]_i$ depletion by FCCP using isolated myocytes seems to resolve this issue.

Concluding remarks

We developed an integrated model of rat ventricular myocytes which could reproduce positive or negative staircase phenomenon, beat-to-beat change in $[\text{Ca}^{2+}]_m$, and effects of metabolic inhibition on Ca^{2+} -dynamics. Previously, there were two important rat myocyte models. One is the rat myocyte models developed by Pandit et al [14] and the other is one developed by Crampin et al [83]. The model by Pandit et al integrated ion channels and Ca^{2+} dynamics but lacks mitochondrial energy metabolism. The model of Crampin et al added cytoplasmic pH regulation to the model of Pandit et al. The rat myocyte model which integrated mitochondrial energy metabolism and ion transport across mitochondrial inner membrane into the preexisting rat myocyte model has not been tried in our scope of research. Our model has also advantages over previous cardiac models in that it incorporates complete ion cycling including Na^+ , K^+ , Ca^{2+} , and H^+ in mitochondria and cytosol. Complete modeling of ions, especially pH, is a key feature to reproduce ischaemia-related change in cardiac function. Modeling of ischaemia-reperfusion injury and ischaemic preconditioning is the future directive of our study.

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APPENDIX

Standard units (unless otherwise noted) are pA for currents, mM for ion concentration and Michaelis constant, mM · ms⁻¹ for ionic flux, mV for membrane potential, ms for time constant, and ms⁻¹ for rate constants. Initial state variables and ion concentrations in Tables 3~5 were obtained after running simulations at a stimulus frequency of 0.2 Hz for 300 sec.

Ca²⁺-independent transient outward K⁺ current (*I*_{to})

$$I_{to} = \bar{G}_{to} \cdot y_1 \cdot (a \cdot y_2 + b \cdot y_3) \cdot (V - E_K) \quad (1)$$

$$\bar{G}_{to} = 17.08 \text{ nS; maximal conductance of } I_{to} \quad (2)$$

$$a = 0.886; \text{ fraction of fast inactivation} \quad (3)$$

$$b = 0.114; \text{ fraction of slow inactivation} \quad (4)$$

$$\bar{y}_1 = \frac{1}{1 + e^{-\frac{V+1.5}{25.8}}}; \text{ voltage-dependence of steady-state activation} \quad (5)$$

$$\tau_{y_1} = 12.3 \cdot e^{-\left(\frac{V+70}{53.4}\right)^2} + 1.15; \text{ time constant of activation (ms)} \quad (6)$$

$$\bar{y}_2 = \bar{y}_3 = \frac{1}{1 + e^{-\frac{V+59.4}{11.9}}}; \text{ voltage-dependence of steady-state inactivation} \quad (7)$$

$$\tau_{y_2} = 195.8 \cdot e^{-\left(\frac{V+36}{18.4}\right)} + 8.7 \cdot e^{-\left(\frac{V-57.3}{23}\right)^2} + 26.7; \text{ time constant of fast inactivation (ms)} \quad (8)$$

$$\tau_{y_3} = 2380 \cdot e^{-\left(\frac{V+70}{27.7}\right)^2} + 36.3; \text{ time constant of slow inactivation (ms)} \quad (9)$$

$$E_K = \frac{R \cdot T}{F} \cdot \ln \left(\frac{[K^+]_e}{[K^+]_i} \right) \quad (10)$$

$$\frac{dy_1}{dt} = \frac{\bar{y}_1 - y_1}{\tau_{y_1}} \quad (11)$$

$$\frac{dy_2}{dt} = \frac{\bar{y}_2 - y_2}{\tau_{y_2}} \quad (12)$$

$$\frac{dy_3}{dt} = \frac{\bar{y}_3 - y_3}{\tau_{y_3}} \quad (13)$$

Steady-state outward K⁺ current (*I*_{ss})

$$I_{ss} = \bar{G}_{Ks} \cdot y_1 \cdot y_2 \cdot (V - E_K) \quad (14)$$

$$\bar{G}_{Ks} = 9.31 \text{ nS; maximal conductance of } I_{Ks} \quad (15)$$

$$\bar{y}_1 = \frac{1}{1 + e^{-\frac{V+11.5}{11.82}}}; \text{ voltage-dependence of steady-state activation} \quad (16)$$

$$\tau_{y_1} = \frac{10000}{45.16 \cdot e^{0.03577 \cdot (V+50)} + 98.9 \cdot e^{-0.1 \cdot (V+38)}}; \text{ time constant of activation} \quad (17)$$

$$\bar{y}_2 = \frac{1}{1 + e^{-\frac{V+87.5}{10.3}}}; \text{ voltage-dependence of steady-state inactivation} \quad (18)$$

$$\tau_{y_2} = 2100; \text{ time constant of inactivation} \quad (19)$$

$$\frac{dy_1}{dt} = \frac{\bar{y}_1 - y_1}{\tau_{y_1}} \quad (20)$$

$$\frac{dy_2}{dt} = \frac{\bar{y}_2 - y_2}{\tau_{y_2}} \quad (21)$$

Delayed rectifier K⁺ current, rapid component (*I*_{Kr})

$$I_{Kr} = G_{Kr} \cdot C_m \cdot (V - E_K) \cdot (0.6 \cdot y_1 + 0.4 \cdot y_2) \cdot y_3 \quad (22)$$

$$G_{Kr} = 0.0127 \cdot \left(\frac{[K^+]_e}{5.4} \right)^{0.2} \text{ nS; maximal conductance of } I_{Kr} \quad (23)$$

$$\alpha_{y_1} = \frac{1}{20 \cdot e^{-11.5} + 5.0 \cdot e^{-300}} \quad (24)$$

$$\beta_{y_1} = \frac{1}{160 \cdot e^{28} + 200 \cdot e^{1000}} + \frac{1}{2500 \cdot e^{20}} \quad (25)$$

$$\alpha_{y_2} = \frac{1}{200 \cdot e^{-13} + 20 \cdot e^{-300}} \quad (26)$$

$$\beta_{y_2} = \frac{1}{1600 \cdot e^{28} + 2000 \cdot e^{1000}} + \frac{1}{10000 \cdot e^{20}} \quad (27)$$

$$\alpha_{y_3} = \frac{1}{10 \cdot e^{17} + 2.5 \cdot e^{300}} \quad (28)$$

$$\beta_{y_3} = \frac{1}{0.35 \cdot e^{-17} + 2 \cdot e^{-150}} \quad (29)$$

$$\frac{dy_1}{dt} = \alpha_{y_1} \cdot (1 - y_1) - \beta_{y_1} \cdot y_1 \quad (30)$$

$$\frac{dy_2}{dt} = \alpha_{y_2} \cdot (1 - y_2) - \beta_{y_2} \cdot y_2 \quad (31)$$

$$\frac{dy_3}{dt} = \alpha_{y_3} \cdot (1 - y_3) - \beta_{y_3} \cdot y_3 \quad (32)$$

Inward rectifier K⁺ current (*I*_{K1})

$$I_{K1} = 60 \cdot \left(\frac{[K^+]_e}{5.4} \right)^{0.4} \cdot \frac{V - E_K - 1.73}{1 + e^{-\frac{1.613 \cdot F}{R \cdot T} \cdot (V - E_K - 1.73)}} \cdot \left(10 + \frac{48}{e^{\frac{V+37}{25}} + e^{-\frac{V+37}{25}}} \right) \cdot \frac{0.0001}{1 + e^{-\frac{V - E_K - 76.77}{-17}}} \quad (33)$$

ATP-sensitive K⁺ current (*I*_{KATP})

$$I_{KATP} = N \cdot \gamma \cdot p(\text{open}) \cdot (V - E_K) \quad (34)$$

$$N = 50000; \text{ total number of channels} \quad (35)$$

$$\gamma = 0.0165 \cdot [K^+]_e^{0.24}; \text{ single channel conductance (nS)} \quad (36)$$

$$p(\text{open}) = \frac{1}{1 + \left(\frac{[ATP]_i}{0.1} \right)^2}; \text{ probability of open} \quad (37)$$

Na⁺ current (*I*_{Na})

$$I_{Na} = I_{Na(Na)} + I_{Na(K)} \quad (38)$$

$$I_{Na(Na)} = 1290 \cdot CF_{Na} \cdot m^3 \cdot h \cdot j; \text{ Na}^+ \text{ component of } I_{Na} \quad (39)$$

$$I_{Na(K)} = 129 \cdot CF_K \cdot m^3 \cdot h \cdot j; \text{ K}^+ \text{ component of } I_{Na} \quad (40)$$

$$\alpha_m = \frac{0.32 \cdot (V+47.13)}{1 - e^{-0.1 \cdot (V+47.13)}}; \text{ forward rate constant of m-gate} \quad (41)$$

$$\beta_m = 0.08 \cdot e^{-\frac{11}{V}}; \text{ backward rate constant of m-gate} \quad (42)$$

For $V \geq -40$,

$$\alpha_h = 0; \text{ forward rate constant of h-gate} \quad (43)$$

$$\beta_h = \frac{7.692}{1 + e^{-11.1}}; \text{ backward rate constant of h-gate} \quad (44)$$

$$\alpha_j = 0; \text{ forward rate constant of j-gate} \quad (45)$$

$$\beta_j = \frac{0.3 \cdot e^{(-2.535 \cdot 10^{-7} \cdot V)}}{1 + e^{-0.1 \cdot (V+32)}}; \text{ backward rate constant of j-gate} \quad (46)$$

For $V \leq -40$,

$$\alpha_h = 0.135 \cdot e^{\frac{V+80}{-6.8}}; \text{ forward rate constant of h-gate} \quad (47)$$

$$\beta_h = 3.56 \cdot e^{0.079 \cdot V} + 3.1 \cdot 10^5 \cdot e^{0.35 \cdot V}; \quad (48)$$

backward rate constant of h-gate

$$\alpha_j = \frac{-1.2714 \cdot 10^5 \cdot e^{0.2444 \cdot V} - 3.474 \cdot 10^{-5} \cdot e^{-0.044 \cdot V}}{1 + e^{0.311 \cdot (V+79.23)}} \cdot (V + 37.78); \quad (49)$$

forward rate constant of j-gate

$$\beta_j = \frac{0.1212 \cdot e^{-0.01052 \cdot V}}{1 + e^{-0.1378 \cdot (V+40.14)}}; \text{ backward rate constant of j-gate} \quad (50)$$

$$\frac{dm}{dt} = \alpha_m \cdot (1 - m) - \beta_m \cdot m \quad (51)$$

$$\frac{dh}{dt} = \alpha_h \cdot (1 - h) - \beta_h \cdot h \quad (52)$$

$$\frac{dj}{dt} = \alpha_j \cdot (1 - j) - \beta_j \cdot j \quad (53)$$

L-type Ca^{2+} current (I_{CaL})

$$I_{\text{CaL}} = \bar{G}_{\text{CaL}} \cdot p(\text{open}_{\text{CaL}}) \cdot (V - E_{\text{Ca}}) \quad (54)$$

$$E_{\text{Ca}} = \frac{R \cdot T}{z_{\text{Ca}} \cdot F} \cdot \ln \left(\frac{[\text{Ca}^{2+}]_e}{[\text{Ca}^{2+}]_i} \right) \quad (55)$$

$$z_{\text{Ca}} = 2; \text{ charge of } \text{Ca}^{2+} \quad (56)$$

$$p(\text{open}_{\text{CaL}}) = d \cdot \left(\left(0.9 + \frac{\text{Ca}_{\text{inact}}}{10} \right) \cdot f_{11} + \left(0.1 - \frac{\text{Ca}_{\text{inact}}}{10} \right) \cdot f_{12} \right); \quad (57)$$

open probability

$$\bar{G}_{\text{CaL}} = 14.32 \text{ nS}; \text{ maximal conductance of } I_{\text{CaL}} \quad (58)$$

$$\bar{d} = \frac{1}{\frac{V+7.9}{1+e^{-7.37}}}; \text{ voltage-dependence of steady-state activation} \quad (59)$$

$$\tau_d = 1.068 \cdot e^{(-0.0045 \cdot (V+7)^2)} + 0.368 \cdot e^{(-0.002 \cdot (V-18)^2)} + 0.088; \text{ time constant of activation in d-gate} \quad (60)$$

$$\bar{f}_{11} = \bar{f}_{12} = \frac{1}{\frac{V+19.24}{1+e^{-7.13}}}; \text{ voltage-dependence of steady-state inactivation in } f_{11}\text{-gate or } f_{12}\text{-gate} \quad (61)$$

$$\tau_{f_{11}} = 36.8 \cdot e^{\left(\frac{V+45}{-12} \right)^2} + \frac{12}{1+e^{-\frac{V+25}{25}}} + \frac{4.5}{1+e^{-\frac{V+75}{25}}} + 6.0; \quad (62)$$

time constant of inactivation in f_{11} -gate

$$\tau_{f_{12}} = 41 \cdot e^{\left(\frac{V+47}{-12} \right)^2} + \frac{80}{1+e^{-\frac{V+55}{-5}}} + \frac{15}{1+e^{-\frac{V+75}{25}}} + 17; \quad (63)$$

time constant of inactivation in f_{12} -gate

$$\bar{\text{Ca}}_{\text{inact}} = \frac{1}{1 + \frac{[\text{Ca} \cdot \text{CMDN}]_i}{0.01}}; \text{ Ca} \cdot \text{CMDN-dependent inactivation}^* \quad (64)$$

$$[\text{Ca} \cdot \text{CMDN}]_i = [\text{Ca}]_i - [\text{Ca}^{2+}]_i; \quad (65)$$

concentration of Ca-calmodulin complex

$$\tau_{\text{Ca}_{\text{inact}}} = 9; \quad (66)$$

time constant of Ca-CMDN-dependent inactivation

$$\frac{dd}{dt} = \frac{\bar{d} - d}{\tau_d} \quad (67)$$

$$\frac{df_{11}}{dt} = \frac{\bar{f}_{11} - f_{11}}{\tau_{f_{11}}} \quad (68)$$

$$\frac{df_{12}}{dt} = \frac{\bar{f}_{12} - f_{12}}{\tau_{f_{12}}} \quad (69)$$

$$\frac{d\text{Ca}_{\text{inact}}}{dt} = \frac{\bar{\text{Ca}}_{\text{inact}} - \text{Ca}_{\text{inact}}}{\tau_{\text{Ca}_{\text{inact}}}} \quad (70)$$

* see ion concentration (Eqs. 159 ~ 172)

Non-selective cation channel (I_{bNSC})

$$I_{\text{bNSC}} = I_{\text{bNSC}(\text{Na})} + I_{\text{bNSC}(\text{K})} \quad (71)$$

$$I_{\text{bNSC}(\text{Na})} = 0.099 \cdot \text{CF}_{\text{Na}}; \text{ Na}^+ \text{ component of } I_{\text{bNSC}} \quad (72)$$

$$I_{\text{bNSC}(\text{K})} = 0.0396 \cdot \text{CF}_{\text{K}}; \text{ K}^+ \text{ component of } I_{\text{bNSC}} \quad (73)$$

Background Ca^{2+} leak current (I_{Cab})

$$I_{\text{Cab}} = P_{\text{Cab}} \cdot \text{CF}_{\text{Ca}} \quad (74)$$

$$P_{\text{Cab}} = 0.6 \text{ pA} \cdot \text{mM}^{-1}; \text{ conversion factor} \quad (75)$$

$\text{Na}^+/\text{Ca}^{2+}$ exchanger

$$I_{\text{NaCa}} = 1.81 \cdot C_m \cdot (k_1 \cdot p(E_1\text{Na}) \cdot y - k_2 \cdot p(E_2\text{Na}) \cdot (1 - y)) \quad (80)$$

$$k_1 = e^{\frac{0.32 \cdot F \cdot V}{R \cdot T}} \quad (81)$$

$$k_2 = e^{\frac{(0.32-1) \cdot F \cdot V}{R \cdot T}} \quad (82)$$

$$k_3 = 1 \quad (83)$$

$$k_4 = 1 \quad (84)$$

$$p(E_1\text{Na}) = \frac{1}{1 + \left(\frac{K_{\text{mNaI}}}{[\text{Na}^+]_i} \right)^3 \cdot \left(1 + \frac{[\text{Ca}^{2+}]_i}{K_{\text{mCaI}}} \right)} \quad (85)$$

$$p(E_2\text{Na}) = \frac{1}{1 + \left(\frac{K_{\text{mNaO}}}{[\text{Na}^+]_e} \right)^3 \cdot \left(1 + \frac{[\text{Ca}^{2+}]_e}{K_{\text{mCaO}}} \right)} \quad (86)$$

$$p(E_1\text{Ca}) = \frac{1}{1 + \left(\frac{K_{\text{mCaI}}}{[\text{Ca}^{2+}]_i} \right) \cdot \left(1 + \frac{[\text{Na}^+]_i}{K_{\text{mNaI}}} \right)^3} \quad (87)$$

$$p(E_2\text{Ca}) = \frac{1}{1 + \left(\frac{K_{\text{mCaO}}}{[\text{Ca}^{2+}]_e} \right) \cdot \left(1 + \frac{[\text{Na}^+]_e}{K_{\text{mNaO}}} \right)^3} \quad (88)$$

$$K_{\text{mNaI}} = 13.125 \text{ mM} \quad (89)$$

$$K_{\text{mCaI}} = 0.000552 \text{ mM} \quad (90)$$

$$K_{\text{mNaO}} = 87.5 \text{ mM} \quad (91)$$

$$K_{mCa_e} = 1.38 \text{ mM} \quad (92)$$

$$\alpha_y = k_2 \cdot p(E_2Na) + k_4 \cdot p(E_2Ca); \quad (93)$$

forward rate constant of y-gate

$$\beta_y = k_1 \cdot p(E_1Na) + k_3 \cdot p(E_1Ca); \quad (94)$$

backward rate constant of y-gate

Na⁺/K⁺ pump current

$$I_{NaK} = 21 \cdot C_m \cdot (k_1 \cdot p(E_1Na) \cdot y - k_2 \cdot p(E_2Na) \cdot (1 - y)) \quad (99)$$

$$k_1 = 0.37 \cdot \frac{1}{1 + \left(\frac{1}{[ATP]_i}\right)^3} \quad (100)$$

$$k_2 = 0.04 \quad (101)$$

$$k_3 = 0.01 \quad (102)$$

$$k_4 = 0.165 \quad (103)$$

$$p(E_1Na) = \frac{1}{1 + \left(\frac{K_{mNa1}}{[Na^+]_i}\right)^{1.06} \cdot \left(1 + \frac{[K^+]_i}{K_{mK1}}\right)^{1.12}} \quad (104)$$

$$p(E_2Na) = \frac{1}{1 + \left(\frac{K_{mNa0}}{[Na^+]_{eff}}\right)^{1.06} \cdot \left(1 + \frac{[K^+]_e}{K_{mK0}}\right)^{1.12}} \quad (105)$$

$$p(E_1K) = \frac{1}{1 + \left(\frac{K_{mK1}}{[K^+]_i}\right)^{1.12} \cdot \left(1 + \frac{[Na^+]_i}{K_{mNa1}}\right)^{1.06}} \quad (106)$$

$$p(E_2K) = \frac{1}{1 + \left(\frac{K_{mK0}}{[K^+]_e}\right)^{1.12} \cdot \left(1 + \frac{[Na^+]_{eff}}{K_{mNa0}}\right)^{1.06}} \quad (107)$$

$$[Na^+]_{eff} = [Na^+]_e \cdot e^{\frac{-0.82 \cdot F \cdot V}{R \cdot T}}; \quad (108)$$

effective concentration at the binding site

$$K_{mNa_e} = 69.8 \text{ mM} \quad (109)$$

$$K_{mK_e} = 1.5 \text{ mM} \quad (110)$$

$$K_{mNa_i} = 8.05 \text{ mM} \quad (111)$$

$$K_{mK_i} = 32.88 \text{ mM} \quad (112)$$

$$\alpha_y = k_2 \cdot p(E_2Na) + k_4 \cdot p(E_2K); \quad (113)$$

forward rate constant of y-gate

$$\beta_y = k_1 \cdot p(E_1Na) + k_3 \cdot p(E_1K); \quad (114)$$

backward rate constant of y-gate

Rate of Na⁺/H⁺ exchanger

$$V_{NHE} = \frac{[H^+]_i^2}{[H^+]_i^2 + K_i^2} \cdot \frac{\alpha_1^+ \cdot \alpha_2^+ - \alpha_1^- \cdot \alpha_2^-}{\alpha_1^+ + \alpha_2^+ + \alpha_1^- + \alpha_2^-} \quad (115)$$

$$\alpha_1^+ = 0.00197 \text{ ms}^{-1} \quad (116)$$

$$\alpha_2^+ = \frac{\frac{[H^+]_i}{K_H} \cdot k_2^+}{\left(1 + \frac{[Na^+]_i}{K_{Na}}\right) \cdot \left(1 + \frac{[H^+]_i}{K_H}\right)} \quad (117)$$

$$\alpha_1^- = \frac{\frac{[Na^+]_i}{K_{Na}} \cdot k_1^-}{\left(1 + \frac{[Na^+]_i}{K_{Na}}\right) \cdot \left(1 + \frac{[H^+]_i}{K_H}\right)} \quad (118)$$

$$\alpha_2^- = 0.000818 \text{ ms}^{-1} \quad (119)$$

$$k_1^- = 1.1724 \text{ ms}^{-1} \quad (120)$$

$$k_2^+ = 0.01415 \text{ ms}^{-1} \quad (121)$$

$$K_{Na} = 21.49 \text{ mM} \quad (122)$$

$$K_H = 1.778 \cdot 10^{-7} \text{ mM} \quad (123)$$

$$K_i = 4.187 \cdot 10^{-2} \text{ mM} \quad (124)$$

Ca²⁺ release through ryanodine receptor (RyR) channel

$$I_{RyR} = 62000 \cdot ([Ca^{2+}]_{rel} - [Ca^{2+}]_i) \cdot p(\text{open}_{RyR}) \quad (128)$$

$$k_1 = 280000 \cdot [Ca^{2+}]_i^2 - 0.507 \cdot CF_{Ca} \cdot p(\text{open}_{CaL}) \quad (129)$$

$$k_2 = \frac{0.08}{1 + \frac{0.36}{[Ca^{2+}]_{rel}}} \quad (130)$$

$$k_3 = 0.000377 \cdot [Ca^{2+}]_{rel}^2 \quad (131)$$

$$k_4 = 0.000849 \quad (132)$$

Ca²⁺ transfer in SR

$$I_{SRT} = 386 \cdot ([Ca^{2+}]_{up} - [Ca^{2+}]_{rel}); \quad (133)$$

Ca²⁺ transfer from the SR uptake site to the release site

Ca²⁺ leak through SR

$$I_{SRL} = 459 \cdot ([Ca^{2+}]_{up} - [Ca^{2+}]_i); \text{ Ca}^{2+} \text{ leak from the SR} \quad (134)$$

Ca²⁺ uptake into SR

$$I_{SRU} = 162500 \cdot (k_2 \cdot p(E_2Ca) \cdot (1 - y) - k_1 \cdot p(E_1Ca) \cdot y) \quad (139)$$

$$k_1 = 0.01 \quad (140)$$

$$k_2 = 1 \quad (141)$$

$$k_3 = \frac{1}{1 + \left(\frac{1}{[\text{ATP}]_i}\right)^3} \quad (142)$$

$$k_4 = 0.01 \quad (143)$$

$$p(E_1\text{Ca}) = \frac{1}{1 + \frac{K_{m\text{Ca}_i}}{[\text{Ca}^{2+}]_{\text{up}}}} \quad (144)$$

$$p(E_2\text{Ca}) = \frac{1}{1 + \frac{K_{m\text{Ca}_o}}{[\text{Ca}^{2+}]_i}} \quad (145)$$

$$p(E_1) = 1 - p(E_1\text{Ca}) \quad (146)$$

$$p(E_2) = 1 - p(E_2\text{Ca}) \quad (147)$$

$$K_{m\text{Ca}_i} = 0.08 \text{ mM} \quad (148)$$

$$K_{m\text{Ca}_o} = 0.0008 \text{ mM} \quad (149)$$

$$\alpha_y = k_2 \cdot p(E_2\text{Ca}) + k_4 \cdot p(E_2);$$

forward rate constant of y-gate

$$\beta_y = k_1 \cdot p(E_1\text{Ca}) + k_3 \cdot p(E_1);$$

backward rate constant of y-gate

Plasmalemma Ca^{2+} pump (PMCA)

$$I_{\text{PMCA}} = \frac{100 \cdot [\text{Ca}^{2+}]_i}{[\text{Ca}^{2+}]_i + 0.0015} \quad (152)$$

Constant field

For $V = 0$,

$$\text{CF}_{\text{Na}} = [\text{Na}^+]_i - [\text{Na}^+]_e; \text{ constant field for } \text{Na}^+ (\text{mM}) \quad (153)$$

$$\text{CF}_{\text{K}} = [\text{K}^+]_i - [\text{K}^+]_e; \text{ constant field for } \text{K}^+ (\text{mM}) \quad (154)$$

$$\text{CF}_{\text{Ca}} = [\text{Ca}^{2+}]_i - [\text{Ca}^{2+}]_e; \text{ constant field for } \text{Ca}^{2+} (\text{mM}) \quad (155)$$

Or,

$$\text{CF}_{\text{Na}} = \frac{F \cdot V}{R \cdot T} \cdot \frac{[\text{Na}^+]_i - [\text{Na}^+]_e \cdot e^{\left(\frac{F \cdot V}{R \cdot T}\right)}}{1 - e^{\left(\frac{F \cdot V}{R \cdot T}\right)}} \quad (156)$$

$$\text{CF}_{\text{K}} = \frac{F \cdot V}{R \cdot T} \cdot \frac{[\text{K}^+]_i - [\text{K}^+]_e \cdot e^{\left(\frac{F \cdot V}{R \cdot T}\right)}}{1 - e^{\left(\frac{F \cdot V}{R \cdot T}\right)}} \quad (157)$$

$$\text{CF}_{\text{Ca}} = \frac{2 \cdot F \cdot V}{R \cdot T} \cdot \frac{[\text{Ca}^{2+}]_i - [\text{Ca}^{2+}]_e \cdot e^{\left(\frac{2 \cdot F \cdot V}{R \cdot T}\right)}}{1 - e^{\left(\frac{2 \cdot F \cdot V}{R \cdot T}\right)}} \quad (158)$$

Ion concentration

$$I_{\text{net(Na)}} = I_{\text{Na(Na)}} + I_{\text{bNSC(Na)}} + 3 \cdot I_{\text{NaK}} + 3 \cdot I_{\text{NaCa}};$$

whole cell current carried by Na^+

$$I_{\text{net(K)}} = I_{\text{Na(K)}} + I_{\text{K1}} + I_{\text{Kr}} + I_{\text{to}} + I_{\text{ss}} + I_{\text{KATP}} +$$

$I_{\text{bNSC(K)}} - 2 \cdot I_{\text{NaK}}$; whole cell current carried by K^+

$$I_{\text{net(Ca)}} = I_{\text{CaL}} + I_{\text{Cab}} - 2 \cdot I_{\text{NaCa}} + I_{\text{PMCA}};$$

whole cell current carried by Ca^{2+}

$$\frac{d[\text{Na}^+]_i}{dt} = \frac{-I_{\text{net(Na)}}}{F \cdot V_i} + V_{\text{Na(mito)}} + V_{\text{NHE}} \quad (162)$$

$$\frac{d[\text{K}^+]_i}{dt} = \frac{-I_{\text{net(K)}}}{F \cdot V_i} + V_{\text{K(mito)}} \quad (163)$$

$$\frac{d[\text{Ca}]_i}{dt} = \frac{-(I_{\text{net(Ca)}} - I_{\text{RyR}} + I_{\text{SRU}} - I_{\text{SRLe}})}{2 \cdot F \cdot V_i} + \frac{d([\text{T}] + [\text{T}^*])}{dt} + V_{\text{Ca(mito)}} \quad (164)$$

$$[\text{Ca}^{2+}]_i = 0.5 \cdot \left(-b_{\text{CMDN}} + \sqrt{b_{\text{CMDN}}^2 + 4 \cdot c_{\text{CMDN}}} \right) \quad (165)$$

$$b_{\text{CMDN}} = [\text{CMDN}]_t - [\text{Ca}]_i + K_{m\text{CMDN}} \quad (166)$$

$$c_{\text{CMDN}} = K_{m\text{CMDN}} \cdot [\text{Ca}]_i$$

$$\frac{dpH_i}{dt} = -\frac{1}{\beta_H} \cdot (V_{\text{H(mito)}} - V_{\text{NHE}}) \quad (167)$$

$$\beta_H = \log_{10} \left([\text{H}^+]_i + 31.2 \cdot \frac{10^{6.4}/[\text{H}^+]_i}{([\text{H}^+]_i + 10^{6.4}/[\text{H}^+]_i)^2} + \right.$$

$6.85 \cdot \frac{10^{7.48}/[\text{H}^+]_i}{([\text{H}^+]_i + 10^{7.48}/[\text{H}^+]_i)^2} \Bigg); \text{ proton buffering factor}$

$$V_{\text{Na(mito)}} = -V_{\text{mito}}/V_i \cdot \frac{d[\text{Na}^+]_m}{dt}^*; \text{Na}^+ \text{ flux (mM} \cdot \text{ms}^{-1})$$

from mitochondria

$$V_{\text{K(mito)}} = -V_{\text{mito}}/V_i \cdot \frac{d[\text{K}^+]_m}{dt}^*; \text{K}^+ \text{ flux (mM} \cdot \text{ms}^{-1})$$

from mitochondria

$$V_{\text{Ca(mito)}} = -V_{\text{mito}}/V_i \cdot \frac{d[\text{Ca}]_m}{dt}^*; \text{Ca}^{2+} \text{ flux (mM} \cdot \text{ms}^{-1})$$

from mitochondria

$$V_{\text{H(mito)}} = -V_{\text{mito}}/V_i \cdot \frac{d[\text{H}]_m}{dt}^*; \text{H}^+ \text{ flux (mM} \cdot \text{ms}^{-1})$$

from mitochondria

* see Eqs. 334 ~ 341.

ATP concentration

$$V_{\text{ATP}_{\text{NaK}}} = \frac{I_{\text{NaK}}}{F \cdot V_i};$$

ATP consumption by Na-K pump (mM $\cdot$ ms $^{-1}$)

$$V_{\text{ATP}_{\text{SRU}}} = \frac{I_{\text{SRU}}}{4 \cdot F \cdot V_i};$$

ATP consumption by SR uptake (mM $\cdot$ ms $^{-1}$)

$$V_{\text{ATP}_{\text{PMCA}}} = \frac{I_{\text{PMCA}}}{4 \cdot F \cdot V_i}; \text{ATP consumption}$$

by plasmalemma Ca^{2+} pump (mM $\cdot$ ms $^{-1}$)

$$V_{\text{ATP}_{\text{constitutive}}} = 1 \cdot 10^{-5} \text{ mM} \cdot \text{ms}^{-1};$$

constitutive cytosolic ATP consumption rate

$$V_{\text{ATP}_{\text{contraction}}} = 0.4 \cdot p(\text{TCa}^*) \cdot [\text{Troponin}]; \text{ATP}$$

ATP consumption by contraction (mM $\cdot$ ms $^{-1}$)

$$V_{\text{ATP}_{\text{util}}} = 0.5 \cdot (V_{\text{ATP}_{\text{NaK}}} + V_{\text{ATP}_{\text{SRU}}} + V_{\text{ATP}_{\text{PMCA}}} + V_{\text{ATP}_{\text{contraction}}});$$

utilization of ATP (mM $\cdot$ ms $^{-1}$)

$$\frac{d[\text{ATP}]_i}{dt} = -V_{\text{CK}}^{\text{mito}*} - V_{\text{ATP}_{\text{util}}} + V_{\text{ATP}_{\text{mito}}} \quad (179)$$

$$\frac{d[\text{ADP}]_i}{dt} = -\frac{d[\text{ATP}]_i}{dt} \quad (180)$$

$$\frac{d[\text{ATP}]_{\text{ic}}}{dt} = -V_{\text{CK}}^{\text{cyto}*} - V_{\text{ATP}_{\text{constitutive}}} \quad (181)$$

$$V_{\text{ATP}_{\text{mito}}} = V_{\text{mito}}/V_i \cdot V_{\text{ANT}}^*;$$

ATP flux (mM $\cdot$ ms $^{-1}$) from mitochondria

* see Eqs. 183 ~ 189.

Creatine kinase

$$V_{CK}^{cyto} = k_{CK}^{cyto} \cdot \left([ATP]_{ic} \cdot [Cr]_{ic} - \frac{[ADP]_{ic} \cdot [CrP]_{ic}}{K_{Eq}} \right) \quad (183)$$

$$k_{CK}^{cyto} = 1.4 \cdot 10^{-4} \text{ ms}^{-1}; \quad (184)$$

forward rate constant of cytoplasmic CK

$$K_{Eq} = 0.095; \text{ equilibrium constant of CK} \quad (185)$$

$$V_{CK}^{mito} = k_{CK}^{mito} \cdot \left([ATP]_i \cdot [Cr]_i - \frac{[ADP]_i \cdot [CrP]_i}{K_{Eq}} \right) \quad (186)$$

$$k_{CK}^{mito} = 1.33 \cdot 10^{-6} \text{ ms}^{-1}; \quad (187)$$

forward rate constant of mitochondrial CK

$$V_{tr}^{CrP} = k_{tr}^{Cr} \cdot ([CrP]_i - [CrP]_{ic}) \quad (188)$$

$$k_{tr}^{Cr} = 2 \cdot 10^{-3} \text{ ms}^{-1}; \text{ transfer rate constant of CrP} \quad (189)$$

Ca²⁺ concentrations in SR

$$\frac{d[Ca]_{rel}}{dt} = \frac{(J_{SR}T - J_{RyR})}{2 \cdot F \cdot V_{rel}} \quad (190)$$

$$[Ca^{2+}]_{rel} = 0.5 \cdot \left(-b_{CSQN} + \sqrt{b_{CSQN}^2 + 4 \cdot c_{CSQN}} \right) \quad (191)$$

$$b_{CSQN} = [CSQN]_t - [Ca]_{rel} + K_{mCSQN} \quad (192)$$

$$c_{CSQN} = K_{mCSQN} \cdot [Ca]_{rel} \quad (193)$$

$$\frac{d[Ca^{2+}]_{up}}{dt} = \frac{(J_{SR}U - J_{SR}T - J_{SR}L)}{2 \cdot F \cdot V_{up}} \quad (194)$$

Muscle model

$$[T] = [T]_t - [TCa] - [TCa^*] - [T^*] \quad (195)$$

T; thin filament site with free troponin C

$$[T]_t = 7 \cdot 10^{-3} \text{ mM}; \text{ total concentration of troponin C} \quad (196)$$

TCa; thin filament site with troponin C bound to Ca²⁺

TCa^{*}; thin filament site with troponin C bound to Ca²⁺ and attached cross-bridge

T^{*}; thin filament site with attached cross-bridge and without Ca²⁺ bound to troponin C

$$Q_b = Y_1 \cdot [Ca^{2+}]_i \cdot [T] - Z_1 \cdot [TCa]; \quad (197)$$

net rate of Ca²⁺ binding to T

$$Y_1 = 39 \text{ nM} \cdot \text{ms}^{-1}; \quad (198)$$

rate parameter of Ca²⁺ binding to troponin C

$$Z_1 = 0.03 \text{ ms}^{-1}; \quad (199)$$

rate parameter of Ca²⁺ unbinding from TCa

$$Q_a = Y_2 \cdot [TCa]_{eff} - Z_2 \cdot [TCa^*]; \quad (200)$$

net rate of cross-bridge attachment

$$Y_2 = 0.0039 \text{ ms}^{-1}; \text{ rate parameter of cross-bridge} \quad (201)$$

attachment to a thin filament site associated with TCa

$$[TCa]_{eff} = [TCa] \cdot e^{-R \cdot (L - L_a)}; \text{ effective [TCa]} \quad (202)$$

where cross-bridge attachment can occur

$$R = 20 (\mu\text{m}^2)^{-1}; \text{ exponential parameter to} \quad (203)$$

characterize the L dependence of $[TCa]_{eff}$

L ; half-sarcomere length

$$L_a = 1.17 \mu\text{m}; \text{ optimal } L \text{ for maximum } [TCa]_{eff} \quad (204)$$

$$Z_2 = 0.0039 \text{ ms}^{-1}; \text{ rate parameter of TCa}^* \text{ detachment} \quad (205)$$

$$Q_r = Y_3 \cdot [TCa^*] - Z_3 \cdot [T^*] \cdot [Ca^{2+}]_i; \quad (206)$$

net rate of Ca²⁺ release from TCa^{*}

$$Y_3 = 0.03 \text{ ms}^{-1}; \quad (207)$$

rate parameter of Ca²⁺ release from TCa^{*}

$$Z_3 = 1560 \text{ nM} \cdot \text{ms}^{-1}; \quad (208)$$

rate parameter of Ca²⁺ rebinding to T^{*}

$$Q_d = Y_4 \cdot [T^*]; \text{ rate of T}^* \text{ detachment to give Ca}^{2+} \text{ and T} \quad (209)$$

$$Y_4 = 0.12 \text{ ms}^{-1}; \text{ rate parameter of T}^* \text{ detachment} \quad (210)$$

$$Q_{d1} = Y_d \cdot \left(\frac{dX}{dt} \right)^2 \cdot [T^*]; \text{ T}^* \text{ detachment process} \quad (211)$$

dependent on the velocity of X change and T^{*}

$$Y_d = 0.027 \text{ ms} \cdot \mu\text{m}^{-2}; \text{ parameter that determines} \quad (212)$$

the influence of dX/dt on Q_{d1} and Q_{d2}

$$Q_{d2} = Y_d \cdot \left(\frac{dX}{dt} \right)^2 \cdot [TCa^*]; \text{ T}^* \text{ detachment process} \quad (213)$$

dependent on the velocity of X change and TCa^{*}

$$\frac{dX}{dt} = B \cdot (L - X - h_c) \quad (214)$$

$$B = 1.2 \text{ ms}^{-1}; \text{ proportionality parameter for } dX/dt \quad (215)$$

$$h_c = 0.005 \mu\text{m}; \text{ cross-bridge elongation at a steady } L \quad (216)$$

$$X; \text{ length composed of half of the thick filament} \quad (217)$$

and the free portion of the thin filament

$$\frac{d[TCa]}{dt} = Q_b - Q_a \quad (218)$$

$$\frac{d[TCa^*]}{dt} = Q_a - Q_r - Q_{d2} \quad (219)$$

$$\frac{d[T^*]}{dt} = Q_r - Q_d - Q_{d1} \quad (220)$$

$$F = F_b + F_p; \text{ force developed by a muscle unit normalized} \quad (221)$$

with respect to muscle cross-sectional area

$$F_b = A \cdot ([TCa^*] + [T^*]) \cdot (L - X); \quad (222)$$

force developed by cross-bridge

$$A = 1800 \text{ mN} \cdot \text{mm}^{-2} \cdot \mu\text{m}^{-1} \cdot \mu\text{M}^{-1}; \text{ stiffness of the} \quad (223)$$

cross-bridge elastic element referred to concentrations

$$F_p = K \cdot (L - L_0)^5 + K_l \cdot (L - L_0); \quad (224)$$

force developed by elastic component in parallel with F_b

$$K = 140000 \text{ mN} \cdot \text{mm}^{-2} \cdot \mu\text{m}^{-5}; \text{ proportionality} \quad (225)$$

parameter of the parallel elastic component of F_p

$$K_l = 200 \text{ mN} \cdot \text{mm}^{-2} \cdot \mu\text{m}^{-5}; \quad (226)$$

linear proportionality parameter equivalent to K

$$L_0 = 0.97 \mu\text{m}; \text{ unstressed } L \text{ that characterizes } F_p \quad (227)$$

Part II. Mitochondrial space**Tricarboxylic acid cycle****Changes in metabolites**

$$\frac{d[\text{AcCoA}]}{dt} = V_{\text{AcCoA}} - V_{\text{CS}} \quad (228)$$

$$V_{\text{AcCoA}} = 1 \cdot 10^{-6} \text{ mM} \cdot \text{ms}^{-1}; \text{ rate of AcCoA influx} \quad (229)$$

$$\frac{d[\text{CIT}]}{dt} = V_{\text{CS}} - V_{\text{ACO}} \quad (230)$$

$$\frac{d[\text{ISOC}]}{dt} = V_{\text{ACO}} - V_{\text{IDH}} \quad (231)$$

$$\frac{d[\alpha\text{KG}]}{dt} = V_{\text{IDH}} - V_{\text{KGDH}} + V_{\text{AAT}} \quad (232)$$

$$\frac{d[\text{SCoA}]}{dt} = V_{\text{KGDH}} - V_{\text{SL}} \quad (233)$$

$$\frac{d[\text{Suc}]}{dt} = V_{\text{SL}} - V_{\text{SDH}} \quad (234)$$

$$\frac{d[\text{FUM}]}{dt} = V_{\text{SDH}} - V_{\text{FH}} \quad (235)$$

$$\frac{d[\text{MAL}]}{dt} = V_{\text{FH}} - V_{\text{MDH}} \quad (236)$$

$$\frac{d[\text{OAA}]}{dt} = V_{\text{MDH}} - V_{\text{CS}} - V_{\text{AAT}} \quad (237)$$

$$\frac{d[\text{ADP}]_m}{dt} = V_{\text{ANT}} - V_{\text{ATPase}}^* - V_{\text{SL}} \quad (238)$$

$$[\text{ATP}]_m = C_A - [\text{ADP}]_m \quad (239)$$

$$\frac{d[\text{NADH}]}{dt} = V_{\text{IDH}} + V_{\text{KGDH}} + V_{\text{MDH}} - V_{\text{O}_2}^{**} \quad (240)$$

$$[\text{NAD}^+] = C_{\text{PN}} - [\text{NADH}] \quad (241)$$

*see Eqs. 326 ~ 328.

**see Eqs. 301 ~ 312

See following section for details of each reaction.

Rate of citrate synthase (CS)

$$V_{\text{CS}} = k_{\text{cat}}^{\text{CS}} \cdot E_{\text{T}}^{\text{CS}} / \left(1 + \frac{K_{\text{M}}^{\text{AcCoA}}}{[\text{AcCoA}]} + \frac{K_{\text{M}}^{\text{OAA}}}{[\text{OAA}]} + \frac{K_{\text{M}}^{\text{AcCoA}}}{[\text{AcCoA}]} \cdot \frac{K_{\text{M}}^{\text{OAA}}}{[\text{OAA}]} \right) \quad (242)$$

$$k_{\text{cat}}^{\text{CS}} = 0.05 \text{ ms}^{-1}; \text{ catalytic constant of CS} \quad (243)$$

$$E_{\text{T}}^{\text{CS}} = 0.4 \text{ mM}; \text{ concentration of CS} \quad (244)$$

$$K_{\text{M}}^{\text{AcCoA}} = 1.26 \cdot 10^{-2} \text{ mM}; \text{ Michaelis constant for AcCoA} \quad (245)$$

$$K_{\text{M}}^{\text{OAA}} = 6.4 \cdot 10^{-3} \text{ mM}; \text{ Michaelis constant for oxaloacetate} \quad (246)$$

Rate of aconitase (ACO)

$$V_{\text{ACO}} = k_{\text{f}}^{\text{ACO}} \cdot \left([\text{CIT}] - \frac{[\text{ISOC}]}{K_{\text{E}}^{\text{ACO}}} \right) \quad (247)$$

$$k_{\text{f}}^{\text{ACO}} = 1.25 \cdot 10^{-2} \text{ ms}^{-1}; \text{ forward rate constant of ACO} \quad (248)$$

$$K_{\text{E}}^{\text{ACO}} = 2.22; \text{ equilibrium constant of ACO} \quad (249)$$

Rate of isocitrate dehydrogenase (IDH)

$$V_{\text{IDH}} = k_{\text{cat}}^{\text{IDH}} \cdot E_{\text{T}}^{\text{IDH}} / \left(1 + \frac{[\text{H}^+]_m}{k_{\text{h},1}} + \frac{k_{\text{h},2}}{[\text{H}^+]_m} + f_{\text{i}}^{\text{IDH}} \cdot \left(\frac{K_{\text{M}}^{\text{NAD}}}{[\text{NAD}^+]} \right) + f_{\text{a}}^{\text{IDH}} \cdot \left(\frac{K_{\text{M}}^{\text{ISOC}}}{[\text{ISOC}]} \right)^{\text{ni}} + f_{\text{i}}^{\text{IDH}} \cdot f_{\text{a}}^{\text{IDH}} \cdot \left(\frac{K_{\text{M}}^{\text{NAD}}}{[\text{NAD}^+]} \right) \cdot \left(\frac{K_{\text{M}}^{\text{ISOC}}}{[\text{ISOC}]} \right)^{\text{ni}} \right) \quad (250)$$

$$k_{\text{cat}}^{\text{IDH}} = 0.03 \text{ ms}^{-1}; \text{ rate constant of IDH} \quad (251)$$

$$E_{\text{T}}^{\text{IDH}} = 0.109 \text{ mM}; \text{ concentration of IDH} \quad (252)$$

$$k_{\text{h},1} = 8.1 \cdot 10^{-5} \text{ mM}; \text{ ionization constant of IDH} \quad (253)$$

$$k_{\text{h},2} = 5.98 \cdot 10^{-5} \text{ mM}; \text{ ionization constant of IDH} \quad (254)$$

$$f_{\text{i}}^{\text{IDH}} = \left(1 + \frac{[\text{NADH}]}{K_{\text{I}}^{\text{NADH}}} \right) \quad (255)$$

$$K_{\text{I}}^{\text{NADH}} = 0.19 \text{ mM}; \text{ inhibition constant by NADH} \quad (256)$$

$$K_{\text{M}}^{\text{NAD}} = 0.923 \text{ mM}; \text{ Michaelis constant for NAD}^+ \quad (257)$$

$$f_{\text{a}}^{\text{IDH}} = \left(\left(1 + \frac{[\text{ADP}]_m}{K_{\text{a}}^{\text{ADP}}} \right) \cdot \left(1 + \frac{[\text{Ca}^{2+}]_m}{K_{\text{Ca}}^{\text{a}}} \right) \right)^{-1} \quad (258)$$

$$K_{\text{ADP}}^{\text{a}} = 0.62 \text{ mM}; \text{ activation constant by ADP} \quad (259)$$

$$K_{\text{Ca}}^{\text{a}} = 0.0005 \text{ mM}; \text{ activation constant for Ca}^{2+} \quad (260)$$

$$K_{\text{M}}^{\text{ISOC}} = 1.52 \text{ mM}; \text{ Michaelis constant for isocitrate} \quad (261)$$

$$\text{ni} = 2.0; \text{ cooperativity for isocitrate} \quad (262)$$

Rate of α -ketoglutarate dehydrogenase (KGDH)

$$V_{\text{KGDH}} = k_{\text{cat}}^{\text{KGDH}} \cdot E_{\text{T}}^{\text{KGDH}} / \quad (263)$$

$$\left(1 + f_{\text{a}}^{\text{KGDH}} \cdot \left(\frac{K_{\text{M}}^{\alpha\text{KG}}}{[\alpha\text{KG}]} \right)^{n_{\alpha\text{KG}}} + f_{\text{a}}^{\text{KGDH}} \cdot \left(\frac{K_{\text{M}}^{\text{NAD}}}{[\text{NAD}^+]} \right) \right)$$

$$k_{\text{cat}}^{\text{KGDH}} = 0.05 \text{ ms}^{-1}; \text{ rate constant of KGDH} \quad (264)$$

$$E_{\text{T}}^{\text{KGDH}} = 0.5 \text{ mM}; \text{ concentration of KGDH} \quad (265)$$

$$f_{\text{a}}^{\text{KGDH}} = \left(\left(1 + \frac{[\text{Mg}^{2+}]_m}{K_{\text{D}}^{\text{Mg}^{2+}}} \right) \cdot \left(1 + \frac{[\text{Ca}^{2+}]_m}{K_{\text{D}}^{\text{Ca}^{2+}}} \right) \right)^{-1} \quad (266)$$

$$K_{\text{D}}^{\text{Mg}^{2+}} = 0.0308 \text{ mM}; \text{ activation constant for Mg}^{2+} \quad (267)$$

$$K_{\text{D}}^{\text{Ca}^{2+}} = 1.27 \cdot 10^{-3} \text{ mM}; \text{ activation constant for Ca}^{2+} \quad (268)$$

$$K_{\text{M}}^{\alpha\text{KG}} = 1.94 \text{ mM}; \text{ Michaelis constant for } \alpha\text{KG} \quad (269)$$

$$K_{\text{M}}^{\text{NAD}} = 38.7 \text{ mM}; \text{ Michaelis constant for NAD}^+ \quad (270)$$

$$n_{\alpha\text{KG}} = 1.2; \text{ Hill coefficient of KGDH for } \alpha\text{KG} \quad (271)$$

Rate of succinyl CoA lyase (SL)

$$V_{\text{SL}} = k_{\text{f}}^{\text{SL}} \cdot \left([\text{SCoA}] \cdot [\text{ADP}]_m - \frac{[\text{SuC}] \cdot [\text{ATP}]_m \cdot [\text{CoA}]}{K_{\text{E}}^{\text{SL}}} \right) \quad (272)$$

$$k_{\text{f}}^{\text{SL}} = 5.0 \cdot 10^{-4} \text{ mM}^{-1} \cdot \text{ms}^{-1}; \quad (273)$$

forward rate constant of SL

$$K_{\text{E}}^{\text{SL}} = 3.115; \text{ equilibrium constant of the SL reaction} \quad (274)$$

Rate of succinic dehydrogenase (SDH)

$$V_{\text{SDH}} = k_{\text{cat}}^{\text{SDH}} \cdot E_{\text{T}}^{\text{SDH}} / \left(1 + \frac{K_{\text{M}}^{\text{Suc}}}{[\text{Suc}]} \cdot \left(1 + \frac{[\text{OAA}]}{K_{\text{I},\text{sdh}}^{\text{OAA}}} \right) \cdot \left(1 + \frac{[\text{FUM}]}{K_{\text{I}}^{\text{FUM}}} \right) \right) \quad (275)$$

$$k_{\text{cat}}^{\text{SDH}} = 3.0 \cdot 10^{-3} \text{ ms}^{-1}; \text{ rate constant of SDH} \quad (276)$$

$$E_{\text{T}}^{\text{SDH}} = 0.5 \text{ mM}; \text{ concentration of SDH} \quad (277)$$

$$K_M^{\text{Suc}} = 0.03 \text{ mM; Michaelis constant for succinate} \quad (278)$$

$$K_{i,\text{sdh}}^{\text{OAA}} = 0.15 \text{ mM; inhibition constant by oxaloacetate} \quad (279)$$

$$K_i^{\text{FUM}} = 1.3 \text{ mM; inhibition constant by fumarate} \quad (280)$$

Rate of fumarate hydratase (FH)

$$V_{\text{FH}} = k_f^{\text{FH}} \cdot \left([\text{FUM}] - \frac{[\text{MAL}]}{K_E^{\text{FH}}} \right) \quad (281)$$

$$k_f^{\text{FH}} = 3.32 \cdot 10^{-3} \text{ ms}^{-1}; \text{ rate constant for FH} \quad (282)$$

$$K_E^{\text{FH}} = 1; \text{ equilibrium constant of FH} \quad (283)$$

Rate of malate dehydrogenase (MDH)

$$V_{\text{MDH}} = k_{\text{cat}}^{\text{MDH}} \cdot E_{\text{T}}^{\text{MDH}} \cdot f_{\text{h,a}} \cdot f_{\text{h,i}} / \left(1 + \frac{K_M^{\text{MAL}}}{[\text{MAL}]} \cdot \left(1 + \frac{[\text{OAA}]}{K_i^{\text{OAA}}} \right) + \frac{K_M^{\text{NAD}}}{[\text{NAD}^+]} + \frac{K_M^{\text{MAL}}}{[\text{MAL}]} \cdot \left(1 + \frac{[\text{OAA}]}{K_i^{\text{OAA}}} \right) \cdot \frac{K_M^{\text{NAD}}}{[\text{NAD}^+]} \right) \quad (284)$$

$$k_{\text{cat}}^{\text{MDH}} = 0.111 \text{ ms}^{-1}; \text{ rate constant of MDH} \quad (285)$$

$$E_{\text{T}}^{\text{MDH}} = 0.154 \text{ mM; concentration of MDH} \quad (286)$$

$$f_{\text{h,a}} = \left(1 + \frac{[\text{H}^+]_{\text{m}}}{k_{\text{h1}}} + \frac{[\text{H}^+]_{\text{m}}^2}{k_{\text{h1}} \cdot k_{\text{h2}}} \right)^{-1} + k_{\text{offset}} \quad (287)$$

$$f_{\text{h,i}} = \left(1 + \frac{k_{\text{h3}}}{[\text{H}^+]_{\text{m}}} + \frac{k_{\text{h3}} \cdot k_{\text{h4}}}{[\text{H}^+]_{\text{m}}^2} \right)^{-2} \quad (288)$$

$$k_{\text{h1}} = 1.13 \cdot 10^{-5} \text{ mM; ionization constant of MDH} \quad (289)$$

$$k_{\text{h2}} = 26.7 \text{ mM; ionization constant of MDH} \quad (290)$$

$$k_{\text{h3}} = 6.68 \cdot 10^{-9} \text{ mM; ionization constant of MDH} \quad (291)$$

$$k_{\text{h4}} = 5.62 \cdot 10^{-6} \text{ mM; ionization constant of MDH} \quad (292)$$

$$k_{\text{offset}} = 3.99 \cdot 10^{-2}; \text{ pH-independent term in the pH activation factor of MDH} \quad (293)$$

$$K_M^{\text{MAL}} = 1.493 \text{ mM; Michaelis constant for malate} \quad (294)$$

$$K_i^{\text{OAA}} = 3.1 \cdot 10^{-3} \text{ mM; inhibition constant for oxaloacetate} \quad (295)$$

$$K_M^{\text{NAD}} = 0.2244 \text{ mM; Michaelis constant for NAD}^+ \quad (296)$$

Rate of aspartate amino transferase (AAT)

$$V_{\text{AAT}} = k_f^{\text{AAT}} \cdot [\text{OAA}] \cdot [\text{GLU}] \cdot \frac{k_{\text{ASP}} \cdot K_E^{\text{AAT}}}{k_{\text{ASP}} \cdot K_E^{\text{AAT}} + [\alpha\text{KG}] / k_i^{\text{AAT}}} \quad (297)$$

$$k_f^{\text{AAT}} = 6.44 \cdot 10^{-4} \text{ ms}^{-1}; \text{ forward rate constant of AAT} \quad (298)$$

$$k_{\text{ASP}} = 1.5 \cdot 10^{-6} \text{ ms}^{-1}; \text{ rate constant of aspartate consumption} \quad (299)$$

$$K_E^{\text{AAT}} = 6.6; \text{ equilibrium constant of AAT} \quad (300)$$

Oxidative phosphorylation reaction rates

Rate of O₂ consumption (V_{O2})

$$V_{\text{O}_2} = 0.5 \cdot \rho^{\text{res}} \cdot \frac{\left(r_a + r_{c1} \cdot e^{-\frac{6 \cdot F \cdot \Delta\psi_B}{R \cdot T}} \right) \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} - r_a \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}} + r_{c2} \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}}{\left(1 + r_1 \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} \right) \cdot e^{-\frac{6 \cdot F \cdot \Delta\psi_B}{R \cdot T}} + \left(r_2 + r_3 \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} \right) \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}} \quad (301)$$

$$r_a = 6.394 \cdot 10^{-13} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (302)$$

$$r_{c1} = 2.656 \cdot 10^{-22} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (303)$$

$$\Delta\psi_B = 50 \text{ mV; phase boundary potential} \quad (304)$$

$$A_{\text{res}} = \frac{R \cdot T}{F} \cdot \ln \left(K_{\text{res}} \cdot \sqrt{\frac{[\text{NADH}]}{[\text{NAD}^+]}} \right) \quad (305)$$

$$K_{\text{res}} = 1.35 \cdot 10^{18}; \text{ equilibrium constant of respiration} \quad (306)$$

$$g = 0.85; \text{ correction factor for voltage} \quad (307)$$

$$\Delta\mu_H = \Delta\psi_m - \frac{R \cdot T}{F} \cdot \ln \left(\frac{[\text{H}^+]_{\text{m}}}{[\text{H}^+]_{\text{i}}} \right) \quad (308)$$

$$r_{c2} = 8.632 \cdot 10^{-30} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (309)$$

$$r_1 = 2.077 \cdot 10^{-18}; \text{ sum of products of rate constants} \quad (310)$$

$$r_2 = 1.728 \cdot 10^{-9}; \text{ sum of products of rate constants} \quad (311)$$

$$r_3 = 1.059 \cdot 10^{-26}; \text{ sum of products of rate constants} \quad (312)$$

Rate of proton efflux driven by NADH oxidation (V_{He})

$$V_{\text{He}} = 6 \cdot \rho^{\text{res}} \cdot \frac{r_a \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} - (r_a + r_b) \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}}{\left(1 + r_1 \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} \right) \cdot e^{-\frac{6 \cdot F \cdot \Delta\psi_B}{R \cdot T}} + \left(r_2 + r_3 \cdot e^{-\frac{F \cdot A_{\text{res}}}{R \cdot T}} \right) \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}} \quad (313)$$

$$r_b = 1.762 \cdot 10^{-16} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (314)$$

Rate of proton efflux driven by FADH₂ oxidation (V_{He(F)})

$$V_{\text{He(F)}} = 4 \cdot \rho^{\text{res(F)}} \cdot \frac{r_a \cdot e^{-\frac{F \cdot A_{\text{res(F)}}}{R \cdot T}} - (r_a + r_b) \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}}{\left(1 + r_1 \cdot e^{-\frac{F \cdot A_{\text{res(F)}}}{R \cdot T}} \right) \cdot e^{-\frac{6 \cdot F \cdot \Delta\psi_B}{R \cdot T}} + \left(r_2 + r_3 \cdot e^{-\frac{F \cdot A_{\text{res(F)}}}{R \cdot T}} \right) \cdot e^{-\frac{g \cdot 6 \cdot F \cdot \Delta\mu_H}{R \cdot T}}} \quad (315)$$

$$A_{\text{res(F)}} = \frac{R \cdot T}{F} \cdot \ln \left(K_{\text{res(F)}} \cdot \sqrt{\frac{[\text{FADH}_2]}{[\text{FAD}]}} \right) \quad (316)$$

$$K_{\text{res(F)}} = 5.765 \cdot 10^{13}; \text{ equilibrium constant of FADH}_2 \text{ oxidation} \quad (317)$$

Rate of proton uptake by mitochondrial F₁F₀-ATPase (V_{Hu})

$$V_{\text{Hu}} = -3 \cdot \rho^{\text{F1}} \cdot \frac{10^2 \cdot p_a \left(1 + e^{-\frac{F \cdot A_{\text{F1}}}{R \cdot T}} \right) - (p_a + p_b) \cdot e^{-\frac{3 \cdot F \cdot \Delta\mu_H}{R \cdot T}}}{\left(1 + p_1 \cdot e^{-\frac{F \cdot A_{\text{F1}}}{R \cdot T}} \right) \cdot e^{-\frac{3 \cdot F \cdot \Delta\psi_B}{R \cdot T}} + \left(p_2 + p_3 \cdot e^{-\frac{F \cdot A_{\text{F1}}}{R \cdot T}} \right) \cdot e^{-\frac{3 \cdot F \cdot \Delta\mu_H}{R \cdot T}}} \quad (318)$$

$$p_a = 1.656 \cdot 10^{-8} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (319)$$

$$A_{\text{F1}} = \frac{R \cdot T}{F} \cdot \ln \left(K_{\text{F1}} \cdot \frac{[\text{ATP}]_{\text{m}}}{[\text{ADP}]_{\text{m}} \cdot [\text{Pi}]} \right) \quad (320)$$

$$K_{\text{F1}} = 1.71 \cdot 10^6; \text{ equilibrium constant of ATP hydrolysis} \quad (321)$$

$$p_b = 3.373 \cdot 10^{-10} \text{ ms}^{-1}; \text{ sum of products of rate constants} \quad (322)$$

$$p_1 = 1.346 \cdot 10^{-8}; \text{ sum of products of rate constants} \quad (323)$$

$$p_2 = 7.739 \cdot 10^{-7}; \text{ sum of products of rate constants} \quad (324)$$

$$p_3 = 6.65 \cdot 10^{-15}; \text{ sum of products of rate constants} \quad (325)$$

Rate of the mitochondrial F_1F_0 -ATPase (V_{ATPase})

$$V_{\text{ATPase}} = -\rho^{F_1} \cdot \frac{\left(10^2 \cdot p_a + p_{c1} \cdot e^{\frac{3 \cdot F \cdot \Delta \psi_m}{R \cdot T}}\right) \cdot e^{\frac{F \cdot A_{F1}}{R \cdot T}} - p_a \cdot e^{\frac{3 \cdot F \cdot \Delta \mu_H}{R \cdot T}} + p_{c2} \cdot e^{\frac{F \cdot A_{F1}}{R \cdot T}} \cdot e^{\frac{3 \cdot F \cdot \Delta \mu_H}{R \cdot T}}}{\left(1 + p_1 \cdot e^{\frac{F \cdot A_{F1}}{R \cdot T}}\right) \cdot e^{\frac{3 \cdot F \cdot \Delta \psi_m}{R \cdot T}} + \left(p_2 + p_3 \cdot e^{\frac{F \cdot A_{F1}}{R \cdot T}}\right) \cdot e^{\frac{3 \cdot F \cdot \Delta \mu_H}{R \cdot T}}} \quad (326)$$

$$p_{c1} = 9.651 \cdot 10^{-17} \text{ ms}^{-1}; \quad (327)$$

sum of products of rate constants

$$p_{c2} = 4.585 \cdot 10^{-17} \text{ ms}^{-1}; \quad (328)$$

sum of products of rate constants

Rate of proton leak (V_{Hleak})

$$V_{\text{Hleak}} = g_H \cdot \Delta \mu_H \quad (329)$$

$$g_H = 1 \cdot 10^{-8} \text{ mM} \cdot \text{ms}^{-1} \cdot \text{mV}^{-1}; \quad (330)$$

leak conductance of proton through inner membrane

Adenine nucleotide translocator (ANT)

$$V_{\text{ANT}} = V_{\text{maxANT}} \cdot \frac{0.75 - 20 \cdot \frac{[\text{ATP}]_i}{[\text{ADP}]_i} \cdot \frac{[\text{ADP}]_m}{[\text{ATP}]_m} \cdot e^{\frac{-F \cdot \Delta \psi_m}{R \cdot T}}}{\left(1 + \frac{10}{9} \cdot \frac{[\text{ATP}]_i}{[\text{ADP}]_i} \cdot e^{\frac{-h \cdot \text{ANT} \cdot F \cdot \Delta \psi_m}{R \cdot T}}\right) \cdot \left(1 + 18 \cdot \frac{[\text{ADP}]_m}{[\text{ATP}]_m}\right)} \quad (331)$$

$$V_{\text{maxANT}} = 0.025 \text{ mM} \cdot \text{ms}^{-1}; \text{ maximal rate of the ANT} \quad (332)$$

$$h^{\text{ANT}} = 0.5; \text{ fraction of } \Delta \psi_m \quad (333)$$

Ion concentrations in mitochondria

Changes in ion concentration in mitochondria

$$\frac{d[\text{Ca}]_m}{dt} = (V_{\text{uni}} - V_{\text{NCX}}) \quad (334)$$

$$\frac{d[\text{Ca}^{2+}]_m}{dt} = \beta_{\text{Ca}} \cdot (V_{\text{uni}} - V_{\text{NCX}}) \quad (335)$$

$$\beta_{\text{Ca}} = 3 \cdot 10^{-2}; \text{ Ca}^{2+} \text{ buffering factor} \quad (336)$$

$$\frac{d[\text{K}^+]_m}{dt} = V_{\text{KATP}} + V_{\text{KH}} \quad (337)$$

$$\frac{d[\text{Na}^+]_m}{dt} = 3 \cdot V_{\text{NCX}} - V_{\text{NHE}} \quad (338)$$

$$\frac{d[\text{H}]_m}{dt} = V_{\text{Hu}} + V_{\text{Hleak}} + V_{\text{NHE}} + V_{\text{ANT}} - V_{\text{KH}} - V_{\text{He}} - V_{\text{HeF}} \quad (339)$$

$$\frac{dpH_m}{dt} = -\frac{1}{\beta_H} \cdot (V_{\text{Hu}} + V_{\text{Hleak}} + V_{\text{NHE}} + V_{\text{ANT}} - V_{\text{KH}} - V_{\text{He}} - V_{\text{HeF}}) \quad (340)$$

$$\beta_H = \log_{10} \left([\text{H}^+]_m + 31.2 \cdot \frac{10^{6.4}/[\text{H}^+]_m}{([\text{H}^+]_m + 10^{6.4}/[\text{H}^+]_m)^2} + 6.85 \cdot \frac{10^{7.48}/[\text{H}^+]_m}{([\text{H}^+]_m + 10^{7.48}/[\text{H}^+]_m)^2} \right); \text{ proton buffering factor} \quad (341)$$

See following section for details of each rate

Rate of Ca^{2+} uniporter (V_{uni})

$$V_{\text{uni}} = P_{\text{Ca}} \cdot \frac{z_{\text{Ca}} \cdot F \cdot \Delta \psi_m}{R \cdot T} \cdot \frac{\alpha_m \cdot [\text{Ca}^{2+}]_m \cdot e^{\frac{-z_{\text{Ca}} \cdot F \cdot \Delta \psi_m}{R \cdot T}} - \alpha_i \cdot [\text{Ca}^{2+}]_i}{e^{\frac{-z_{\text{Ca}} \cdot F \cdot \Delta \psi_m}{R \cdot T}} - 1} \quad (342)$$

$$P_{\text{Ca}} = 2.159 \cdot 10^{-2} \text{ ms}^{-1}; \quad (343)$$

permeability of the Ca^{2+} uniporter

$$z_{\text{Ca}} = 2; \text{ charge of } \text{Ca}^{2+} \quad (344)$$

$$\alpha_m = 0.2; \text{ mitochondrial activity coefficient for } \text{Ca}^{2+} \quad (345)$$

$$\alpha_i = 0.341; \text{ extramitochondrial (cytosolic) activity coefficient for } \text{Ca}^{2+} \quad (346)$$

Rate of $\text{Na}^+/\text{Ca}^{2+}$ exchanger (V_{NCX})

$$V_{\text{NCX}} = V_{\text{NCXmax}} \cdot \quad (347)$$

$$\frac{\frac{[\text{Na}^+]_i^3 \cdot [\text{Ca}^{2+}]_m}{K_{\text{Na}}^3 \cdot K_{\text{Ca}}} \cdot e^{\frac{0.5 \cdot F \cdot \Delta \psi_m}{R \cdot T}} \cdot \frac{[\text{Na}^+]_m^3 \cdot [\text{Ca}^{2+}]_i}{K_{\text{Na}}^3 \cdot K_{\text{Ca}}} \cdot e^{\frac{-0.5 \cdot F \cdot \Delta \psi_m}{R \cdot T}}}{1 + \frac{[\text{Na}^+]_i^3}{K_{\text{Na}}^3} + \frac{[\text{Ca}^{2+}]_m}{K_{\text{Ca}}} + \frac{[\text{Na}^+]_i^3 \cdot [\text{Ca}^{2+}]_m}{K_{\text{Na}}^3 \cdot K_{\text{Ca}}} + \frac{[\text{Na}^+]_m^3}{K_{\text{Na}}^3} + \frac{[\text{Ca}^{2+}]_i}{K_{\text{Ca}}} + \frac{[\text{Na}^+]_m^3 \cdot [\text{Ca}^{2+}]_i}{K_{\text{Na}}^3 \cdot K_{\text{Ca}}}}$$

$$V_{\text{NCXmax}} = 1.863 \cdot 10^{-4} \text{ mM} \cdot \text{ms}^{-1}; \text{ maximal rate of NCX} \quad (348)$$

$$K_{\text{Na}} = 8.0 \text{ mM}; \text{ Michaelis constant for } \text{Na}^+ \quad (349)$$

$$K_{\text{Ca}} = 0.008 \text{ mM}; \text{ Michaelis constant for } \text{Ca}^{2+} \quad (350)$$

Rate of K^+ flux through mitochondrial ATP-sensitive potassium channel (V_{KATP})

$$I_{\text{KATP}} = \bar{G}_{\text{KATP}} \cdot \frac{[\text{K}^+]_m^{0.24}}{1 + ([\text{ATP}]_i/0.1)^2} \cdot (\Delta \psi_m - E_K) \quad (351)$$

$$\bar{G}_{\text{KATP}} = 4.4 \cdot 10^{-4} \text{ nS}; \quad (352)$$

maximal conductance ATP-sensitive potassium channel

$$E_K = \frac{R \cdot T}{F} \cdot \ln \left(\frac{[\text{K}^+]_m}{[\text{K}^+]_i} \right) \quad (353)$$

$$V_{\text{KATP}} = \frac{I_{\text{KATP}}}{F \cdot V_{\text{mito}}} \quad (354)$$

Rate of K^+/H^+ exchanger (V_{KH})

$$V_{\text{KH}} = X_{\text{KH}} \cdot ([\text{K}^+]_i \cdot [\text{H}^+]_m - [\text{K}^+]_m \cdot [\text{H}^+]_i) \quad (355)$$

$$X_{\text{KH}} = 5.1344 \cdot 10^2; \text{ conversion factor} \quad (356)$$

Rate of Na^+/H^+ exchanger (V_{NHE})

$$V_{\text{NHE}} = V_{\text{NHEmax}} \cdot \quad (357)$$

$$\frac{\frac{[\text{H}^+]_m}{[\text{H}^+]_m + K_{\text{H,reg}}} \cdot \left(\frac{[\text{H}^+]_i}{K_{\text{H,NHE}}} \cdot \frac{[\text{Na}^+]_m}{K_{\text{Na,NHE}}} \cdot \frac{[\text{H}^+]_m}{K_{\text{H,NHE}}} \cdot \frac{[\text{Na}^+]_i}{K_{\text{Na,NHE}}} \right)}{1 + \frac{[\text{H}^+]_i}{K_{\text{H,NHE}}} + \frac{[\text{Na}^+]_m}{K_{\text{Na,NHE}}} + \frac{[\text{H}^+]_i}{K_{\text{H,NHE}}} \cdot \frac{[\text{Na}^+]_m}{K_{\text{Na,NHE}}} + \frac{[\text{H}^+]_m}{K_{\text{H,NHE}}} + \frac{[\text{Na}^+]_i}{K_{\text{Na,NHE}}} + \frac{[\text{H}^+]_m}{K_{\text{H,NHE}}} \cdot \frac{[\text{Na}^+]_i}{K_{\text{Na,NHE}}}}$$

$$V_{\text{NHEmax}} = 0.762 \text{ mM} \cdot \text{ms}^{-1}; \quad (358)$$

maximal rate of Na^+/H^+ exchanger

$$K_{\text{H,reg}} = 75.7 \cdot 10^{-6} \text{ mM}; \quad (359)$$

Michaelis constant of allosteric binding for H^+

$$K_{\text{H,NHE}} = 1 \cdot 10^{-7} \text{ mM}; \text{ Michaelis constant for } \text{H}^+ \quad (360)$$

$$K_{\text{Na,NHE}} = 20 \text{ mM}; \text{ Michaelis constant for } \text{Na}^+ \quad (362)$$

Mitochondrial membrane potential ($\Delta \Psi_m$)

$$\frac{d\Delta \Psi_m}{dt} = \frac{V_{\text{He}} + V_{\text{HeF}} - V_{\text{Hu}} - V_{\text{Hleak}} - V_{\text{ANT}} - V_{\text{NCX}} - 2 \cdot V_{\text{uni}} - V_{\text{KATP}}}{C_{\text{mito}}} \quad (362)$$