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The influence of different $InsP_3$ receptor isoforms on Ca^{2+} signaling in tracheal smooth muscle cells

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Abstract

In airway myocytes, like in many cells, Ca^{2+} signaling is controlled by inositol 1,4,5-trisphosphate ($InsP_3$) via $InsP_3$ receptors ($InsP_3R$) located in the sarco–endoplasmic reticulum. Three types of $InsP_3R$ exist, labeled Types 1, 2, and 3, which differ in their gating kinetics. We analyze a possible impact of the different gating kinetics of Type 1 and Type 3 $InsP_3R$ on the time course of cytosolic Ca^{2+} concentration in tracheal smooth muscle cells upon agonist stimulation. Previous experimental data in rat tracheal myocytes showed that upon gradually increased stimulation with acetylcholine (ACh), a contractile agonist that acts via $InsP_3$ production, signal spikes, several spikes with declining maxima, and sustained oscillations appear. Our model reproduces the time courses of cytosolic Ca^{2+} measured in tracheal myocytes. Moreover, by postulating slight variations in the model parameters which determine the total number of receptors expressed and the ratio between Type 1 and Type 3 $InsP_3R$, it offers an explanation to the experimental observation of qualitatively different responses of cells within a presumably homogeneous tissue.

Keywords: $InsP_3$ receptor subtypes; Ca^{2+} oscillations; Smooth muscle cells

1. Introduction

In many cells, including smooth muscle cells, Ca^{2+} signaling is controlled by inositol 1,4,5-trisphosphate ($InsP_3$) via $InsP_3$ receptors ($InsP_3R$), Ca^{2+} channels located in the sarco- or endoplasmic reticulum membrane [1]. The opening of this channel depends on Ca^{2+} and $InsP_3$ binding to its respective binding sites, which is referred to as Ca^{2+}-induced Ca^{2+} release (CICR) and $InsP_3$-induced Ca^{2+} release (IICR), respectively. We prefer to stress the fact that there is actually only one process which is dependent on both Ca^{2+} and $InsP_3$ by using the term Ca^{2+}- and $InsP_3$-induced Ca^{2+} release (CIICR). When stimulated by $InsP_3$ or by agonists acting via $InsP_3$ production, cells exhibit a variety of Ca^{2+} response patterns. Stimulation may result in a biphasic response, i.e. a single transient Ca^{2+} increase, followed by a steady-state phase, during which cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) remains stable above the resting $[Ca^{2+}]_{cyt}$ value. In cells with adequate physiological properties, sustained oscillations also may occur upon stimulation. Under other conditions, the Ca^{2+} concentration rests at a low level, but a small increase in $[Ca^{2+}]_{cyt}$ triggers a large pulse of $[Ca^{2+}]_{cyt}$. This property is known as Ca^{2+} excitability [2–4] and is responsible for the propagation of Ca^{2+} waves at sub-, intra-, and intercellular levels [5].

There is no doubt that at least three types (isoforms) of $InsP_3R$ exist, labeled Types 1, 2, and 3 ($InsP_3R1$, $InsP_3R2$, and $InsP_3R3$) [6–9]. They differ in their gating kinetics, i.e. in their opening probability, which depends on cytosolic Ca^{2+} and $InsP_3$ concentrations [10–15]. All receptor subtypes are activated by Ca^{2+} in the cytoplasm at concentrations higher than about 0.05 μM. Only Type 1 receptor is inactivated by $[Ca^{2+}]_{cyt}$ of more than 0.3 μM, whereas Types 2 and 3 receptors stay open at high $[Ca^{2+}]_{cyt}$.

The physiological significance of the expression of different receptor subtypes remains largely unknown, although

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recent experiments on genetically engineered B cells have shown that cells expressing different choices of $InsP_3R$ subtypes react differently to stimulation by Ca^{2+}, $InsP_3$, and ATP [15]. It has been hypothesized that the oscillatory response of cells requires the presence of receptors that are inhibited at high Ca^{2+}, thus Type 1 $InsP_3$ receptors. However, this is not a mathematical necessity. Several theoretical models may explain calcium oscillations without an inhibitory effect on $InsP_3Rs$ [3,16–18]. This should, in principle, allow to observe calcium oscillations in tissues (or cells) that express only noninhibited $InsP_3R2$ and/or $InsP_3R3$ or different fractions of $InsP_3R1$. It should be expected that the characteristics of calcium oscillation (frequency and amplitude) will depend upon the ratio between Ca^{2+}-inhibited and -noninhibited $InsP_3$ receptors.

2. Experimental evidences

We have recently published results of experiments done in freshly isolated tracheal smooth muscle cells [19], in which individual cells were stimulated with graded concentrations of acetylcholine (ACh). Fig. 1 shows examples of typical cellular responses to such stimulation: upon weak stimulation, a transient increase of $[Ca^{2+}]_{cyt}$ is followed by a stable state at an elevated level of Ca^{2+} concentration. Then damped oscillations with two, three, or even more spikes of increased Ca^{2+} are observed. At rather high $[Ca^{2+}]_{cyt}$, some cells show an initial phase of oscillations with declining amplitudes, followed by sustained oscillations with lower amplitudes (not shown). The oscillation frequency increases with the agonist concentration [19]. Such oscillat-

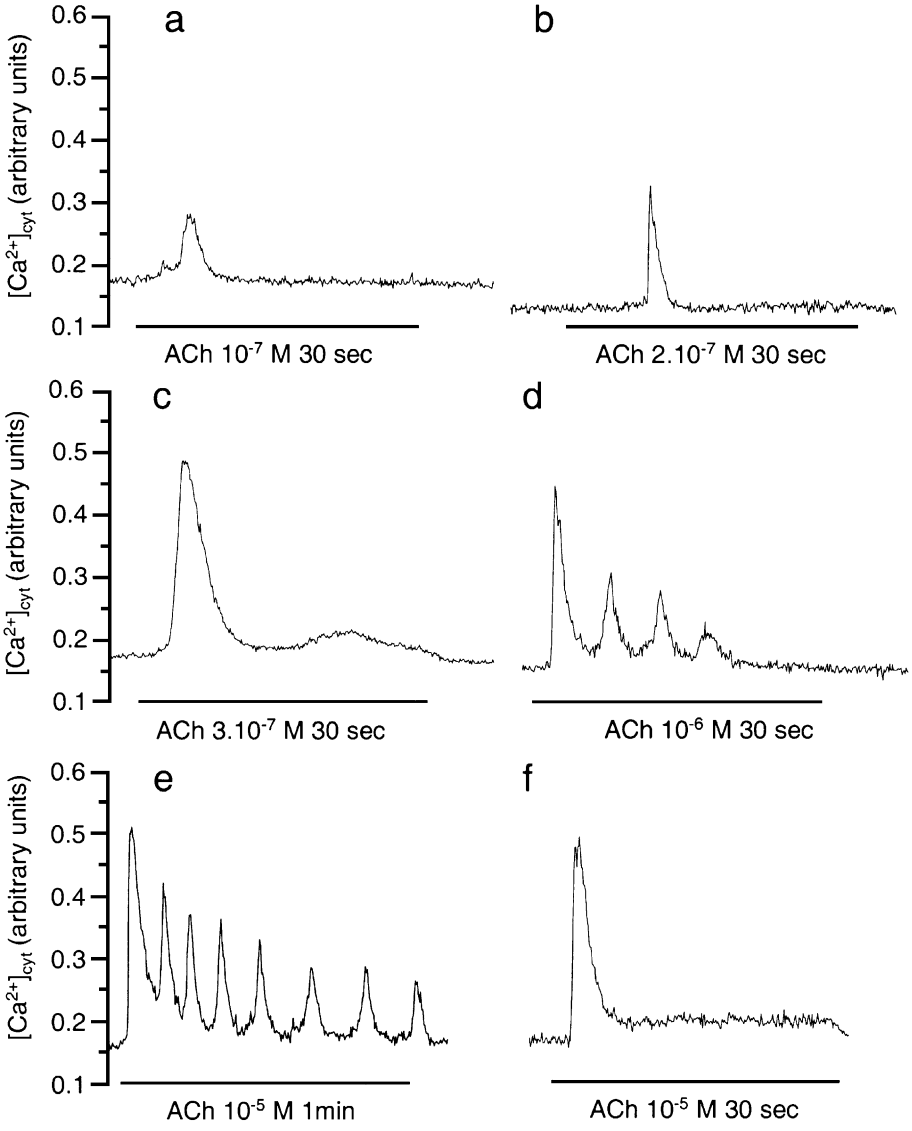

Fig. 1. Examples of typical cellular Ca^{2+} responses to stimulation with acetylcholine at (a) 10^{-7} M, (b) 2.10^{-7} M, (c) 3.10^{-7} M, (d) 10^{-6} M, and (e and f) 10^{-5} M. Note that trace (e) is a typical oscillatory response, whereas trace (f) is non-oscillatory despite of the same intensity of stimulation.

ing patterns have been observed in airway myocytes in response to various agonists acting via G-protein-coupled receptors and InsP3 production in a variety of species [20–22]. In our measurements, several cells under identical experimental conditions did not show the same responses on agonist stimulation. Different levels of stimulation were needed for different cells to oscillate. Some cells did not oscillate even upon very strong stimulation. Plotting the fraction of oscillating cells with respect to agonist concentration gives a Michaelis–Menton-like curve, with a maximum around 60% [19]. We showed that the Ca^{2+} response pattern, including the occurrence of sustained Ca^{2+} oscillations, did not depend on extracellular Ca^{2+} influx, but on the release of intracellular calcium involving $InsP_3$ receptors [19].

The question arises on how cells of an identical cell type from the same tissue, i.e. the tracheal smooth muscle, in which Ca^{2+} responses involve largely identical structure, can show such different dynamical behaviors. One possibility is that we have actually measured two subpopulations of cells with different physiological properties. However, one should consider that even a homogenous biological population is characterized by interindividual variations. Our hypothesis is that one or more parameters, which have a crucial role on the mechanisms involved in the generation of Ca^{2+} oscillations, may differ slightly within a cell population so that slight quantitative changes between cells in these parameters may lead to qualitatively and quantitatively different behaviors. An obvious candidate to account for the various Ca^{2+} responses lies in the ratio of different $InsP_3R$ subtypes. This assumption is strongly supported by the central role of $InsP_3$ receptors in the dynamics of Ca^{2+} concentration and by the essentially different gating properties of the different subtypes, especially the inhibition of only $InsP_3R1$ by high $[Ca^{2+}]_{cyt}$. In addition, a varied overall density of receptors expressed may change the Ca^{2+} response pattern significantly. The following study has been done to examine the sensitivity of Ca^{2+} dynamics resulting from changes in these quantities in order to determine whether such a mathematical model may account for the characteristics of Ca^{2+} response of airway myocytes, i.e. the characteristics of the various response patterns and the respective proportion of cells exhibiting them, depending on the level of cell stimulation.

3. Mathematical model

The model we propose is sketched in Fig. 2. It describes the exchange of Ca^{2+} between the cytosol, the sarcoplasmic reticulum (SR), and buffering protein-binding sites in airway smooth muscle cells. The origin for the buffering is the ligands to which Ca^{2+} binds. Examples are Ca^{2+}-binding proteins, such as calreticulin and calsequestrin, but also certain phospholipids, and virtually any compound containing oxyanionic groups [23]. As in Goldbeter's models [3,4], Ca^{2+} is pumped into the SR by sarco- or endoplasmic

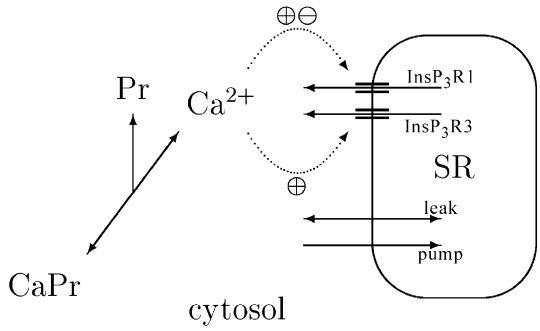

Fig. 2. Schematic presentation of the model system.

reticulum ATPase (SERCA) pumps and released back into the cytosol through Ca^{2+} channels, which are governed by a CIICR mechanism. We also include Ca^{2+} binding proteins in the same way as it has been done before in various models [24,25]. In this first approach, we do not take exchange of Ca^{2+} with the extracellular medium into account. This is supported by our experimental results showing that extracellular calcium influx is not directly involved in Ca^{2+} signaling in the cell types analyzed here. Furthermore, there is no experimental evidence of any mitochondrial significance in the calcium-signaling pathway in airway smooth muscle cells; thus, we also neglect calcium uptake and release by mitochondria. We wished to limit the number of equations in our model in order to bring out the mathematical mechanisms necessary to explain our observations. Pursuing the idea of the great influence of the expression of different receptor subtypes, we concentrated our study on the SR membrane processes and, more particularly, on the release of Ca^{2+} from the SR through $InsP_3$ receptor Ca^{2+} channels.

For the flux of Ca^{2+} through these channels, we assumed that both Ca^{2+}-inhibited (Type 1) and Ca^{2+}-noninhibited (Type 3) $InsP_3$ receptors are present in the cell, with a given ratio. We omit in this first approach Type 2 receptors, which have a similar gating kinetics as $InsP_3R3$, i.e. no inhibitory effect at high $[Ca^{2+}]_{cyt}$ on the open probability. Thus, we do not expect significant qualitative differences in the results in case of the inclusion of $InsP_3R2$. The limitation to only two receptor types, one with and one without inhibition, allows us to emphasize the model predictions based on the variety of $InsP_3R$ subtypes variability.

In our model, the cytosolic and reticular Ca^{2+} concentrations, which we denote as Ca_{cyt} and Ca_{SR}, respectively, are changed according to the following differential equations:

$$\frac{dCa_{cyt}}{dt} = -J_{SERCA} + (1-r)J_{R1} + rJ_{R3} + J_{leak} + k_{-}CaPr - k_{+}Ca_{cyt}Pr \quad (1)$$

$$\frac{dCa_{SR}}{dt} = \frac{\beta_{SR}}{\rho_{SR}}(J_{SERCA} - (1-r)J_{R1} - rJ_{R3} - J_{leak}) \quad (2)$$

Here, the parameter r stands for the fraction of Type 3 receptors expressed, with respect to the total number of receptors. The concentrations of free and occupied sites on buffering proteins are denoted as Pr and CaPr, respectively. These variables are not independent because exchange of Ca^{2+} and buffering proteins with the extracellular medium is neglected; hence, conservation relations apply for the concentration of total cellular calcium, Ca_{tot},

$$Ca_{tot} = Ca_{cyt} + \frac{\rho_{SR}}{\beta_{SR}} Ca_{SR} + CaPr, \tag{3}$$

and for the total concentration of protein-binding sites, Pr_{tot},

$$Pr_{tot} = Pr + CaPr. \tag{4}$$

With k_- and k_+, the off- and on-rate constants are addressed. The parameter ρ_{SR} represents the ratio of the reticular with respect to the cytosolic volume. A constant factor β_{SR} describes the effect of buffering in the SR.

For the Ca^{2+} uptake into the SR, J_{SERCA}, we assume a sigmoid dependency on cytosolic Ca^{2+}, following the approach proposed by Lytton et al. [26],

$$J_{SERCA} = k_{SERCA} \frac{Ca_{cyt}^2}{d_{SERCA}^2 + Ca_{cyt}^2}. \tag{5}$$

In this equation, k_{SERCA} is the maximal rate of the ATPase and d_{SERCA} represents the half-saturation for Ca^{2+}.

The flux through Type 1 $InsP_3$ receptor channels is determined by the expression

$$J_{R1} = k_{dens} c_{R1} \frac{P^2}{d_{R1}^2 + P^2} \frac{Ca_{cyt}^3}{(K_{diss} + Ca_{cyt})^3} h^3 (Ca_{SR} - Ca_{cyt}). \tag{6}$$

Basically, Eq. (6) is identical to the expression of Li and Rinzel [2]. However, in accordance with the most recent experimental results for the activity of a single Type 1 receptor in dependence on $InsP_3$ concentration published by Moraru et al. [11], we take into account factor $c_{R1}P^2/({d_{R1}}^2 + P^2)$, with c_{R1} and d_{R1} being the maximal rate and the half-saturation for Ca^{2+}, respectively, and P stands for the $InsP_3$ concentration. The parameter k_{dens} reflects the total number of channels in the SR membrane. The sigmoid dependency on Ca_{cyt}, with the dissociation constant K_{diss}, corresponds to the instant activation of the channel, whereas the delayed inactivation, i.e. the inhibitory effect of $InsP_3R1$ at high concentrations of Ca^{2+}, is taken into account by an extra variable h. It is determined by the equation

$$\frac{dh}{dt} = q_1 \frac{P + q_2}{P + q_3}(1 - h) - q_4 h Ca_{cyt}. \tag{7}$$

The constants q_1, q_2, q_3, and q_4 intrinsically contain constants determined by the velocities of binding and unbinding of Ca^{2+} and $InsP_3$ to the receptor subunits. The derivation of the kinetics applied here from a physiological model of Ca^{2+} and $InsP_3$ binding to the receptor published by De Young and Keizer [27] is comprehensively explained in the work of Li and Rinzel [2].

The Type 3 $InsP_3$ receptor has a different gating kinetics than that of the $InsP_3R1$. First, there is no inactivation of the channel by high $[Ca^{2+}]_{cyt}$, which is taken into account for by omitting the respective variable h in the description of $InsP_3R1$ Ca^{2+} flux. Second, its activity has a different dependency on $InsP_3$. Here, the measurements of Hagar and Ehrlich [14] are the source of the maximal rate c_{R3} and half-saturation for Ca^{2+} d_{R3}. Copying the rest of the term from above (Eq. (6)), the $InsP_3R3$ flux reads

$$J_{R3} = k_{dens} c_{R3} \frac{P^2}{d_{R3}^2 + P^2} \frac{Ca_{cyt}^3}{(K_{diss} + Ca_{cyt})^3} (Ca_{SR} - Ca_{cyt}). \tag{8}$$

Experiments with thapsigargin have also unmasked a rather small Ca^{2+} leak from the SR that is independent of $InsP_3$

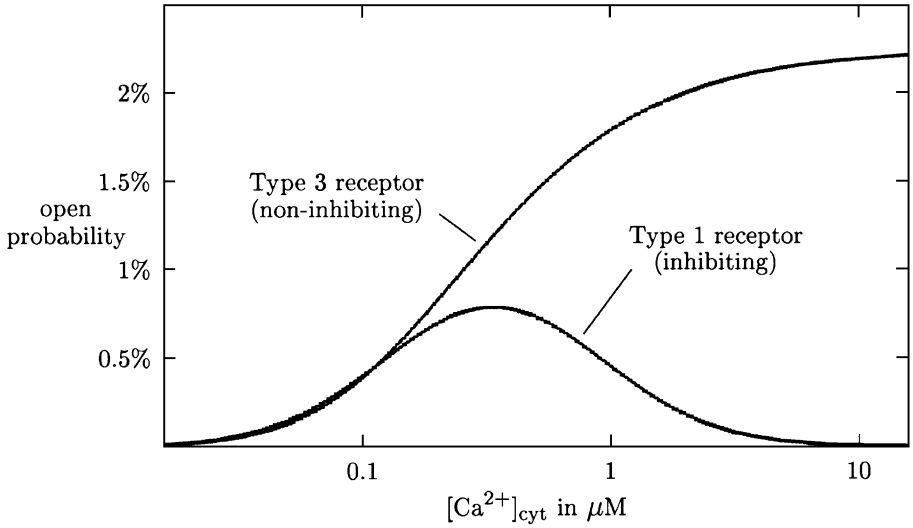

Fig. 3. Ca^{2+} fluxes through Types 1 and 3 $InsP_3$ receptors at equilibrium.

Table 1
Model parameters for which all results are calculated

Parameter		Value
Ca_{tot}	total cellular Ca^{2+} concentration	9 μM
Pr_{tot}	total concentration of cytosolic proteins	12 μM
k_{dens}	overall density of $InsP_3$ receptors in the SR membrane	(see text)
r	fraction of Type 3 $InsP_3$ receptors	(see text)
ρ_{SR}	volume ratio between the SR and the cytosol	0.01
β_{SR}	ratio of free Ca^{2+} to total Ca^{2+} in the SR	0.0025
P	concentration of $InsP_3$	(see text)
k_{SERCA}	rate constant of ATPases	0.9 s^{-1}
d_{SERCA}	half-saturation for Ca^{2+} of ATP pumps	0.1 μM
k_{leak}	rate constant of Ca^{2+} leak flux through the SR membrane	0.02 s^{-1}
c_{R1}	Ca^{2+} permeability of Type 1 receptor	0.03
c_{R3}	Ca^{2+} permeability of Type 3 receptor	0.08
d_{R1}	half-saturation for $InsP_3$ of Type 1 receptor	0.5 μM
d_{R3}	half-saturation for $InsP_3$ of Type 3 receptor	3.2 μM
K_{diss}	1/8 saturation for Ca^{2+} of Types 1 and 3 receptors	0.08 μM
q_1	kinetic constant determining $InsP_3R1$ inhibition	0.2048 μM^{-1}
q_2	kinetic constant determining $InsP_3R1$ inhibition	0.13 μM^{-1}
q_3	kinetic constant determining $InsP_3R1$ inhibition	0.9434 μM^{-1}
q_4	kinetic constant determining $InsP_3R1$ inhibition	0.2 μM^{-1}
k_+	on-rate constant of Ca^{2+} binding to proteins	0.005 μM^{-1} s^{-1}
k_-	off-rate constant of Ca^{2+} binding to proteins	0.0024 s^{-1}

Values for k_{dens}, r, and P are given in the text and in the figure captions.

activation [19,28]. In the model, this is taken into account by the term

$$J_{leak} = k_{leak}(Ca_{SR} - Ca_{cyt}), \quad (9)$$

with k_{leak} being the leak rate constant.

Assuming an equilibrium state, the Ca^{2+} release fluxes from the sarcoplasmic reticulum into the cytosol through the two types of $InsP_3$ receptors, J_{R1} and J_{R3}, show the bell-shaped ($InsP_3R1$) and sigmoid ($InsP_3R3$) dependencies on Ca_{cyt} depicted in Fig. 3, which correspond to the curves experimentally yielded by Moraru et al. [11] for $InsP_3R1$ and by Hagar and Ehrlich [14] for $InsP_3R3$.

4. Results

The model equations have been integrated numerically using the set of parameter values given in Table 1, but varying the parameters that stand for the overall density of receptors on the SR surface (k_{dens}), the fraction of channels that correspond to the kinetics of $InsP_3R3$ with respect to the total number of $InsP_3$ receptors (r), and the concentration of $InsP_3$ (P). Of these control parameters, k_{dens} and r represent the quantities which we assumed to vary among different cells. The behavior of these slightly varying cells upon various intensities of stimulation, expressed by the parameter P, was the focal point of our study.

Within the parameter region analyzed, each choice of parameters leads to only one steady state. The regions of stability and instability are separated by Hopf bifurcations which, in the subspace of the three bifurcation parameters, r, k_{dens}, and P, form two-dimensional surfaces. Fig. 4 shows projections of them, with the value of r fixed at 0.2 (solid lines) and 0.4 (dotted lines), respectively, i.e. Hopf bifurcation lines in different planes of P and k_{dens}. In our model, the Hopf bifurcations are rather close to the boundaries of limit cycle oscillatory behavior, which means that sustained oscillations are found between the Hopf bifurcation lines, and only there. This holds for a large region of pairs of k_{dens} and r, so in the following, we use the terms "region of (sustained)

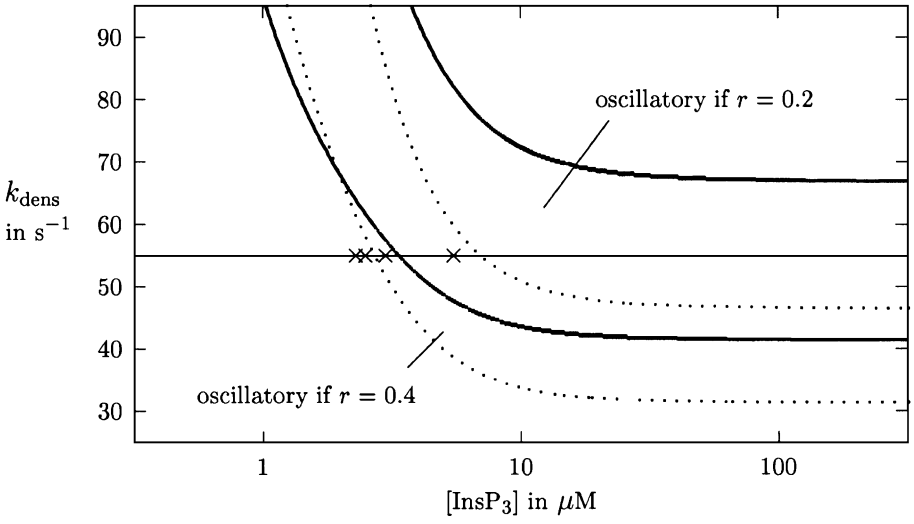

Fig. 4. Hopf bifurcation lines with respect to $InsP_3$ concentration (P) and overall receptor channel density (k_{dens}), with two different values for the ratio of differential receptor subtype expressions, $r = 0.2$ (solid lines) and $r = 0.4$ (dotted lines). The horizontal line corresponds to a constant $k_{dens} = 55\ s^{-1}$. The crosses represent parameter values for which the resulting Ca^{2+} time courses (with $r = 0.2$) are displayed in Fig. 5.

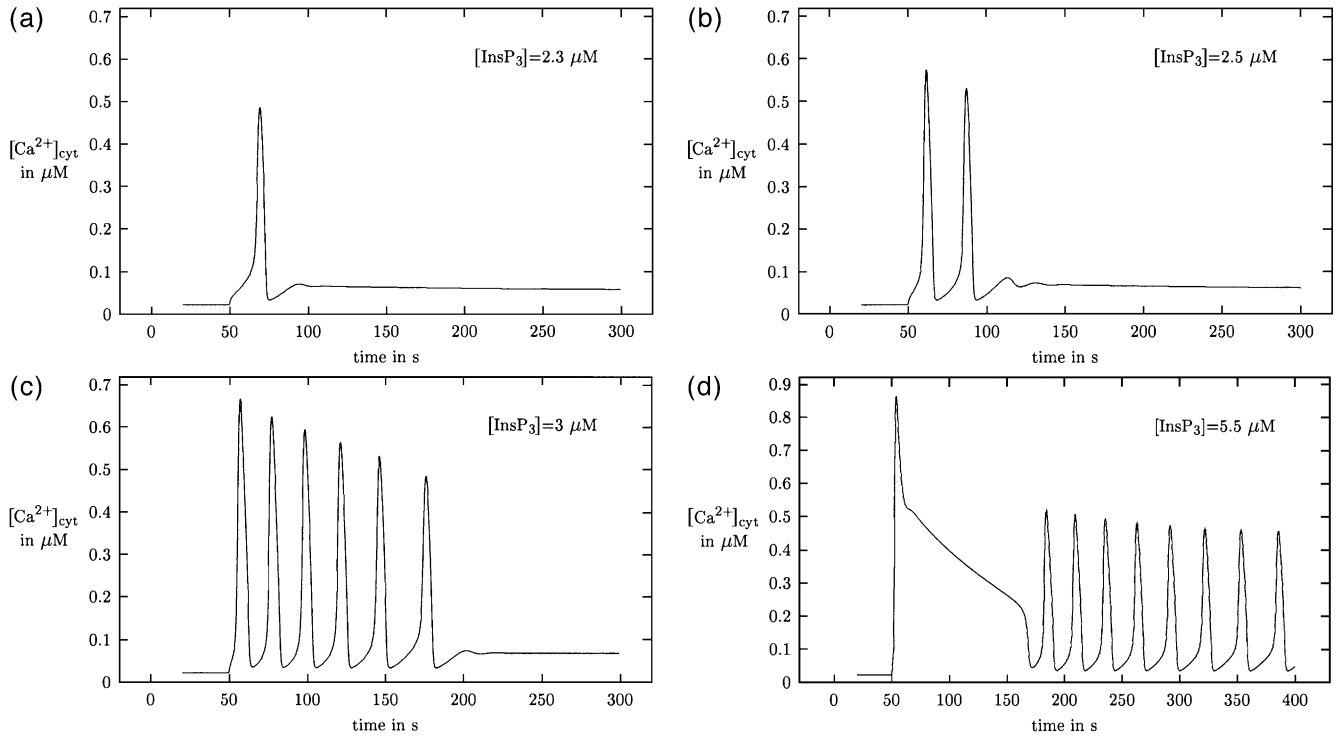

Fig. 5. Exemplary time courses of $[Ca^{2+}]_{cyt}$ upon $InsP_3$ stimulation of different intensities. (a) $[InsP_3]=2.3$ μM, (b) $[InsP_3]=2.5$ μM (c) $[InsP_3]=3$ μM, (d) $[InsP_3]=5.5$ μM.

oscillatory behavior" and "outside stable steady-state behavior" synonymously.

A closer look at Fig. 4 illustrates the different response patterns of model cells that differ in the quantities k_{dens} and r. The horizontal line in the diagram corresponds to a constant overall density of $InsP_3$ receptors, $k_{dens}=55\ s^{-1}$. If the system is calculated with $r=0.4$, which means 60% Type 1 and 40% Type 3 receptors, there is a transition from a stable focus, i.e. damped oscillations, to limit cycle, i.e. sustained oscillations, at $P=2.68$ μM. At $P=6.91$ μM, there is a transition back to a stable steady state at which $[Ca^{2+}]_{cyt}$ stays at a higher level than in the lower stable region. This $[InsP_3]$ dependency of the dynamical behavior of the Ca^{2+} concentration has been observed in a variety of cell types, but not in tracheal smooth muscle cells. There, some cells also show oscillations above a certain threshold of $InsP_3$ stimulation, but they retain this oscillatory behavior also at very high stimulation. Therefore, in the framework of this model, they rather correspond to a situation with either a lower r (lower fraction of $InsP_3R3$) or a lower

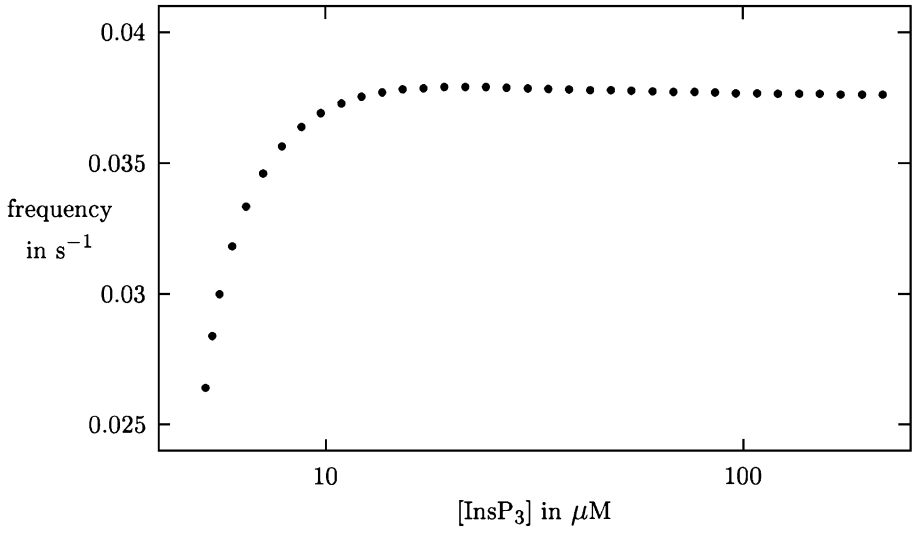

Fig. 6. Frequency of $[Ca^{2+}]$ spikes as a function of $InsP_3$ concentration in the region of sustained oscillations with $r=0.2$.

k_{dens} (lower overall receptor density). For example, for $k_{dens} = 55\ s^{-1}$ and $r = 0.2$, the system shows sustained oscillations for any $InsP_3$ concentrations higher than 3.36 μM; there is no transition back to stable steady-state behavior even at very high concentrations of $InsP_3$. Leaving $r = 0.4$, the receptor density would have to be lower than $k_{dens} = 31.4\ s^{-1}$ to explain the experimental observation that there is no transition to a stable steady state at high [$InsP_3$] in tracheal smooth muscle cells.

We have calculated the model equations with $r = 0.2$ and $k_{dens} = 55\ s^{-1}$ at different levels of $InsP_3$ concentration (P), which are marked by crosses on the horizontal line in Fig. 4. The transition between stable focus and sustained oscillation behaviors takes place at the intersection of this line with the solid curve, standing for Hopf bifurcations in the case of $r = 0.2$. In our calculations, we set $P = 0$ at the beginning, so the system was at rest. Then the $InsP_3$ concentration was set to a given fixed value, and the resulting time course of

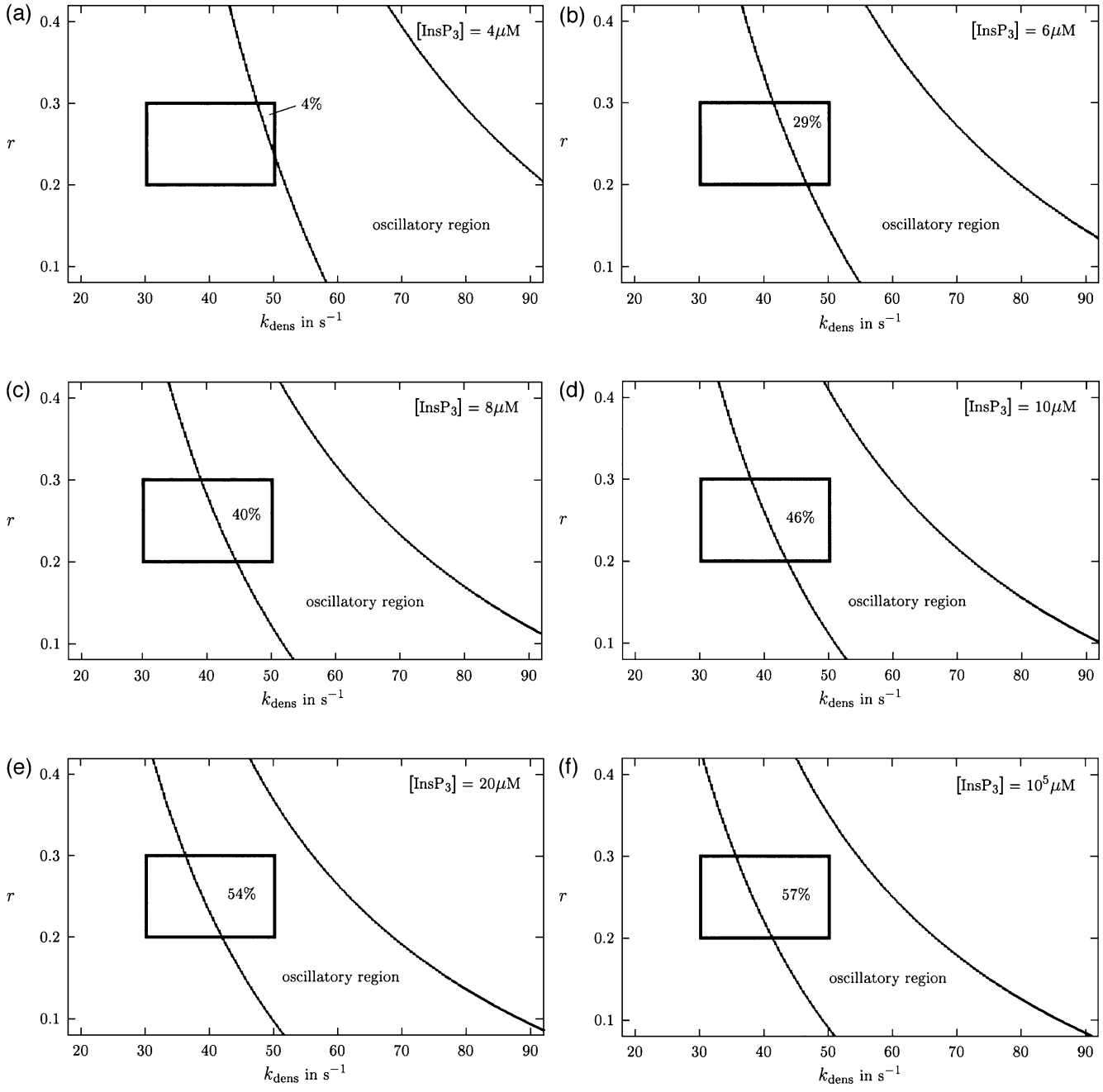

Fig. 7. Situation of the Hopf bifurcation lines (i.e. boundaries of sustained oscillatory behaviour) in the r–k_{dens} plane with varying intensity of stimulation P. The rectangles represent a population of cells that are equally distributed in overall $InsP_3$ receptor density and fraction of Type 1 receptor expressed between $k_{dens} = 30, \ldots, 50\ s^{-1}$ and $r = 0.2, \ldots, 0.3$. Percentages of the cells that lie in the oscillatory regime at the given $InsP_3$ stimulations are indicated.

cytosolic Ca^{2+} was plotted. This procedure was repeated with different values of P. Fig. 5a–d shows some exemplary time courses of $[Ca^{2+}]_{cyt}$, with increasing $[InsP_3]$ applied in the described manner. The system shows a behavior which is in very good agreement with our experimental observations [19]: very low $[InsP_3]$ has almost no effect on the Ca^{2+} trajectory; the system is slightly distorted, but falls back to a resting state immediately (not shown). An $InsP_3$ concentration of 2.3 μM leads to a single Ca^{2+} spike and a decay back to elevated resting level (see Fig. 5a). This behavior is due to the excitability of the system at this value of P, as described by Li et al. [29]. Further increase of $[InsP_3]$ leads to two, three, and even more spikes (Fig. 5b and c) so we speak of damped oscillations due to a stable focus. At higher $InsP_3$ concentrations, an initial phase of declining spikes leads to an infinite phase of sustained limit cycle oscillations (see Fig. 5d). The frequency of the limit cycle oscillations as a function of $InsP_3$ concentration is shown in Fig. 6: it rises sharply at lower $[InsP_3]$ and remains almost constant after reaching a certain saturation level. This is also in accordance with our observations [19].

With the different behaviors of $[Ca^{2+}]_{cyt}$ explained by our model in the various parameter regions, we give an answer to the question why in experiments with cells that are presumed to exist under identical conditions show different behaviors upon stimulation with $InsP_3$. We propose that cells differ slightly both in the density of $InsP_3$ receptors as well as in the ratio of Type 3 to Type 1 receptors, which corresponds to individual variability in these parameters in a homogeneous population of tracheal smooth muscle cells. Within the framework of our model, this means that cells occupy a parameter region in the r–k_{dens} plane, in which some part leads to sustained oscillation above a threshold of P and some part always remains at stable steady-state behavior for all $InsP_3$. The regions of this plane which lead to oscillations for a confined interval of P and to stability for lower and higher P do not belong to the physiological situation of tracheal smooth muscle cells.

It is imaginable that the cells are distributed around some average values for r and k_{dens}, for example, with a Gaussian distribution. A quantitative example of the simpler case of equal distribution is demonstrated in Fig. 7a–f. In each of these diagrams, a section of the r–k_{dens} plane is shown, where the $InsP_3$ concentration P is fixed at 4, 6, 8, 10, 20, and 10^5 μM, respectively. The limits between stable and limit cycle behavior are plotted. The rectangle appearing in each of these pictures represents a cell population with parameter values of $0.2 < r < 0.3$ and $30 < k_{dens} < 50\ s^{-1}$. Assuming a constant distribution of cells within these parameter intervals, the fraction of cells showing sustained oscillations at the given $InsP_3$ concentrations correspond to those parts of the rectangles that lie in the parameter region of oscillatory behavior in the respective diagram. According to this, no cells show sustained oscillations for stimulations with $[InsP_3]$ below 2.9 μM. Then the number of oscillating cells increases sharply. At an $InsP_3$ concentration of 20 μM, the saturation level is almost reached. Further increase of $[InsP_3]$, even to very high values, does not yield further significant changes. This is in agreement with the results of our experiments [19] (see also Fig. 1).

5. Discussion

In this paper, an explanation is given to the question why cells of an identical cell type from the same tissue, in which Ca^{2+} responses involve a largely identical structure, can show qualitatively different dynamical behaviors in response to agonist stimulation. Therefore, we employ a minimal model based on recent measurements of the gating kinetics of two isoforms of the $InsP_3$ receptor [11,14]. The meaningfulness of this model is underlined by the fact that it reproduces the different patterns of cytosolic Ca^{2+} curves which we have measured on individual cells [19], like single spikes followed by an elevated rest state, two and more spikes with declining amplitudes (damped oscillations), and sustained oscillations. The experimentally observed phenomenon that, with increasing agonist concentration, the number of cells responding with oscillations of cytosolic Ca^{2+} concentration grows in the beginning, but does not exceed a certain saturation limit, whatever the intensity of stimulation, which is sufficiently explained by the assumption that individual cells differ slightly in the total density of $InsP_3$ receptors as well as in the ratio of the two isoforms $InsP_3R1$ and $InsP_3R3$ expressed.

The idea to combine the kinetics of the opening and closing of different $InsP_3$ receptor subtypes in a model to analyze their effect on the dynamics of cytosolic Ca^{2+} concentration is unprecedented. Although there is a much more detailed theoretical study on the different receptor isoform published by Moraru et al. [11], that work does not deliver a model of the whole cell. Our model contains only a simplified version of the description of $InsP_3R$ subtypes, but it includes a minimal choice of processes that suffice to explain oscillations of the cytosolic Ca^{2+} concentration; thus, the time courses yielded by the model can be compared to Ca^{2+} curves which we have measured [19].

Indeed, assuming that increase in agonist concentration results in an increase in $InsP_3$ concentration, it is relevant to compare our experimental data with modeling predictions. Although the model is a simplified one, its predictions are rather close to our experimental observations on the characteristics of a single cell response as well as on the behaviors of a population of cells. In our experiments, the percentage of oscillating cells increases with agonist concentration up to a maximum of 50–60% of oscillating cells. The prediction of the model is in accordance with these experimental data. In other species, however, the occurrence of both oscillating and non-oscillating responses in cells from the same airway smooth muscle has not been evidenced, though similar structures seem to be involved in these different calcium responses. In bovine trachea, all cells

exhibit non-oscillating profile [30]. In contrast, oscillating patterns have been described in pig and guinea pig tracheal cells [21,22]. The interest of our theoretical approach, i.e. the analysis of the behavior of a population of cells, is that a single model may account for different qualitative and quantitative characteristics of the calcium response including from different species.

The differences in calcium response between airway myocytes from various species may be explained by differences in the regions occupied by the cell populations in the space of the parameters. The consequence of such an approach is that experiments should not focus only on the identification of specific structures associated with specific responses, but also on the determination of the parameters that may play a critical role in the occurrence of oscillations. In our case, the total density and the relative ratios of the $InsP_3$ receptor subtypes are critical parameters. Northern blot analysis and in situ hybridization in mouse tissues demonstrated that $InsP_3$ receptor mRNA was located in great amount in smooth muscle including in bronchioles [31]. However, to our knowledge, there is no data available about $InsP_3$ receptor density and expression of the various isoforms in airway smooth muscle. Some results have been published about other smooth muscle. In artery myocytes, immunolocalization showed that the three isoforms of $InsP_3$ receptors were expressed and that their relative amount varied during development and cell proliferation [32,33]. Both $InsP_3$ receptor Types 1 and 3 were detected by immunoblotting in rat aorta; Type 3 has been predominant in neonatal (4–6 days) and Type 1 in developed (6 weeks) aorta [34]. Ratio polymerase chain reaction in vascular [35] and myometrial [36] smooth muscle showed that the mRNA of all three isoforms were present, with Type 1 representing about 70–85%. This ratio is in the range that has been chosen in our theoretical study. It is likely that in airway smooth muscle, various $InsP_3R$ isoforms may be expressed in a similar range and that their relative amount may also vary between species, location in the respiratory track, and pathophysiological conditions. Since our model showed that $InsP_3R$ isoform expression may be critical in Ca^{2+} response, it would be of great interest to determine the level of expression of these isoforms in airway smooth muscle cells. Varying the strength of stimulation with fixed k_{dens} and r, which corresponds to the experimental stimulation of a single cell with various agonist concentrations, results in our model an increase in the amplitude of the first Ca^{2+} rise and in the frequency of oscillations. This is in accordance with our experimental data in response to increasing concentrations of ACh. However, the maximum oscillation frequency predicted by our model, though in the same range of order, is a little smaller than that experimentally observed in a variety of species, which is about 10 oscillations per minute [37].

The simple model presented here has a great potential for improvement to yield more realistic predictions. Future studies should concentrate on the inclusion of several structures that have not been taken into account. In our model, we have included only two types of $InsP_3$ receptors, Type 1 as Ca^{2+}-inhibited receptor and Type 3 as non-Ca^{2+}-inhibited one. However, a second Ca^{2+}-noninhibited $InsP_3$ receptor exists, and though its gating kinetics is close to that of Type 3, it should be included in the model. Moreover, in parallel with $InsP_3$ receptors, Ca^{2+} may also be released from the sarcoplasmic reticulum through another calcium channel, the ryanodine-sensitive channel (RyR). Functional RyRs have been shown to be present in a variety of airway smooth muscle cells including human bronchi. However, its physiological implication in the calcium response to agonist stimulation remains unclear since controversial results have been published on this topic [22,37,38]. Therefore, it would be of interest to include the RyR in the model in order to evaluate its possible effect on calcium signaling. Another structure that we have not included in the model, and whose potential role in calcium response is a matter of debate, is represented by mitochondria. Experimental as well as theoretical studies have demonstrated that mitochondria may be of great importance in calcium signaling [39,40], in particular, as an excitable organelle capable of fast calcium uptake. However, to the best of our knowledge, the putative implication of mitochondria in calcium signaling in airway smooth muscle cells has not yet been studied. Some results have been published on other smooth muscle cells like gastric, intestinal, and arterial myocytes [41–44]. Taken together, these experimental results indicate that mitochondria may act as a low kinetic buffer compartment with the ability to increase calcium concentration upon cell stimulation, but without evidence of any mitochondrial CICR. These results suggest that mitochondria may not play a primary role in calcium response to agonist stimulation in airway smooth muscle cells. However, further experimental studies are needed to investigate the involvement of mitochondria in calcium dynamics in airway myocytes and whether it is relevant to include mitochondrial terms in the model.

In conclusion, our model gives an explanation of the occurrence of various Ca^{2+} response patterns in airway smooth muscle cells, i.e. oscillating and non-oscillating responses. Though the model used is a simplified one, its qualitative and quantitative predictions are close to experimental data. Future development is oriented into the study of other structures in airway smooth muscle cells and is associated with further experiments in order to determine experimental values that are presently missing.

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