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Review

Modelling of calcium handling in airway myocytes

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Abstract

Airway myocytes are the primary effectors of airway reactivity which modulates airway resistance and hence ventilation. Stimulation of airway myocytes results in an increase in the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and the subsequent activation of the contractile apparatus. Many contractile agonists, including acetylcholine, induce $[\text{Ca}^{2+}]_i$ increase via Ca^{2+} release from the sarcoplasmic reticulum through InsP_3 receptors. Several models have been developed to explain the characteristics of InsP_3 -induced $[\text{Ca}^{2+}]_i$ responses, in particular Ca^{2+} oscillations. The article reviews the modelling of the major structures implicated in intracellular Ca^{2+} handling, i.e., InsP_3 receptors, SERCAs, mitochondria and Ca^{2+} -binding cytosolic proteins. We developed theoretical models specifically dedicated to the airway myocyte which include the major mechanisms responsible for intracellular Ca^{2+} handling identified in these cells. These biocomputations pointed out the importance of the relative proportion of InsP_3 receptor isoforms and the respective role of the different mechanisms responsible for cytosolic Ca^{2+} clearance in the pattern of $[\text{Ca}^{2+}]_i$ variations. We have developed a theoretical model of membrane conductances that predicts the variations in membrane potential and extracellular Ca^{2+} influx. Stimulation of this model by simulated increase in $[\text{Ca}^{2+}]_i$ predicts membrane depolarisation, but not great enough to trigger a significant opening of voltage-dependant Ca^{2+} channels. This may explain why airway contraction induced by cholinergic stimulation does not greatly depend on extracellular calcium. The development of such models of airway

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myocytes is important for the understanding of the cellular mechanisms of airway reactivity and their possible modulation by pharmacological agents.

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Keywords: Airways; Smooth muscle cell; Calcium; Theoretical model; Biocomputation

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1. Characteristics of calcium signalling in airway myocytes

Airway myocytes are the primary effectors of airway reactivity which modulates airway resistance and hence ventilation. An increase in airway reactivity, i.e., hyperreactivity, is observed in several respiratory diseases like asthma and chronic obstructive pulmonary diseases (COPD), and can lead to respiratory distress and eventually death. Stimulation of airway myocytes results in an increase in the cytosolic Ca²⁺ concentration ([Ca²⁺]_i) and the subsequent activation of the contractile apparatus. Many studies have been performed in several species in freshly isolated cells, in cultured cells and, more recently, in lung slices, to characterise the [Ca²⁺]_i response to several contractile agonists, in particular acetylcholine, the major physiological mediator of airway reactivity (Bergner and Sanderson, 2002a; Roux et al., 1998b). The [Ca²⁺]_i response appears to be biphasic: a first Ca²⁺ peak is followed either by a progressive decay to a plateau phase or by [Ca²⁺]_i oscillations usually superimposed on the plateau. It seems that the occurrence of Ca²⁺ oscillations depends on species. Oscillating responses to acetylcholine have been reported in mouse, swine and guinea-pig airway smooth muscle cells, while the response observed in bovine and human myocytes are non-oscillating ones (Bergner and Sanderson, 2002a; Hyvelin et al., 2000a; Liu and Farley, 1996; Roux et al., 1998a). In rat, both oscillating and non-oscillating responses have been observed (Roux et al., 1997). Fig. 1 shows typical [Ca²⁺]_i response to acetylcholine in rat and human freshly isolated myocytes. The characteristics of the [Ca²⁺]_i response depends on the amplitude of stimulation. In particular, several authors have evidenced

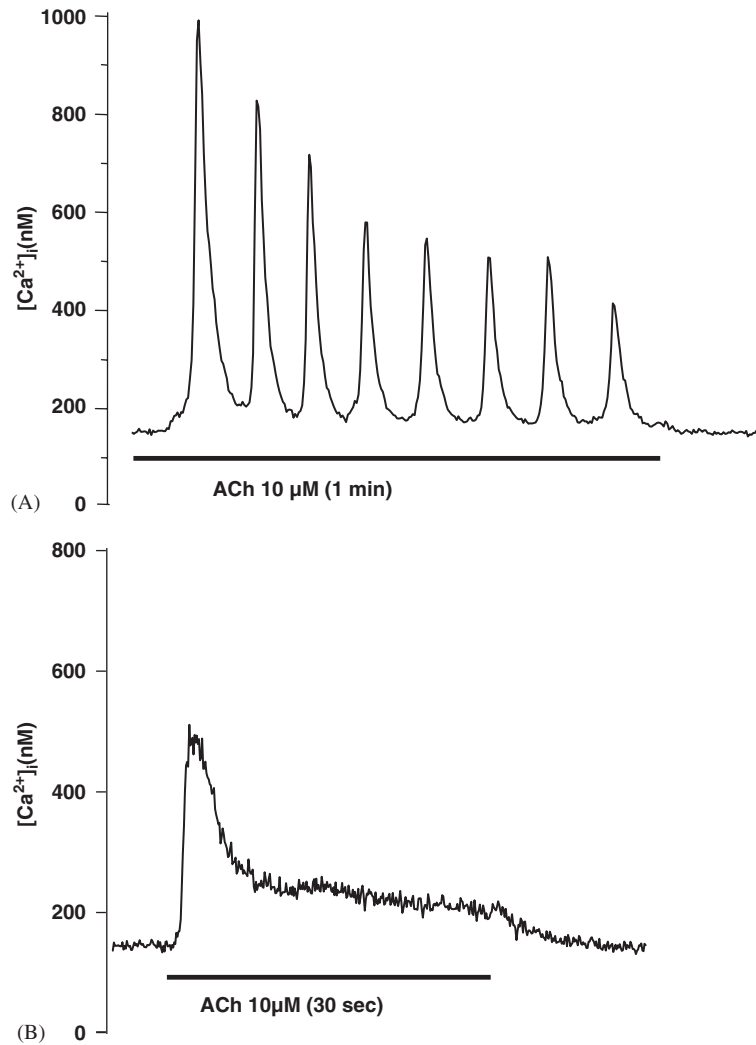


Fig. 1. $[Ca^{2+}]_i$ response to ACh stimulation in rat and human freshly isolated airway myocytes. Original traces of calcium response to $10\ \mu\text{M}$ acetylcholine in rat tracheal myocytes (A) and human bronchial myocytes (B). Cells were obtained from fresh tissues by enzymatic dissociation followed by mechanical dispersion and used within one day. $[Ca^{2+}]_i$ was measured by microspectrofluorimetry using the fluorescent dye Indo 1, as previously described (Roux and Marhl, 2004).

that the frequency of oscillations depends on the level of stimulation and correlates with the amplitude of the contractile response, both in physiological and pathophysiological conditions (Bergner and Sanderson, 2002a, b; Liu and Farley, 1996; Roux et al., 1997, 1998a). This suggests that the oscillation frequency may encode for contraction.

Acetylcholine and several other contractile agonists are InsP_3 -mediated agonists and induce $[Ca^{2+}]_i$ increase via Ca^{2+} release from the sarcoplasmic reticulum through InsP_3 receptors. Though some authors have concluded that the sustained phase on the response depended on

extracellular calcium influx (Kajita and Yamaguchi, 1993; Liu and Farley, 1996), studies performed in freshly isolated cells and tissues have shown, in rat, mouse, guinea-pig, swine and human airways, that the calcium response to ACh did not primarily depend on extracellular calcium influx (Bergner and Sanderson, 2002a; Roux et al., 1998b). Hence, the relationship between cholinergic stimulation and the $[Ca^{2+}]_i$ response pattern, in particular $[Ca^{2+}]_i$ oscillations primarily depends on InsP-induced Ca^{2+} release from the sarcoplasmic reticulum and intracellular Ca^{2+} handling, as in many non-excitabile cells.

In the following sections, we will first review the existing modelling of the main intracellular structures involved in Ca^{2+} dynamics in non-excitabile cells, i.e. the InsP₃ receptors of the sarcoplasmic reticulum, the SERCAs, the mitochondria as intracellular Ca^{2+} stores, and the Ca^{2+} -binding cytosolic proteins. We will then present two models which we have already published specifically dedicated to the modelling of Ca^{2+} handling in airway myocytes. The last part of the article is a new model of membrane conductances in airway myocytes which we have developed to assess the relationship between plasma membrane ion currents and intracellular Ca^{2+} signalling.

1.1. Models of InsP₃-receptors

InsP₃-receptors (IP₃R) play a crucial role in calcium dynamics. In particular InsP₃-receptors are involved in Ca^{2+} release from intracellular stores, mainly from the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR). One of the main features of the IP₃R, which has been found experimentally, is that in a wide range of cell types, the activation of the IP₃R is much faster than the inactivation (Parker and Ivorra, 1990; Oancea and Meyer, 1996; LeBeau et al., 1999). Hence, fast activation of the IP₃R, followed by slow inactivation, is also an important characterisation of mathematical models for Ca^{2+} oscillations. The basic mechanism of Ca^{2+} release from the ER/SR is the inhibition of IP₃R, which causes termination of the Ca^{2+} spikes. Two mechanisms of IP₃R inactivation have been suggested: (i) transition of the IP₃R into an inactive conformation upon Ca^{2+} binding (Parker and Ivorra, 1990; Bezprozvanny et al., 1991; Finch et al., 1991; Moraru et al., 1999); and (ii) inactivation of the IP₃R by phosphorylation (LeBeau et al., 1999).

The first of the two possibilities was studied in theoretical models by Poledna (1991, 1993) and Atri et al. (1993). The mathematical description of the mechanism of the IP₃R inactivation was motivated by analogy to the Hodgkin-Huxley model of nerve excitation (cf. Keener and Sneyd, 1998). A more sophisticated, eight-dimensional model was developed by De Young and Keizer (1992). This is one of the most influential models in the field. In later studies the model has been simplified, by using time scale arguments, to two-dimensional models (Li and Rinzel, 1994; Keizer and De Young, 1994; Tang et al., 1996), which have a structure reminiscent of the Hodgkin-Huxley models.

In the model by De Young and Keizer (1992), IP₃R is assumed to consist of three equivalent and independent subunits. Each subunit has an IP₃ binding site, an activating Ca^{2+} binding site, and an inactivating Ca^{2+} binding site, each of which can be either occupied or unoccupied, and thus each subunit can be in one of eight states. Other studies use a simplified or even more sophisticated description of possible states. For example, two states (Li and Rinzel, 1994), five states (Bezprozvanny and Ehrlich, 1994), 125 states (Moraru et al., 1999) are distinguished

according to the binding of Ca^{2+} and/or IP_3 (cf. Schuster et al., 2002). The transitions between the states are modelled by mass-action kinetics.

The IP_3R is affected both by cytosolic Ca^{2+} concentration and IP_3 . The dual effect of the cytosolic calcium and the IP_3 on the IP_3R can be considered to be an allosteric effect. This approach is used in the model by Laurent and Claret (1997). In agreement with the previous models, this model is again able to mimic the bell-shaped curve of the dependence of Ca^{2+} release from the vesicular compartments on $\text{Ca}_{\text{cyt}}^{2+}$ (Bezprozvanny et al., 1991), whereas the IP_3 binding process itself is not cooperative. Further models describing the kinetics of IP_3 -sensitive Ca^{2+} channels include Swillens et al. (1994), Dupont and Swillens (1996) and Moraru et al. (1999).

The IP_3R can be phosphorylated (with one phosphate per receptor subunit) by protein kinases A and C and Ca^{2+} /calmodulin-dependent protein kinase II (CaM kinase II) (cf. Bezprozvanny and Ehrlich, 1995). The phosphorylation of subtype III of the IP_3R is included in the mathematical models proposed by LeBeau et al. (1999) and Sneyd et al. (2000). The model proposed by LeBeau et al. (1999) for pancreatic acinar cells includes four different states of the receptor with one of these being phosphorylated. The open probability curve of the IP_3R is calculated to be an increasing function of the cytosolic Ca^{2+} concentration, as found for type-III IP_3R (Hagar et al., 1998). The model can explain long-period baseline spiking typical for cholecystokinin stimulation, which is accompanied by receptor phosphorylation, as well as short-period, raised baseline oscillations.

More recently, a model has been developed by Sneyd and Dufour (2002) that incorporates several important experimental findings: sequential binding of IP_3 and activating Ca^{2+} , modulation of InsP_3 binding by Ca^{2+} , a fit of the model to dynamical data instead of steady-state data only, time-dependent inactivation upon IP_3 binding, and saturating binding rates of Ca^{2+} . A very recent model, which takes into account the allosteric effects with the goal of elucidating adaptation and incremental responses by the IP_3R , has been developed by Dawson et al. (2003). A comparison of the Sneyd–Dufour model, the Dawson–Lea–Irvine model, and the traditional DeYoung–Keizer model (De Young and Keizer, 1992) was given by Sneyd et al. (2004). For recent reviews of mathematical models of Ca^{2+} oscillations which take into account the kinetics of IP_3R see Schuster et al. (2002) and Falcke (2004).

In the modelling of the kinetics of IP_3R , it is worth taking into account the existence of three different subtypes of the IP_3R (subtypes I, II, and III) in more detail because experimental work points to a physiological significance of the differential expression of IP_3R subtypes (Hagar et al., 1998; Moraru et al., 1999; Miyakawa et al., 1999; Hagar and Ehrlich, 2000).

1.2. Models of SERCAs

After Ca^{2+} release from the ER or SR, the reuptake of Ca^{2+} from the cytosol into the ER or SR is accomplished by Ca^{2+} pumps, the so-called sarco-endoplasmic Ca^{2+} ATP-ases (SERCA). The consumption of ATP provides the energy for Ca^{2+} transport from the cytosol into the ER or SR. The energy is needed for pumping Ca^{2+} against a large concentration gradient that exists between the cytosol and the ER or SR.

There are three main different isoforms of SERCA (Lytton et al., 1992). In fast-twitch skeletal muscle cells only SERCA1 is expressed. SERCA2 is characteristic for cardiac muscle cells and

adult slow-twitch skeletal muscle cells. SERCA3 was found in several non-muscle cells (cf. Falcke, 2004). Lytton et al. (1992) found, however, that for all isoforms of SERCA, the Hill coefficient is ≈ 2 . Therefore, in most mathematical models describing intracellular Ca^{2+} signalling, the kinetics of Ca^{2+} pumping from the cytosol into the ER or SR is modelled by the Hill kinetics with the Hill coefficient 2 (for review see Schuster et al., 2002; Falcke, 2004).

The mathematical modelling of Ca^{2+} signal transduction in non-excitabile cells has always included the SERCA as an important component. In fact, there exists no mathematical model of intracellular Ca^{2+} handling, in which Ca^{2+} release from the ER or SR is considered and in which SERCA has not been taken into account. For example, the SERCA represents a vital part already in the earliest models for Ca^{2+} oscillations in non-excitabile cells, e.g. in the model by Meyer and Stryer (1988), or in the model by Goldbeter et al. (1990).

It should be emphasised, however, that for Ca^{2+} signalling in tracheal smooth muscle cells a traditional dogma of a direct immediate Ca^{2+} reuptake by SERCA was broken. The experimental and theoretical results indicate (see Roux and Marhl, 2004) that after Ca^{2+} release from the SR into the cytosol, though Ca^{2+} pumping back by SERCA is active, it is not primarily involved in removing Ca^{2+} from the cytosol. We showed that this is due, in part, to mitochondrial Ca^{2+} uptake and Ca^{2+} binding to cytosolic proteins, which is also in agreement with experimental results in other cells (see e.g. Babcock and Hille, 1998, cf. Schuster et al., 2002). More about the role of mitochondria and the Ca^{2+} -binding proteins in the cytosol can be found in the following paragraphs.

1.3. Modelling of Ca^{2+} sequestration in mitochondria

Mitochondria are not just important powerhouses in the cells. For several decades, it has been known that mitochondria also play an important role in Ca^{2+} sequestration (Slater and Cleland, 1953; Vasington and Murphy, 1962; see also Carafoli, 1987; Gunter and Pfeiffer, 1990; for review see Schuster et al., 2002; Falcke, 2004). There are several Ca^{2+} transport processes across the mitochondrial inner membrane: the Ca^{2+} uniporter and the permeability transition pore (PTP) (Pozzan et al., 1994; Bernardi and Petronilli, 1996), the $\text{Na}^+/\text{Ca}^{2+}$ and $\text{H}^+/\text{Ca}^{2+}$ exchangers (Hehl et al., 1996; Babcock et al., 1997).

For a long time, mitochondria were not included in the modelling of calcium dynamics in non-excitabile cells. With the exception of the model of Meyer and Stryer (1988), mitochondria had at first been neglected. The experimental data have mainly shown that the accumulation of Ca^{2+} starts at Ca^{2+} concentrations of about 5–10 μM (cf. Pozzan et al., 1994), which is much higher than the physiological Ca^{2+} concentration in the cytosol. Later experiments, however, re-evaluated the role of mitochondria, showing that mitochondria start to take up Ca^{2+} at average cytosolic Ca^{2+} concentrations in a range between 0.5 and 1 μM (cf. Jouaville et al., 1995; Bernardi and Petronilli, 1996; Babcock et al., 1997). This is not in contradiction with the earlier experiments, since it has also been shown that mitochondria are located near the mouths of channels across the ER/SR membrane (Rizzuto et al., 1999; Rutter and Rizzuto, 2000). In these microdomains the Ca^{2+} concentrations could be 100–1000 times larger than that of the average Ca^{2+} concentration in the cytosol (cf. Petersen et al. 1994; Pozzan et al., 1994). Therefore, mitochondria can indeed sequester Ca^{2+} released from the ER (Hehl et al., 1996; Babcock et al., 1997; Simpson and Russell, 1998a, b; Rizzuto et al., 1998; Drummond and Tuft, 1999). For

example, in chromaffin cells, around 80% of the Ca^{2+} released from the ER is cleared first into mitochondria (Babcock and Hille, 1998).

These experimental results have stimulated the inclusion of mitochondria in the modelling of Ca^{2+} oscillations (Meyer and Stryer, 1988; Chay 1996; Marhl et al., 1998a, 2000; Selivanov et al., 1998; Haberichter et al., 2001) and Ca^{2+} homeostasis (Magnus and Keizer, 1997, 1998a, b). In the theoretical study by Marhl et al. (1998a), it has been shown that mitochondria could play an important role in modulating the Ca^{2+} signals and, in particular, could regulate the amplitude of Ca^{2+} oscillations (see also Grubelnik et al., 2001). The mitochondrial sequestration of Ca^{2+} leads to fairly constant amplitudes over wide ranges of oscillation frequency due to clipping the peaks at about the threshold of fast Ca^{2+} uptake (see also Meyer and Stryer, 1988). This is in agreement with the idea of frequency encoding of Ca^{2+} signals (cf. Schuster et al., 2002).

1.4. Modelling of Ca^{2+} binding to cytosolic proteins

Cytosolic Ca^{2+} -binding proteins are usually considered only as target proteins in the role of information receivers. In addition to the sensing of the Ca^{2+} signal, Ca^{2+} -binding proteins also exert a feedback on the process of Ca^{2+} oscillations itself. Therefore, the inclusion of Ca^{2+} -binding proteins into the modelling of calcium dynamics is of particular importance, and is actually done in all newer models of Ca^{2+} handling. In several models, a rapid-equilibrium approximation for Ca^{2+} binding to proteins is used (Wagner and Keizer, 1994; Jafri and Keizer, 1995, 1997; Höfer et al., 2001). However, the rapid-equilibrium approximation is not always justified (Smith et al., 1996; Naraghi and Neher, 1997), and therefore, several other mathematical models include the dynamics of Ca^{2+} binding to proteins (Jafri et al., 1992; Jafri and Gillo, 1994; Jafri and Keizer, 1995, 1997; Marhl et al. 1997, 1998a, b, 2000). All these models, taking into account the dynamics of Ca^{2+} binding to proteins, show that the cytosolic proteins can be essential components of the oscillatory mechanism. The proteins can play an important role in temporary Ca^{2+} sequestration and hence influence the frequency and the amplitude of Ca^{2+} oscillations.

Cytosolic Ca^{2+} -binding proteins are characterised by a wide range of values of the binding and dissociation rate constants (Falke et al., 1994; Smith et al., 1996; Naraghi and Neher, 1997). Roughly, two main classes of proteins can be distinguished (Falke et al., 1994; Berlin et al., 1994; Skelton et al., 1994; Maurer et al., 1996). The first class represents the so-called buffering proteins (also known as “storage” proteins) such as parvalbumin, calbindin, and also C-domains of calmodulin or troponin C, which bind calcium relatively slowly but with a high affinity (Falke et al., 1994; Smith et al., 1996). The second class, the so-called signalling proteins (also known as “regulatory” proteins) are characterised by binding sites that have very high rate constants of binding and dissociation with respect to calcium, but low affinity. Examples are provided by the N-domains of calmodulin or troponin C.

In the mathematical model by Marhl et al. (Marhl et al., 1998a, b) both buffering and signalling proteins have been considered. In the model the rapid-equilibrium approximation is used for signalling proteins, whereas the dynamics of Ca^{2+} binding to the buffering proteins is modelled by differential equations. An important model prediction is the transfer of Ca^{2+} from signalling to buffering proteins. This is in agreement with experimental observations both in Ca^{2+} oscillations and Ca^{2+} transients. In skeletal muscle, for example, the Ca^{2+} released into the

cytosol first binds to troponin C and, after a brief lag phase, the bound Ca^{2+} population shifts to parvalbumin (Falke et al., 1994; Taylor, 1995). There, the buffering proteins play an important role in terminating the Ca^{2+} transients evoking muscle contraction. In other biological systems, this mechanism may play a role in the termination of spikes in oscillations.

1.5. Mathematical modelling of intracellular Ca^{2+} dynamics in airway myocytes

1.5.1. Model of InsP_3 -induced Ca^{2+} response in rat tracheal cell

Recently, we have developed a model of ACh-induced $[\text{Ca}^{2+}]_i$ response in rat tracheal myocytes (Haberichter et al., 2002). In these cells, experimental studies have shown that the Ca^{2+} response to cholinergic stimulation in rat tracheal smooth muscle cell was a complex one. For low level of stimulation, the calcium response was a non-oscillating one. For increased concentration, both oscillating and non-oscillating responses were observed, and the $[\text{Ca}^{2+}]_i$ response was concentration-dependent both for the amplitude of the first peak and the frequency of oscillations, but not in relation to the percentage of oscillating cells, which was about 50–60%. Similar results were obtained when permeabilised cells were directly stimulated by InsP_3 (Hyvelin et al., 2000b; Roux et al., 1997).

Our model is a cell model that includes not only the InsP_3 receptors but also the SR as a compartment which can store, buffer and release Ca^{2+} . We also considered the possible important role of cytosolic Ca^{2+} -binding proteins by incorporating in the model one class of proteins, the buffering proteins (cf. Fig. 2A). Cholinergic stimulation was simulated by increasing the intracellular InsP_3 concentration. We used it to analyse the impact of different gating kinetics of subtype I and subtype III IP_3R on the calcium dynamics in tracheal smooth muscle cells upon InsP_3 stimulation. In accordance with our previous experimental data, we showed that for different concentrations of InsP_3 several spikes with declining maxima, and sustained calcium oscillations appear (cf. Fig. 2B). Moreover, an explanation as to the experimental observation of qualitatively different responses of cells within a presumably homogenous tissue is given by the model. The model predictions show that this could be a consequence of different proportions of subtypes I and III IP_3R s in the cells. The model also showed that, if we took into account the buffering properties of the cytosol, oscillations can be observed with and without IP_3R type I, and that, reciprocally, non-oscillating responses can be observed with IP_3R type I alone. The model hence pointed out the importance of the relative expression of InsP_3 receptor subtypes but also the role of the cellular environment in the $[\text{Ca}^{2+}]_i$ response profile, so that the occurrence of oscillations cannot be attributed to the properties of the IP_3R alone. Interestingly, Morel et al. (2003) published later that in vascular smooth muscle cell type, the $[\text{Ca}^{2+}]_i$ response was characterised by both oscillating and non-oscillating response in a fixed proportion that was about 50%. The authors demonstrated that the non-oscillating responses were associated with the expression of IP_3R type I alone, and that oscillations required the expression of both IP_3R types I and II. The authors' explanation hypothesised in the discussion was that IP_3R type II may have, in contrast with in vitro observation, a biphasic regulation by Ca^{2+} in vivo. Although this may be true, it does not explain the absence of oscillation in cells expressing only IP_3R type I, which is also supposed to be biphasically regulated by Ca^{2+} , and also points out that Ca^{2+} oscillations are a complex phenomenon that cannot be attributed to a single intracellular structure.

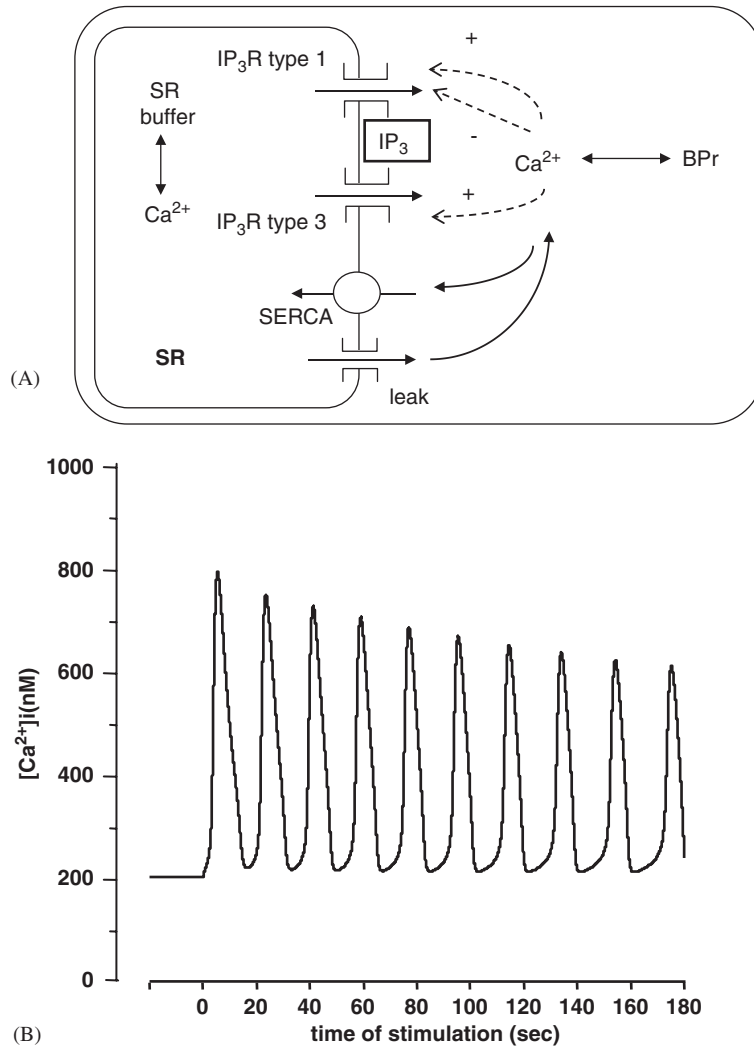


Fig. 2. Model of InsP₃-induced [Ca²⁺]_i response in rat tracheal cells. (A) Schematic presentation of the model. Abbreviations: BPr : buffering cytosolic proteins; SR: sarcoplasmic reticulum. (B) Predicted [Ca²⁺]_i response to 4 μM InsP₃, for a ratio IP₃R subtype 1/subtype 2 equal to 0.15. Stimulation begins at time 0.

1.5.2. Model of cytosolic Ca²⁺ clearance upon Ca²⁺ release from the SR

As explained above, both experimental studies in several cell types and theoretical investigations has pointed out the importance of the mechanisms responsible for the cytosolic Ca²⁺ clearance, the so-called “OFF” mechanisms, in Ca²⁺ handling upon cell stimulation. To determine in airway myocytes the relative role of the different “OFF” mechanisms in the cytosolic Ca²⁺ clearance following Ca²⁺ release from the SR, we developed a theoretical model combined with experimental investigation (Roux and Marhl, 2004). Since caffeine was used in the experimental studies to trigger Ca²⁺ release from the SR, the model included ryanodine receptors (RyR) and, in addition with Ca²⁺ pumping back into the SR by SERCAs, mitochondria and

Ca^{2+} -binding cytosolic proteins. The schema of the model is presented in Fig. 3A. Such a model reproduces the pattern of the Ca^{2+} response to caffeine observed in experimental recordings in freshly isolated rat tracheal cells. This $[\text{Ca}^{2+}]_i$ response is characterised by a first transient increase followed by a progressive decay to a plateau phase. For short time stimulation (1 s), there is no plateau and we showed that the RyRs are closed during the decay phase following the initial transient. Hence, the analysis of the decay phase gives information on the “OFF” mechanisms without participation of any “ON” mechanism (Roux and Marhl, 2004). Simulation of inhibition of SERCA activity predicted that the kinetics of the $[\text{Ca}^{2+}]_i$ decay phase did not depend on SERCA activity, and the theoretical predictions were confirmed by experiments using cyclopiazonic acid as SERCA inhibitor.

The model shows that Ca^{2+} uptake by mitochondria is more important than Ca^{2+} pumping back by SERCA for the immediate cytosolic Ca^{2+} clearance after Ca^{2+} release from the SR. The model predictions of Ca^{2+} dynamics with active versus blocked mitochondrial Ca^{2+} uptake has been verified experimentally. In the presence of FCCP, a quick mitochondrial uptake inhibitor, the decay in $[\text{Ca}^{2+}]_i$ concentration was slower than that in control condition, and ANOVA analysis showed that the difference was statistically significant (Roux and Marhl, 2004). In this model, we also considered the role of cytosolic Ca^{2+} -binding proteins. Whereas in the model by Haberichter et al. (2002) only one class of proteins (buffering proteins) is taken into account, in this later study both signalling and buffering proteins are included in the model. The model predictions indicate a possible scenario for Ca^{2+} distribution in the cytosol after Ca^{2+} release from the SR. The first process that may take place is Ca^{2+} binding to signalling proteins, quickly followed by Ca^{2+} sequestration into mitochondria. After a short time delay, Ca^{2+} is shifted to the buffering proteins, and finally, after a considerably longer time delay, Ca^{2+} is pumped back into the SR. The predictions of the model for Ca^{2+} distribution upon 30 s caffeine stimulation is shown in Fig. 3B.

1.6. Mathematical modelling of membrane conductances

Although models of electrophysiology of other muscle cells have been developed there are no models of airway smooth muscle. In the case of other organs, particularly the heart and neurones, such models have proved to be valuable in permitting integrative interpretations of experimental work, and in enabling multi-level modelling of organs to be constructed. It is important therefore to develop an electrophysiological model of the airway myocyte both for integration with the calcium signalling model and for future incorporation into organ models of the lung. In this section we describe the first such model.

1.6.1. Computational

An airway smooth muscle cell model of membrane conductances was constructed using the OXSOFT HEART 4.X programme (Noble, 1999) and experimental data obtained from the literature. Individual currents were modelled separately, either by incorporating new equations into the programme or by adjusting the parameter values of existing components; voltage-dependence and kinetics in HEART were then adjusted to fit the experimental findings. The parameter values were stored in individual input files for each current. These parameters were then incorporated into a single input file to produce a model of membrane potential in airway

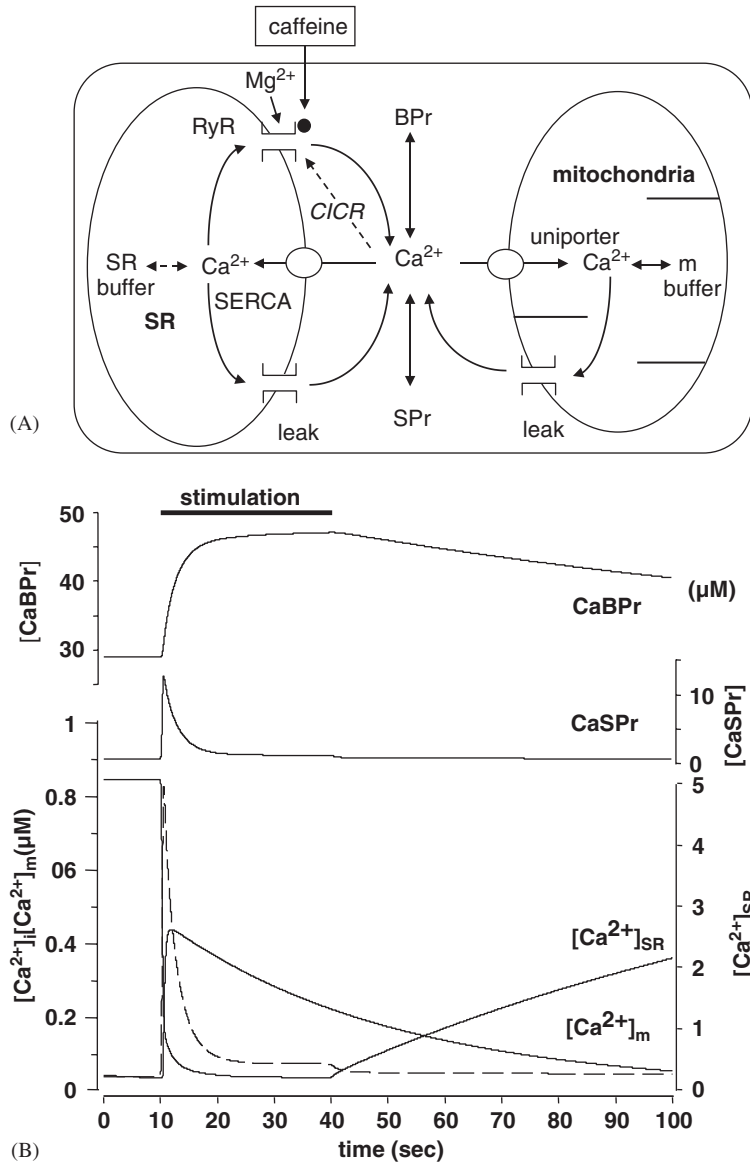


Fig. 3. Model of cytosolic Ca^{2+} clearance upon caffeine stimulation. (A) Schematic presentation of the model. Abbreviations: BPr: buffering cytosolic proteins; SPr: signalling cytosolic proteins; RyR: ryanodine receptor; CICR: Ca^{2+} -induced Ca^{2+} release; SR: sarcoplasmic reticulum; m buffer: mitochondrial buffer. (B) Predicted Ca^{2+} distribution upon caffeine-induced Ca^{2+} release from the SR. The simulated application of caffeine was 30 s, and began at time 10. Dashed line represents $[Ca^{2+}]_i$. Full lines represent the concentrations of free Ca^{2+} in the SR ($[Ca^{2+}]_{SR}$), in the mitochondria ($[Ca^{2+}]_m$), and bound to signalling (CaSPr) and buffering (CaBPr) proteins.

smooth muscle cells (ASMC). Simulations were also performed with the MADONNA software (University of Berkeley, Berkeley, CA), using the Runge–Kutta routine for numerical solving of differential equations.

1.6.2. Presentation of the model

The model includes the major membrane ion currents identified in airway smooth muscle cells (ASMC) that may be activated upon cholinergic stimulation. Basically, these ion currents are the L-type voltage-dependent Ca^{2+} current (I_{CaL}), the Ca^{2+} -activated Cl^- current (I_{ClCa}), the delayed rectifier K^+ current (I_{Kdr}), the Ca^{2+} -activated K^+ current (I_{KCa}), and a non-specific cationic current (I_{cationic}). Active ion fluxes through the plasma membrane incorporated in the model are Na^+ , K^+ and Ca^{2+} fluxes through the Na^+-K^+ ATPase (NKA) and the plasma membrane Ca^{2+} ATPase (PMCA). The $\text{Na}^+-\text{Ca}^{2+}$ exchanger is not incorporated in the model on the basis of experimental studies which showed that, in contrast with cardiac myocytes, it is not significantly involved in Ca^{2+} extrusion upon ASMC stimulation (Janssen et al., 1997). The model also includes background currents for Ca^{2+} , K^+ and Na^+ (cf. Fig. 4).

The equilibrium potential of Na^+ , K^+ , Ca^{2+} and Cl^- is determined using the Nernst equation, written, respectively:

$$E_{\text{Ca}} = (RT/2F) \ln(\text{Ca}_o/\text{Ca}_i), \quad (1)$$

$$E_{\text{Na}} = (RT/F) \ln(\text{Na}_o/\text{Na}_i), \quad (2)$$

$$E_{\text{K}} = (RT/F) \ln(\text{K}_o/\text{K}_i), \quad (3)$$

$$E_{\text{Cl}} = (RT/F) \ln(\text{Cl}_i/\text{Cl}_o), \quad (4)$$

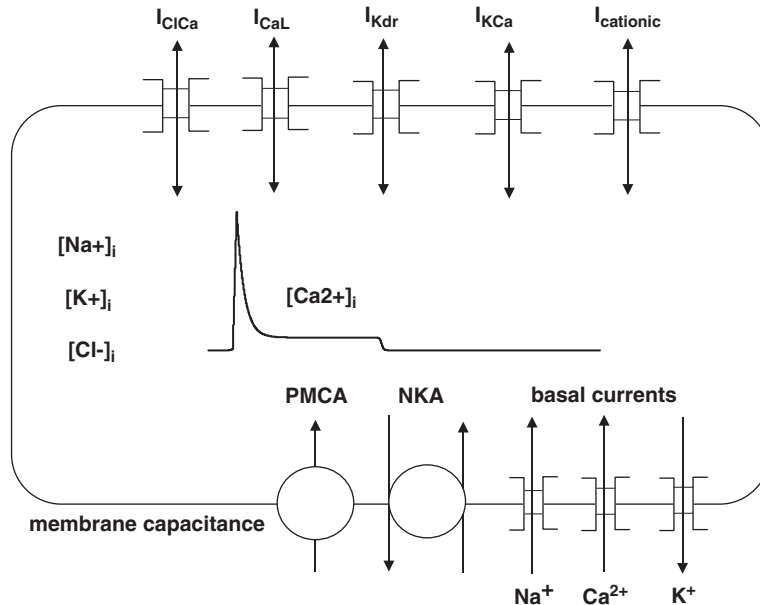


Fig. 4. Model of membrane conductances. Schematic presentation of the model. The model includes L-type voltage-operated Ca^{2+} current (I_{CaL}), Ca^{2+} -activated Cl^- current (I_{ClCa}), Ca^{2+} -activated K^+ current (I_{KCa}), delayed rectifier K^+ current (I_{Kdr}), non-specific cationic current (I_{cationic}), basal currents for Ca^{2+} , Na^+ and K^+ , the plasma membrane Ca^{2+} ATPase (PMCA) and the Na^+-K^+ ATPase (NKA). Extracellular and intracellular concentrations of Na^+ , K^+ , Ca^{2+} and Cl^- are fixed or forced.

where Ca_o , Ca_i , Na_o , Na_i , K_o , K_i , Cl_o and Cl_i are the extracellular and intracellular concentrations of calcium, sodium, potassium and chloride ions respectively. The model constants were: F (Faraday constant) = 96485.3415 C/mol; R (gas constant) = 834.472 mJ/(mol K); and temperature (T) = 310.0 K.

The delayed rectifier K^+ current (I_{Kdr}) was modelled by using a set of equations based on those previously determined by Kotlikoff (1990) and Boyle et al. (1992) with the parameter values adjusted according to the experimental electrophysiological characterisation from canine and porcine tracheal cells (Boyle et al., 1992; Kotlikoff, 1990). The current was given by the following equation:

$$I_{Kdr} = G_{Kdr}(\gamma_{KSS} + (x_{i1} + x_{i2})(1 - \gamma_{KSS}))x_a^2(E - E_K). \quad (5)$$

in which E is the membrane potential, G_{Kdr} (0.035 μ S) is the conductance of the delayed rectifier channel, γ_{KSS} (0.15) is the ratio of G_{Kdr} on the steady state conductance, x_a is the voltage-dependent activation gate and x_{i1} and x_{i2} the voltage-dependent inactivation gates, as described below:

$$\frac{dx_a}{dt} = (x_{a,\infty} - x_a)/\tau_{xa} \quad (6)$$

$$\text{with } x_{a,\infty} = 1/(1 + e^{(5.5-E)/6.0}) \quad (7)$$

$$\text{and } \tau_{xa} = -0.00003552E + 0.003262 \text{ s}, \quad (8)$$

$$\frac{dx_{i1}}{dt} = (x_{i1,\infty} - x_{i1})/\tau_{xi1} \quad (9)$$

$$\text{with } x_{i1,\infty} = 1/(1 + e^{(E+4.3)/7.5}) \quad (10)$$

$$\text{and } \tau_{xi1} = 0.25 \text{ s},$$

$$\frac{dx_{i2}}{dt} = (x_{i2,\infty} - x_{i2})/\tau_{xi2} \quad (11)$$

$$\text{with } x_{i2,\infty} = 1/(1 + e^{(E+4.3)/7.5}) \quad (12)$$

and $\tau_{xi2} = 1.98$ s. Initial values were $x_a = 0.001$; $x_{i1} = x_{i2} = 0.0$.

The L-type voltage-dependent Ca^{2+} current (I_{CaL}) was modelled by using equations inherited from OXSOF cardiac modelling. Parameter values were fitted according to the experimental results published from canine trachea for the permeability (P_{CaL}) and the voltage-dependent activation gate (d_L) (Kotlikoff, 1988) and from human bronchus for the voltage-dependent inactivation gate (f_L) (Marthan et al., 1989).

$$I_{CaL} = 4P_{CaL}d_Lf_L(E - V_{surf}) \frac{(F/RT)}{(1 - e^{-(E-V_{surf})2F/RT})} ((Ca_i e^{-(E-V_{surf})2F/RT}) - (Ca_o e^{-(E-V_{surf})2F/RT})), \quad (13)$$

$$\frac{dd_L}{dt} = \alpha_d - (\alpha_d + \beta_d)d_L, \quad (14)$$

$$\alpha_d = 30(E + 18)/(1 - e^{0.25(E+18)}) \text{mV}^{-1} \text{s}^{-1}, \quad (15)$$

$$\beta_d = 12(E + 18)/(e^{0.1(E+18)} - 1) \text{mV}^{-1} \text{s}^{-1}, \quad (16)$$

$$\frac{df_L}{dt} = (f_\infty - f_L)/\tau_f, \quad (17)$$

$$\text{with } f_\infty = 1/(1 + e^{(E+23)/6.6}) \quad (18)$$

and $\tau_f = 0.0173$ s.

Constant values were taken for the permeability of the L-type channels $P_{\text{CaL}} = 0.0030 \text{ nA mM}^{-1}$, and V_{surf} (the surface charge of the channel) = 150 mV. Initial values were $d_L = 0.0$, $f_L = 1.0$.

The Ca^{2+} -activated K^+ current (I_{KCa}) description was based on the equations from the model of Vergara and Latorre (1983), and data were from canine tracheal cells (McCann and Welsh, 1986). I_{KCa} was given by the equation:

$$I_{\text{KCa}} = G_{\text{KCa}}x_{\text{Ca1}}B(E - E_K), \quad (19)$$

where G_{KCa} (2.45 μS) is the conductance, and x_{Ca1} and B are gating variables.

$$\frac{dx_{\text{Ca1}}}{dt} = (x_{\text{Ca1},\infty} - x_{\text{Ca1}})/\tau_{x_{\text{Ca1}}}, \quad (20)$$

$$\text{where } \tau_{x_{\text{Ca1}}} = 1 \text{ s}, \quad (21)$$

$$x_{\text{Ca1},\infty} = \frac{(\text{Ca}_i^2 + K_4\text{Ca}_i)}{(\text{Ca}_i^2 + (K_4\text{Ca}_i(1 + (\alpha/\beta))) + K_4K_2(\alpha/\beta))}, \quad (22)$$

where $\alpha = 280 \text{ s}^{-1}$, $\beta = 480 \text{ s}^{-1}$ are rate constants.

$$K_4 = 0.0000125 e^{(-1.99(EF/RT))} \text{mM} \quad (23)$$

$$\text{and } K_2 = 0.000275 e^{(-1.51(EF/RT))} \text{mM}. \quad (24)$$

$$\text{For } \text{Ca}_i \geq 0.1 \text{ mM}, \quad dB/dt = K_1\text{Ca}_ix_{\text{Ca1}} - K_{-1}B, \quad (25)$$

$$\text{where } K_{-1} = 0.24e^{(-0.012E)} \text{s}^{-1} \quad (26)$$

$$\text{and } K_1 = 0.85e^{(0.04E)} \text{mM}^{-1} \text{s}^{-1}. \quad (27)$$

For other values of Ca_i : $B = 1$.

Initial values in the above differential equations were: $x_{\text{Ca1}} = 1.0$, $B = 0.001$.

The Ca^{2+} -activated Cl^- current (I_{ClCa}) was described by using the already published model by Roux et al. (2001) and experimental characterisation by Janssen and Sims (1995):

$$I_{\text{ClCa}} = G_{\text{Cl}}(E - E_{\text{Cl}})(1/1 + (1 + (\text{Ca}_{\text{CT}}/\text{Ca}_i)^h)), \quad (28)$$

where $G_{\text{Cl}} = 0.01 \mu\text{S}$ is the conductance of the channel, $\text{Ca}_{\text{CT}} = 500 \text{ nM}$, and $h = 3$.

The non-specific cationic current (I_{cationic}) was described by the sum of each ionic current through the channel:

$$I_{\text{cationic}} = I_{\text{nsNa}} + I_{\text{nsK}} + I_{\text{nsCa}}, \quad (29)$$

with

$$I_{\text{nsNa}} = \text{ns} \alpha_{\text{Ca}} P_{\text{ns(Ca)}} EF^2/RT \frac{(0.75\text{Na}_i e^{(EF/RT)}) - 0.75\text{Na}_o}{e^{(EF/RT)} - 1}, \quad (30)$$

$$I_{\text{nsK}} = \text{ns} \alpha_{\text{Ca}} P_{\text{ns(Ca)}} EF^2/RT \frac{(0.75\text{K}_i e^{(EF/RT)}) - 0.75\text{K}_o}{e^{(EF/RT)} - 1}, \quad (31)$$

$$I_{\text{nsCa}} = 4\text{ns} \alpha_{\text{Ca}} P_{\text{ns(Ca)}} EF^2/RT \frac{(1.5\text{Ca}_i e^{(EF/RT)}) - 1.5\text{Ca}_o}{e^{(EF/RT)} - 1}. \quad (32)$$

In these equations, inherited from Luo-Rudy cardiac model,

$$\alpha_{\text{Ca}} = 1/(1 + (K_{\text{m,ns(Ca)}}/\text{Ca}_i)^3), \quad (33)$$

with $K_{\text{m,ns(Ca)}} = 0.0012 \text{ mM}$.

The gating variable ns is given by the equations:

$$\frac{\text{dns}}{\text{dt}} = (\text{ns}_\infty - \text{ns})/\tau_{\text{ns}}, \quad (34)$$

$$\text{ns}_\infty = 1/(1 + e^{((E-69.8)/-11.9)}), \quad (35)$$

$$\tau_{\text{ns}} = -0.00089875E + 0.20025 \text{ s}, \quad (36)$$

$$P_{\text{ns(Ca)}} = 0.0000000175 \text{ nA mM}^{-1} \text{ mol C}^{-1}.$$

Values were obtained by fitting the model with experimental data from equine and porcine tracheal cells (Fleischmann et al., 1997; Wang et al., 1997; Yamashita and Kokubun, 1999). Initial value for ns was 1.0.

Models of PMCA and the NKA were taken from models of pumps and exchanger developed in OXSOF. Parameter values for PMCA current were derived from experimental data obtained in dog trachea (Janssen et al., 1997). In the absence of precise available data in airway smooth muscle, data for NKA was taken from gastric smooth muscle (Skinner et al., 1993). As explained above, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger was not incorporated in the model on the basis of experimental studies indicating that it was not significantly involved in Ca^{2+} handling upon ASMC stimulation (Janssen et al., 1997).

Equations were as follows:

$$I_{\text{pCa}} = I_{\text{pCa max}}(\text{Ca}_i/(Kmp_{\text{Ca}} + \text{Ca}_i)), \quad (37)$$

where $I_{pCa_{max}}$ (1.15 nA) is the maximal current and Kmp_{Ca} (0.05 mM) the half-saturation constant of the PMCA.

$$I_{NaK} = I_{NaK_{max}}(K_o/(Km_K + K_o))(Na_i/(Km_{Na} + Na_i)), \quad (38)$$

where $I_{NaK_{max}}$ (0.7 nA) is the maximal current and Km_K (1 mM) and Km_{Na} (40 mM) the half-saturation constants of the NKA for K^+ and Na^+ , respectively.

Background Ca^{2+} , K^+ and Na^+ currents (I_{bCa} , I_{bK} and I_{bNa} , respectively) were described by the following conductance equations, as in DiFrancesco and Noble's model (DiFrancesco and Noble, 1985):

$$I_{bCa} = G_{bCa}(E - E_{Ca}), \quad (39)$$

$$I_{bK} = G_{bK}(E - E_K), \quad (40)$$

$$I_{bNa} = G_{bNa}(E - E_{Na}). \quad (41)$$

In the absence of available experimental data in airway smooth muscle cell on these background currents, conductances were determined on the assumption of ionic homeostasis at rest, i.e., that, for each ion, the sum of the current was equal to zero. The constant values were then $G_{bCa} = 1.194 \times 10^{-5} \mu S$, $G_{bNa} = 0.003263 \mu S$ and $G_{bK} = 0.008729 \mu S$.

The membrane potential was calculated from the different ionic currents and the capacitance (cap) according to the following differential equation:

$$\frac{dE}{dt} = \frac{-1}{cap} (I_{CaL} + I_{Kdr} + I_{KCa} + I_{ClCa} + I_{cationic} + I_{NaK} + I_{pCa} + I_{bCa} + I_{bK} + I_{bNa}). \quad (42)$$

The capacitance, estimated from experiments and in accordance with the literature (McCulloch et al., 2000; Zhang et al., 2004), was 0.00002 μF . The initial value for E was -60 mV.

1.6.3. Predictions of the model

The model was used with fixed extracellular and intracellular ion concentrations. With ion concentrations corresponding to generally accepted physiological values in smooth muscle cells, the model predicts a stable value of the membrane potential about -60 mV, a value close to the physiological one determined in patch-clamp experiment and in measurement using microelectrodes.

As explained previously, the cholinergic response of ASMC does not appear to depend significantly on the influx of extracellular calcium, though L-type Ca^{2+} channels are present and functional in ASMC (Marthan et al., 1989; Roux et al., 1997). Hence we used our model to predict the amplitude of Ca^{2+} influx upon ACh stimulation. Since cholinergic agonists act primarily by the production of $InsP_3$ and calcium release from the RS, cholinergic activation of the membrane conductances was mimicked by simulating a $[Ca^{2+}]_i$ increase similar to that observed in ASMC. We used forced equations to simulate a non-oscillating $[Ca^{2+}]_i$ increase similar to that experimentally recorded in freshly isolated ASMCs (see Fig. 5A). As shown in Fig. 5B, this $[Ca^{2+}]_i$ increase induces a membrane depolarisation from -60 to -46 mV. This depolarisation is mainly due to the activation of the Ca^{2+} -activated Cl^- current, and tends to be limited by the activation of the Ca^{2+} -activated K^+ current, without any significant opening of the delayed rectifier K^+ current (cf. Fig. 5C). Ion fluxes through the non-specific cationic channel are

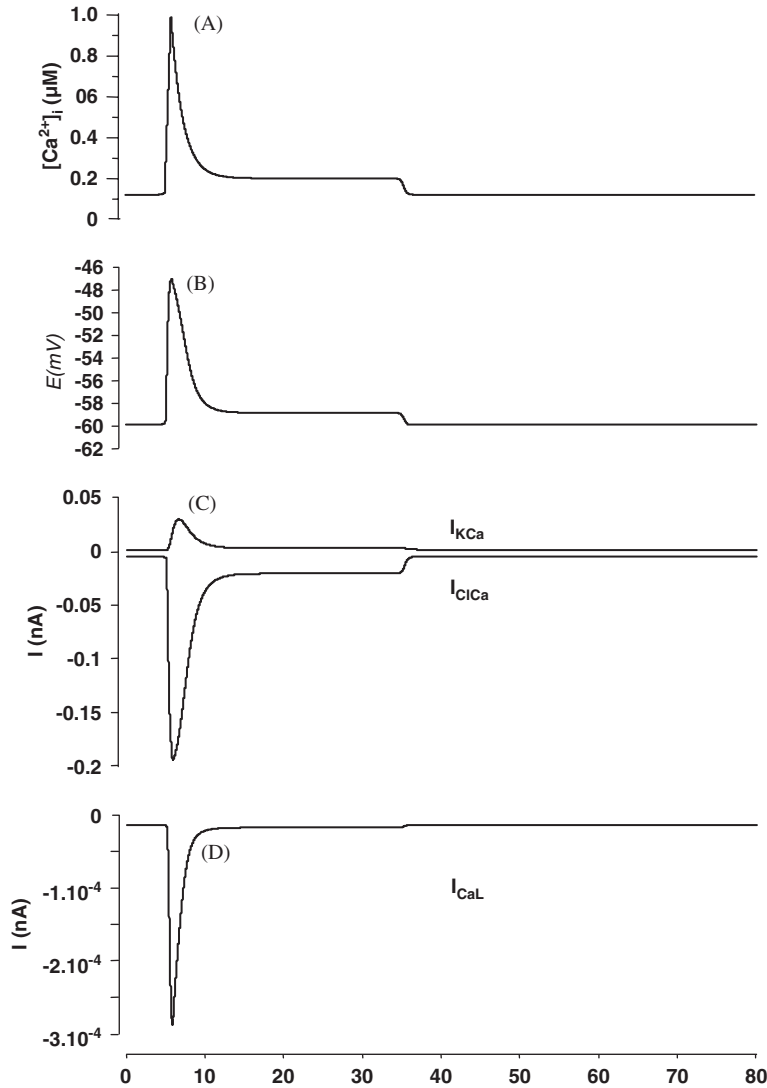


Fig. 5. Effect of $[Ca^{2+}]_i$ increase on membrane potential and membrane conductances. (A) Forced $[Ca^{2+}]_i$ corresponding to a simulated non-oscillating $[Ca^{2+}]_i$ response to ACh (30 s). Equations of forced $[Ca^{2+}]_i$ were as used previously (Roux et al., 2001). (B) Predicted variations in membrane potential (E), in mV. (C) Predicted I_{ClCa} and I_{KCa} , in nA. (D) Predicted I_{CaL} , in nA. Extracellular and intracellular K^+ , Na^+ and Cl^- were: $[K^+]_o = 5$ mM; $[K^+]_i = 150$ mM; $[Na^+]_o = 130$ mM; $[Na^+]_i = 12$ mM; $[Cl^-]_o = 140$ mM; $[Cl^-]_i = 55$ mM. $[Ca^{2+}]_o$ was 2 mM.

negligible. Due to the limited depolarisation, there is negligible activation of Ca^{2+} current, as shown in Fig. 5D, and hence no significant extracellular Ca^{2+} influx.

The model predicts that the amplitude of the depolarisation depends mainly on the conductance of the Ca^{2+} -activated Cl^- current. We hence carried out studies to investigate the sensitivity of the model predictions to variation in the conductance of this channel. Fig. 6A shows the predicted variation in membrane potential induced by a 5 s Ca^{2+} puff up to 1 μM for G_{Cl} varying from

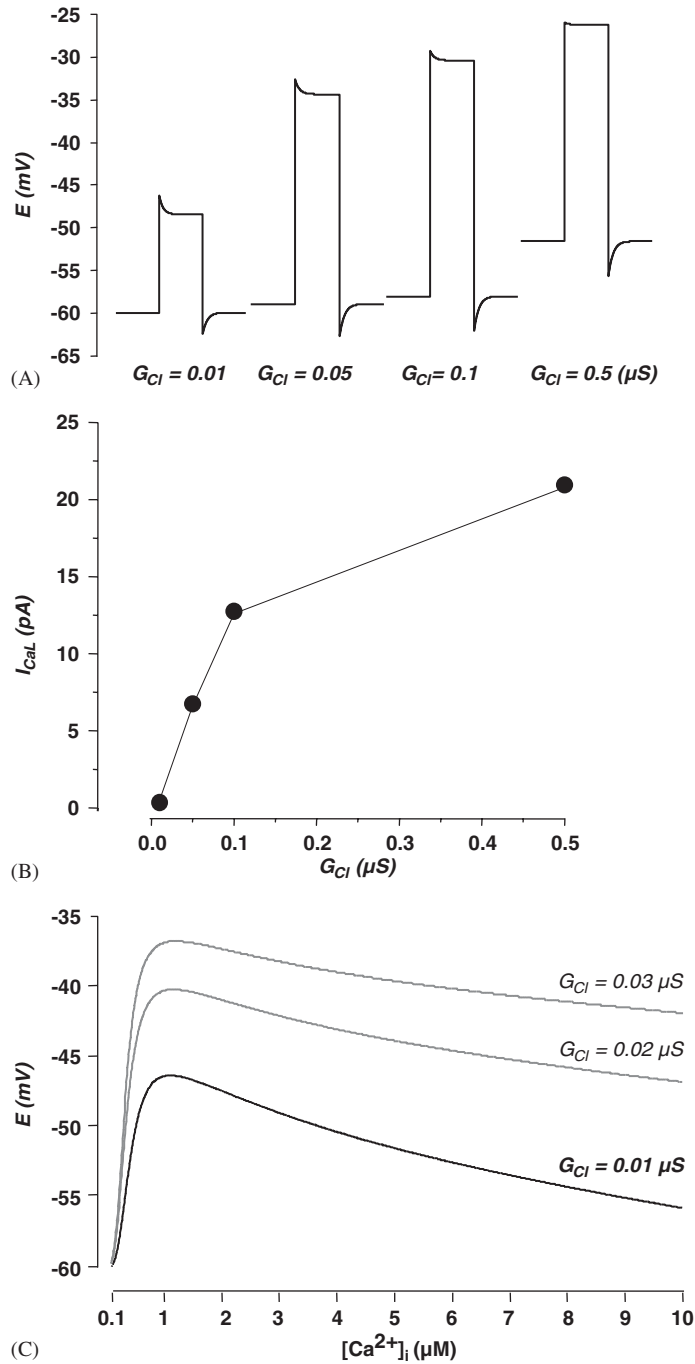


Fig. 6. Influence of G_{Cl} and $[Ca^{2+}]_i$ on membrane potential and Ca^{2+} influx. (A) Predicted variations in membrane potential (E , in mV) induced by a 5 s increase in $[Ca^{2+}]_i$ up to $1 \mu\text{M}$, for various G_{Cl} (in μS). (B) Predicted value of voltage-activated Ca^{2+} current (I_{CaL} , in pA) for various G_{Cl} (in μS). Predicted relationship between membrane potential (E , in mV) and $[Ca^{2+}]_i$ (in μM) for $G_{Cl} = 0.01 \mu\text{S}$ (value corresponding to experimental data), $G_{Cl} = 0.02$ and $0.03 \mu\text{S}$.

0.01 μS , the value introduced in the model on the basis of experimental data (Roux et al., 2001), up to 0.5 μS . The predictions of the model indicate that increasing G_{Cl} increases membrane depolarisation, but not in a rectilinear manner, so the voltage remains below -25 mV . As a consequence, Ca^{2+} current through L-type Ca^{2+} current, the main source on extracellular Ca^{2+} entry in ASMCs, remains weak, as shown in Fig. 6B.

In the series of simulations mentioned above and illustrated in Fig. 6A and B, stimulation was mimicked by a Ca^{2+} puff of 1 μM amplitude. This is in the range of experimentally recorded values in ASMCs (Roux et al., 1997; Hyvelin et al., 2000a,b). However, $[\text{Ca}^{2+}]_i$ may be heterogeneous and local Ca^{2+} concentration close to the SR may be much higher than the average $[\text{Ca}^{2+}]_i$. Hence, Ca^{2+} release from the SR close to the plasma membrane may lead to higher local $[\text{Ca}^{2+}]_i$ than that recorded in microspectrofluorimetry. We have used our model to test the effect of such high local $[\text{Ca}^{2+}]_i$ values by increasing the forced $[\text{Ca}^{2+}]_i$ up to 10 μM . According to the model, the maximum depolarisation is observed between 1 and 2 μM Ca^{2+} and is about -46 mV , a value for which there is only a small opening of the L-type voltage-operated Ca^{2+} current and no significant extracellular Ca^{2+} influx. We tested the predictions of the model for various values both for $[\text{Ca}^{2+}]_i$ and G_{Cl} , the two main agents of membrane depolarisation. Results are shown in Fig. 6C. For $[\text{Ca}^{2+}]_i$ higher than 1–2 μM , the depolarisation does not increase because the Ca^{2+} -activated Cl^- current is already maximum and even tends to decrease. This is also observed for G_{Cl} values higher than that initially introduced in the model. According to the model, this decrease in membrane depolarisation for high $[\text{Ca}^{2+}]_i$ is due to a significant increase in Ca^{2+} extrusion by the PMCA which tends to hyperpolarise the plasma membrane. This is an interesting prediction of the model, because it points out the importance the PMCA may have in Ca^{2+} homeodynamics in the case of high subplasmalemmal Ca^{2+} concentration. At the same time, this prediction should be considered carefully because if the PMCA participates in a critical manner in the membrane potential in the hypothesis of high local $[\text{Ca}^{2+}]_i$, another mechanism of Ca^{2+} extrusion, i.e., the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) functionally associated with the NKA, may interfere. In the present model, NCX was not introduced on the basis of previous experimental data showing that it was not significantly involved in Ca^{2+} handling upon cell stimulation (Janssen et al., 1997). On the basis of the predictions of our model, it appears that the role of NCX in Ca^{2+} handling in ASMCs should be re-examined both experimentally and theoretically. Further experiments should be carried out on CNX in ASMCs, and future development of our model should include NCX. However, introduction of CNX in the model needs previously, to be relevant, available experimental data. Moreover, though the predictions of the model for very high $[\text{Ca}^{2+}]_i$ should be taken very carefully, this does not invalidate its predictions for lower $[\text{Ca}^{2+}]_i$ values.

Taken together, all this may explain why, in airway smooth muscle cells, though L-type Ca^{2+} channels are present and may be activated by depolarisation, intracellular Ca^{2+} influx does not significantly participate to the $[\text{Ca}^{2+}]_i$ response upon cholinergic stimulation, even in the hypothesis of high subplasmalemmal Ca^{2+} concentration upon cholinergic stimulation. However, these explanations should not be considered as complete or definitive. Rather, this model should be understood as a first step in the modelling of the electrophysiological properties of ASMCs that may help in pointing out some privileged directions for further investigation of airway smooth muscle physiology. For example, the predictions of the model for high $[\text{Ca}^{2+}]_i$ points out that the mechanisms responsible for Ca^{2+} extrusion, even though not directly implicated in cytosolic Ca^{2+} clearance, may play a significant role in the plasma membrane electrophysiology. Also, a

more systematic experimental characterisation of the different membrane conductances in several species, at least in those most used as animal models, and if possible in human ASMC, would be useful. Such studies are usually not well considered, because they are qualified as “only descriptive” in cells like ASMCs in which Ca^{2+} influx is not an important signalling pathway. However, these conductances actually exist in these cells, and the development of theoretical models highlights that their characterisation is not only descriptive but fully explicative. Some electrophysiological studies would also be useful to test the predictions of the model. Voltage recording in current-clamp configuration would allow to measure the membrane potential at rest and when an increase in $[\text{Ca}^{2+}]_i$ concentration is triggered. Since the model predicts that the depolarisation depends mainly on the activation of the Ca^{2+} -activated Cl^- current, experiments should also be performed in experimental conditions in which this current is inhibited.

1.7. Conclusion

In contrast with what has been done in cardiac physiology, few theoretical models of smooth muscle cells have been developed to understand the excitation-contraction coupling in these cells. On the basis of experimental characterisation of the Ca^{2+} signal in airway myocytes, we have built some mathematical models specifically dedicated to investigate the mechanisms responsible for Ca^{2+} signalling in airway smooth muscles. These models are incomplete and somehow “composite”, since they incorporate experimental data from several species and sometimes several smooth muscle cell types. However, these models give interesting information on Ca^{2+} dynamics in airway myocytes. For example, our theoretical investigation of cytosolic Ca^{2+} clearance has broken the traditional intuitive dogma of a direct and immediate Ca^{2+} reuptake by SERCA following Ca^{2+} release from the SR, and pointed out the critical role of Ca^{2+} -binding proteins in the shape of the Ca^{2+} signal. Our model of membrane conductances may explain why Ca^{2+} influx is not significantly involved in the Ca^{2+} signal upon cholinergic stimulation in ASMCs, though L-type channels are present and functional, and also indicates that the role of NCX in these cells should be re-examined. The development of such models of airway myocytes is important for the understanding of the cellular mechanisms of airway reactivity and their possible modulation by pharmacological agents. This requires further development of the model that includes the contractile apparatus in order to have a stimulation-contraction coupling model. Such a model would help in the development of therapeutic targets against airway hyperreactivity. Additionally, such models can be used as a basis for the building of theoretical models of other smooth muscle cells, in particular vascular myocytes involved in vascular reactivity.

Editor's note: Please see also related communications in this volume by Moskvin et al. (2005) and Hinch et al. (2005). For further downloadable content please see <http://www.physio-me.org.nz/publications/PBMB-2005-89/Roux/>

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