Mathematical Modelling of Ca^{2+} Oscillations in Airway Smooth Muscle Cells

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Abstract. The action of different agonists such as acetylcholine on the membrane of airway smooth muscle cells may induce cytosolic Ca^{2+} oscillations which can be a part of the Ca^{2+} signalling pathway, eventually leading to cell contraction. The aim of the present study is to present a mathematical model of the possible effect of the initial Ca^{2+} distribution within the cell on the form and frequency of induced Ca^{2+} oscillations. It takes into account intracellular Ca^{2+} stores such as sarcoplasmic reticulum and cytosolic proteins as well as Ca^{2+} exchange across the plasma membrane. We are able to demonstrate a closer agreement of model predictions with observed Ca^{2+} traces for a significantly wider range of parameter values, as was previously reported. We show also that the total cellular Ca^{2+} content is an important system parameter especially because of the content in sarcoplasmic reticulum. At a total Ca^{2+} increase of about 20%, the oscillation frequency increases by 25%; also, damped oscillations become sustained. Cases are indicated in which such a situation could occur.

Key words: calcium oscillations, airway smooth muscle cells, mathematical modelling, sarcoplasmic reticulum

1. Introduction

A large variety of eukaryotic cell types display oscillations of cytosolic Ca^{2+} concentration [1]. A central process in the initiation of these oscillations is the release of Ca^{2+} ions from the sarcoplasmic or endoplasmic reticulum to the cytosol. The Ca^{2+} release may occur via channels sensitive to inositol 1,4,5-trisphosphate (IP_3) known as IP_3 receptor (IP_3R) channels [1]. The opening of the IP_3R channels is triggered by IP_3 and Ca^{2+} when they are bound to the respective binding sites of the channel, whereas Ca^{2+} concentration in the vicinity of the channel controls the open-probability of the channel. As both Ca^{2+} and IP_3 are required for opening the channel, this mechanism is named Ca^{2+} -and- IP_3 -induced Ca^{2+} release (CIICR) [2]. On the other hand, Ca^{2+} can be released through the channels sensitive only

to Ca^{2+} itself; these channels are termed ryanodine receptor (RyR) channels and the corresponding transport mechanism is known as Ca^{2+} -induced Ca^{2+} release (CICR) [3].

In the airway smooth muscle cells, both types of Ca^{2+} channels are expressed in the membrane of sarcoplasmic reticulum (SR). For example, stimulation of the cells with caffeine triggers Ca^{2+} release from the SR through the RyR channels; however, it gives a non-oscillatory response [4]. Calcium oscillations in airway smooth muscle cells are commonly linked to the production of the intracellular second messenger IP_3 and, concomitantly, to the release of Ca^{2+} from the SR through the IP_3R channels [5–7]. Metabolite IP_3 is produced after an agonist action on the cell receptors coupled with G-protein which is later transferred to a rapid breakdown of phosphatidyl inositol 4,5 biphosphate (PIP_2) under the influence of phospholipase C (PLC). Release of IP_3 is then followed by Ca^{2+} mobilization from the SR. The harmonic interplay between the mechanisms of Ca^{2+} release, binding of Ca^{2+} to proteins, and pumping back into the SR may result in Ca^{2+} oscillations. An increase in cytosolic free Ca^{2+} is followed by the $\text{Ca}^{2+}/\text{CaM}$ dependent activation of myosin light chain kinase (MLCK) that triggers phosphorylation of myosin light chains (MLC). This results in an increase in myosin ATPase activity and, consequently, in cross-bridge cycling. The extent of phosphorylated MLC (MLC-P) and, hence, the amplitude of force production depends on the balance of the activities of MLCK and myosin light chain phosphatase (MLCP). As activity of MLCK is coupled to Ca^{2+} signalling pathway, force production is Ca^{2+} dependent as well. However, the relation between force, MLC phosphorylation and intracellular free Ca^{2+} concentration is not unique because both MLCK and MLCP are targets of intracellular signalling irrespective of changes in Ca^{2+} [8]. Although smooth muscle tone appears to be dependent on a network of several activating and inhibiting intracellular signals, the Ca^{2+} signal is still one of the most important ones as it can be efficiently modulated by the amplitude and the frequency of oscillations [1, 8]. Basic elements of excitation-contraction coupling in airway smooth muscle cells mediated by Ca^{2+} are schematically presented in Figure 1.

Stimulation of the cell with acetylcholine (ACh) is a typical example where a signalling pathway occurs via IP_3 production; moreover, ACh is also a common agonist for the initiation of Ca^{2+} oscillations in airway smooth muscle cells [5, 6]. Experiments show a typical oscillatory pattern where the first spike with higher amplitude is followed by several smaller spikes. The frequency of oscillations changes with the agonist level and might be from 4 to 15 spikes/minute at low and at high ACh concentration, respectively [7, 10, 11]. The amplitude of the first spike is around $1\ \mu\text{M}$, whereas the amplitude of the successive spikes is around $0.4\ \mu\text{M}$ [7, 10, 11]. Recent experimental data show that ACh-induced Ca^{2+} oscillations in airway smooth muscle cells are responsible for sustained muscle contraction and the associated reduced airway calibre [7]. These authors also hypothesise that the

Figure 1. Schematic presentation of Ca^{2+} mediated pathway from excitation to contraction in the airway smooth muscle cells.

magnitude of the sustained contraction may be regulated by the frequency of Ca^{2+} oscillations.

The aim of the present work is to study the influence of Ca^{2+} overload in the cell that may occur after the blockade of the plasma membrane NaK–ATPases. The idea arose after the presentation of a new conception of micro domains of junctional space between the plasma and the SR membrane in the smooth muscle cells [12, 13] that bears the hypothesis of loading of the SR with Ca^{2+} , whereas mean cytosolic Ca^{2+} concentration remains almost unaffected [14]. Our intention was to simulate the initial redistribution by using a simplified mathematical model and then to observe the influence of the overload on the frequency and the amplitude of Ca^{2+} oscillations. For this purpose we constructed a mathematical model which takes into account calcium exchange between intra- and extracellular space as well as calcium exchange between intracellular stores. All important calcium fluxes across the plasma and SR membrane and the binding to cytosolic proteins are included; however, some of them are given in a very simplified form. We analysed different parameter values to gain better agreement with the experimentally observed Ca^{2+} oscillations within a broad range of stimulation. We showed that the calcium overload increases the frequency of oscillations significantly. Furthermore, the overload can change the dynamic behaviour of the system.

2. Mathematical Model

Transport and binding processes of Ca^{2+} in airway smooth muscle cells which are taken into account by the mathematical modelling of cytosolic Ca^{2+} concentration are schematically presented in Figure 2. The model cell is an open system with respect to the exchange of Ca^{2+} between the cytoplasm and the extracellular medium across the plasma membrane. In a non-oscillating situation the resting Ca^{2+}

Figure 2. Schematic presentation of the model of the airway smooth muscle cell. Fluxes and concentrations of Ca^{2+} considered in the mathematical model are denoted by the same symbols as in the model equations. SR is the sarcoplasmic reticulum, SERCA is the sarco-/endo-plasmic reticulum Ca^{2+} ATPase and PMCA is the plasma membrane Ca^{2+} ATPase.

influx is counterbalanced by the Ca^{2+} effluxes mediated by the plasma membrane Ca^{2+} -ATPase (PMCA) and $\text{Na}^+/\text{Ca}^{2+}$ exchanger, whereas sodium and potassium ions are not taken explicitly into consideration. The sarcoplasmic reticulum (SR) and the Ca^{2+} binding cytosolic proteins serve as intracellular Ca^{2+} stores. The Ca^{2+} fluxes across the SR membrane, the leak, the influx mediated by the SR Ca^{2+} -ATPases (SERCA), and the efflux through the IP_3R channel are the fluxes already considered by the mathematical model of Haberichter *et al.* [2].

A basic framework of the mathematical model consists of four first-order ordinary differential equations (1)–(4) describing the time evolution of the system variables. These are free cytosolic Ca^{2+} concentration (Ca_{cyt}), free Ca^{2+} concentration in the SR (Ca_{SR}), concentration of the free binding sites for Ca^{2+} on proteins (Pr) as well as the quantity h , which has a meaning of a probability of opening the IP_3R channel with respect to IP_3 , and cytosolic Ca^{2+} concentration:

$$\frac{d\text{Ca}_{\text{cyt}}}{dt} = -J_{\text{SERCA}} + J_{\text{leak}} - J_+ + J_- + J_R - J_{\text{PMCA}} + J_{\text{in}} - J_o \quad (1)$$

$$\frac{d\text{Ca}_{\text{SR}}}{dt} = \frac{\beta_{\text{SR}}}{\rho_{\text{SR}}} (J_{\text{SERCA}} - J_R - J_{\text{leak}}) \quad (2)$$

$$\frac{d\text{Pr}}{dt} = J_- - J_+ \quad (3)$$

$$\frac{dh}{dt} = q_1 \frac{I P_3 + q_2}{I P_3 + q_3} (1 - h) - q_4 h \text{Ca}_{\text{cyt}}. \quad (4)$$

In addition, the total concentration of Ca^{2+} binding sites on the protein is conserved:

$$Pr_{\text{tot}} = Pr + CaPr, \quad (5)$$

where the first and the second term denote concentration of Ca^{2+} free and bound sites, respectively.

The Ca^{2+} fluxes considered in the model equations (1)–(3) are explained in the following section.

J_R is the Ca^{2+} flux through the IP_3R channel:

$$J_R = k_{\text{dens}} c_R \frac{IP_3^2}{d_R^2 + IP_3^2} \frac{Ca_{\text{cyt}}^3}{(K_{\text{diss}} + Ca_{\text{cyt}})^3} h^3 (Ca_{\text{SR}} - Ca_{\text{cyt}}), \quad (6)$$

where k_{dens} reflects the overall density of the IP_3R channel in the SR membrane and c_R the permeability of the channel.

J_{SERCA} is the Ca^{2+} flux mediated by the ATP-dependent Ca^{2+} pumps across the SR membrane with the corresponding rate constant k_{SERCA} :

$$J_{\text{SERCA}} = k_{\text{SERCA}} \frac{Ca_{\text{cyt}}^2}{d_{\text{SERCA}}^2 + Ca_{\text{cyt}}^2}. \quad (7)$$

J_{leak} is the passive Ca^{2+} -leak flux from the SR into the cytosol with the corresponding rate constant k_{leak} :

$$J_{\text{leak}} = k_{\text{leak}} (Ca_{\text{SR}} - Ca_{\text{cyt}}). \quad (8)$$

J_+ and J_- are the Ca^{2+} fluxes of Ca^{2+} binding to and releasing from protein binding sites with rate constants k_+ and k_- , respectively:

$$J_+ = k_+ Ca_{\text{cyt}} \cdot Pr, \quad (9)$$

$$J_- = k_- CaPr. \quad (10)$$

The binding and release of Ca^{2+} to a single protein-binding site is assumed as a simple bimolecular reaction.

J_{PMCA} is the Ca^{2+} flux mediated by the ATP-dependent Ca^{2+} pumps across the plasma membrane with the corresponding rate constant k_{PMCA} :

$$J_{\text{PMCA}} = k_{\text{PMCA}} \frac{Ca_{\text{cyt}}^2}{d_{\text{PMCA}}^2 + Ca_{\text{cyt}}^2}. \quad (11)$$

Table I. The model parameters for oscillatory response in reference state

Symbol	Description	Value
Pr_{tot}	Total concentration of cytosolic proteins	100 μM
k_{dens}	Overall density of the IP_3R channels	130 s^{-1}
IP_3	IP_3 concentration	5 μM
ρ_{SR}	Volume ratio between the SR and the cytosol	0.01
β_{SR}	Ratio of free vs. total Ca^{2+} in the SR	0.0025
k_{SERCA}	Rate constant of the SERCA pumps	3.5 s^{-1}
d_{SERCA}	Half saturation for Ca^{2+} on SERCA pumps	0.1 μM
k_{leak}	Rate constant for leak	0.1 s^{-1}
c_{R}	Open probability of the IP_3R channels	0.03
d_{R}	Half saturation for IP_3 on the IP_3R channels	0.5 μM
K_{diss}	1/8 saturation constant for Ca^{2+} on the IP_3R	0.08 μM
q_1	Kinetic constant determining IP_3R inhibition	0.2048 μM^{-1}
q_2	Kinetic constant determining IP_3R inhibition	0.13 μM^{-1}
q_3	Kinetic constant determining IP_3R inhibition	0.9434 μM^{-1}
q_4	Kinetic constant determining IP_3R inhibition	0.2 μM^{-1}
k_+	On-rate constant for Ca^{2+} binding to proteins	0.015 $\mu\text{M}^{-1}\text{s}^{-1}$
k_-	Off-rate constant for Ca^{2+} binding to proteins	0.08 s^{-1}
k_{PMCA}	Rate constant of the PMCA pumps	0.5 s^{-1}
d_{PMCA}	Half saturation for Ca^{2+} on PMCA pumps	0.38 μM
J_{in}	Resting Ca^{2+} influx across the PM	0.12 μMs^{-1}
J_0	Ca^{2+} efflux through the $\text{Na}^+/\text{Ca}^{2+}$ exchangers	0.11 μMs^{-1}

J_{in} is the resting Ca^{2+} influx across the plasma membrane from the extracellular medium into the cytosol and is considered as constant. This assumption is in accordance with some previous models of Ca^{2+} oscillations (for a review see [9]).

J_0 is the Ca^{2+} efflux across the plasma membrane from the cytosol into the extracellular medium mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. This flux is also considered as constant ensuring the steady state of the cell.

The model parameters and their values are presented in Table I. The system of differential equations was solved numerically. First, the simulation was performed for a non-stimulated cell, which means that we take IP_3 concentration to be zero. After some time (in simulation we took 30 sec) the action of acetylcholine to stimulate Ca^{2+} oscillations was modelled by a step-wise increase of IP_3 concentration which then remains constant.

3. Model Predictions and Discussion

The mathematical model of Ca^{2+} oscillations in airway smooth muscle cells is simple in its description of Ca^{2+} fluxes across the plasma membrane. The model is

applied to two different cases. The first deals with a stimulated cell and consequent appearance of Ca^{2+} oscillations as a reference to the second case, in which the total cellular Ca^{2+} content increased before stimulation. Results and discussion are presented below with respect to these two cases.

It is worthwhile to compare our model with the model of Ca^{2+} oscillations in airway smooth muscle cell proposed by Haberichter *et al.* [2]. The latter is a closed model with respect to Ca^{2+} , as no exchange with extracellular medium is included in the model. In this closed model, Ca^{2+} oscillations appear due to Ca^{2+} exchange between cytosol, SR and the Ca^{2+} binding proteins, whereby a close inspection of the model shows that the bell-shaped IP_3 induced Ca^{2+} release from the SR and the oppositely directed Ca^{2+} pump are the basic nonlinearities necessary for the occurrence of Ca^{2+} oscillations. Although it has been hypothesised that the oscillatory response requires the presence of receptors that are inhibited at high Ca^{2+} , this is not a mathematical necessity. It is widely accepted that there exist three isoforms of IP_3R channels, labelled as type 1, 2 and 3 [15]. The main differences between them are in their gating kinetics and thus in their open probabilities with respect to the concentrations of IP_3 and Ca^{2+} [16, 17]. There is opposing evidence showing which isoform is non-inhibitory [17], and furthermore, it is not clear which isoforms are expressed to a larger extent in the airway smooth muscle cells [6]. It has been also hypothesised that IP_3R of type 1 is the actual molecular basis of Ca^{2+} oscillations and that the oscillating pattern of Ca^{2+} signalling in smooth muscle cells is related to the relative expression of three different isoforms in these cells [6]. These are the main reasons why we omitted the non-inhibitory type of IP_3R channel from our modelling and took into account only one type of IP_3R channels.

Moreover, a detailed examination and analysis of the model presented by Haberichter *et al.* [2] shows that the pattern of Ca^{2+} oscillations is far from experimentally observed oscillations, especially at larger IP_3 concentrations. That is mostly due to a plateau, i.e. the very slow and almost linearly decreasing cytosolic Ca^{2+} concentration that appeared at IP_3 concentrations of about $5.5 \mu\text{M}$ after the first spike and lasted approximately 100 s before sustained oscillations began. Extension of the plateau strongly depends on IP_3 concentration and can last for 200 s at IP_3 $30 \mu\text{M}$.

The complete list of the model parameters also shows some other differences in the values chosen in comparison to those used by the model of Haberichter *et al.* [2]. The main differences are, roughly speaking, in faster exchange of Ca^{2+} with Ca^{2+} binding proteins, higher total concentration of proteins in the cytosol, higher SR Ca^{2+} -ATPase activity and larger IP_3R channel density. In particular, the model parameters k_+ and k_- were studied carefully, searching for those values which enabled Ca^{2+} to oscillate without a plateau in the first spike. We achieved this within a range of IP_3 concentration which significantly exceeds reported values; in vascular smooth muscles IP_3 concentration between 0.5 and $30 \mu\text{M}$ was observed to cause Ca^{2+} release and concomitant creation of tension [18]. Since the relevant IP_3 concentration for action of IP_3 as a second messenger is difficult to demonstrate, the

Figure 3. Oscillations of Ca^{2+} concentration in SR (Ca_{SR}) (above) and oscillations of cytosolic Ca^{2+} concentration (Ca_{cyt}) (below) calculated by the model. Oscillations in the reference case with model parameters values given in Table I (dashed lines), oscillations in the case of Ca^{2+} overload with parameter values as in Table I except $J_0 = 0$ (solid lines). The arrow indicates the simulation of initiation of the agonist action; in numerical integration, from this time on $\text{IP}_3 = 5 \mu\text{M}$.

above broadening of the parameter value space for obtaining the oscillations with respect to IP_3 concentration should be viewed as an improvement in mathematical modelling of Ca^{2+} oscillations in airway smooth muscle cells.

The oscillations predicted by our model are presented in Figure 3. The parameter values are those listed in Table I. IP_3 concentration of $5 \mu\text{M}$ was applied to simulate the initiation of the agonist action, and damped Ca^{2+} oscillations appear (dashed lines in Figure 3). The initial Ca^{2+} concentrations in the SR and in the cytosol are approximately 5 and $0.05 \mu\text{M}$, respectively. The frequency of oscillations is approximately 9 min^{-1} . This is in accordance with the frequency observed in the airway smooth muscle cells [7, 10].

The mathematical model was applied to studies of the possible effect of an increase of Ca^{2+} content in SR on Ca^{2+} oscillations. An increase of Ca^{2+} concentration in SR also means a corresponding increase in the total cell Ca^{2+} . This overload is simulated to appear in the model before cytosolic Ca^{2+} oscillations are initiated. Such a case could occur after a blockade of the plasma membrane NaK-ATPases. Consequently, sodium concentration in the cell increases and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger is inhibited, and Ca^{2+} fluxes across the plasma membrane are then shifted from the counterbalance attained in the resting state. In the model, this situation is simulated simply by omitting the Ca^{2+} efflux J_0 from consideration. Without changing any values of the other model parameters, the model predictions show that the capability of transporting Ca^{2+} into SR by SERCA is such that neither cytosol nor the cytosolic proteins can compete for increased Ca^{2+} influx. In the simulation, we terminated the Ca^{2+} overload after the Ca^{2+} increase in the SR reached 20% of the total cell Ca^{2+} content. Then, oscillations were initiated by applying

IP_3 concentration of $5 \mu\text{M}$ as is described in the previous section. The complete time course of Ca^{2+} loading into SR as well as of oscillating Ca^{2+} are given in Figure 3 (solid lines). It is clearly shown that during the time SR is being loaded the cytosolic Ca^{2+} is not elevated; the same is predicted for Ca^{2+} binding proteins (not shown). An increase of frequency of oscillations upon Ca^{2+} overload in SR was simulated by the model. In the case treated, the 20% overload elevates the frequency by about 25%. The amplitude of the spikes does not change significantly, but on the other hand, the mean Ca^{2+} content in the cytosol is evidently increased, as the concentration minima are higher in comparison to the reference oscillations. Furthermore, it is interesting to observe that the calcium overload changes the dynamic characteristics of the system; namely, in this case, oscillations become sustained after a few spikes. This result suggests that the total Ca^{2+} content in the cell, and especially in the SR, might be of great importance for the occurrence of sustained oscillations.

As concluding remarks, the present model does not take into account diffusion of Ca^{2+} through the cytosolic space which should be an important feature of a mechanism of Ca^{2+} overload in SR. There are hypotheses of Ca^{2+} entry from extracellular space into the SR across the microdomains of the junctional space between the two membranes [14]. There is also evidence referring to arterial smooth muscles that high ouabain affinity isoforms of Na^+ pumps located in the plasma membrane at the site where the SR and plasma membranes are in close proximity, and that these isoforms may regulate the local concentration of sodium and, indirectly, Ca^{2+} in these regions [12, 13, 19]. We must point out that this evidence has not been directly obtained in airway smooth muscle cells for which our mathematical model was designed. However, studies of ouabain inhibitory action on sodium pumps in airway smooth muscle cells may give an insight in to relations between the Ca^{2+} overload and cytosolic Ca^{2+} oscillations and eventual muscle contraction in smooth muscle cells.

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