Temporal Aspects of Ca^{2+} Signaling in Airway Myocytes

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Contents

Abstract This chapter discusses how variation in intracytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) over time can be considered as a signal for the contractile machinery. It presents an overview of the literature that describes the Ca^{2+} response pattern to different contractile or relaxant agonists in several types of airway smooth muscle cells (ASMCs) depending on the species and location in the airway tree and recording methods, insisting on the temporal aspects of this response. Since the dynamics of the Ca^{2+} signal depends on the dynamics of the mechanisms

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Y.-X. Wang (ed.), *Calcium Signaling In Airway Smooth Muscle Cells*,
DOI 10.1007/978-3-319-01312-1_8,

responsible for this signal, the chapter presents an overview of the main mechanisms responsible for Ca^{2+} homeodynamics in ASMCs. By analyzing some examples, it shows how the kinetics of these mechanisms determine the pattern of the Ca^{2+} signal. The consequence of cell-to-cell variations in the Ca^{2+} signal is also discussed, with special attention to oscillatory versus nonoscillatory responses. The last part of the chapter presents the relationship between the parameter of the Ca^{2+} signal and the pattern of the contractile response. The mechanisms of the contractile apparatus itself will not be detailed, this question being beyond the scope of the chapter, but the temporal relationship between the Ca^{2+} signal and the subsequent contraction is analyzed.

Keywords Calcium dynamics • Oscillations • Kinetics • Model • Contraction

1 $[Ca^{2+}]_i$ Variations as a Signal

1.1 What Makes Ca^{2+} Variation a Signal?

A Ca^{2+} signal is, by definition, a variation over time or space of the intracellular Ca^{2+} concentration that is detected by a given intracellular machinery, so that the behavior of the cell is altered according to the variation in Ca^{2+} concentration. In this chapter, we consider only temporal variation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), which is a major determinant of airway smooth muscle cells (ASMC) contraction. Though the Ca^{2+} signal may encode for several cell functions, such as contraction, proliferation, and secretion, this chapter will consider only the contractile response of ASMCs as a cell function.

Time-dependent variations in $[Ca^{2+}]_i$ is considered a signal insofar as they carry information that the intracellular machinery is able to decipher, i.e., is able to demonstrate a behavior specifically dependent on these $[Ca^{2+}]_i$ variations, and that is why biologists study temporal variation in $[Ca^{2+}]_i$. Hence, what is of interest for biologists is what makes sense for the cell. This determines which parameters should be used to characterize Ca^{2+} signals and the relevant correlations that can be established between the variation in the intensity of stimulation and the $[Ca^{2+}]_i$ response on the one hand, and between the parameters of the Ca^{2+} signal and the cellular functional response on the other hand. The scheme of the signaling pathway can be summarized as follows:

$$[\text{agonist}]\ (1) \rightarrow Ca^{2+}\text{signal}\ (2) \rightarrow \text{contractile response},$$

where the first step (1) corresponds to the encoding of the Ca^{2+} signal and the second step (2) to the decoding of the Ca^{2+} signal. A signal can be basically defined as the oriented relationship through a communication channel between an issuing system (source) and a recipient system (receptor). The information is what modifies the state or the evolution of the system, and the message is the expression of this information. A given Ca^{2+} signal may carry several distinct messages, insofar as the Ca^{2+} signal can be deciphered by distinct recipient systems. For example, a Ca^{2+} signal may induce contraction, proliferation, or secretion, for example, each behavior being determined by a given effector system capable of deciphering the Ca^{2+} signal. This chapter focuses on the contractile apparatus.

According to this scheme, in ASMCs, the source of the contractile information is the agonist/agonist receptor complex and the recipient system or final receptor is the contractile apparatus. A time-dependent variation in $[Ca^{2+}]_i$ is the signal that carries the message, i.e., the contractile information. Hence, the temporal aspects of Ca^{2+} signals are at the heart of the notion of Ca^{2+} signal.

1.2 Signal and Message

The signal is the change in time or space of the cytosolic Ca^{2+} concentration. The message is the contractile information encoded in the signal. Hence, it is important to determine what makes a time-dependent variation in the concentration of free Ca^{2+} in the cytosol a message for the contractile machinery. Whatever the complexity of the mechanisms activated by an increase in $[Ca^{2+}]_i$, Ca^{2+} ions by themselves carry a message only if they bind to a target molecule. Hence, variation in $[Ca^{2+}]_i$ is a message only if it induces a correlated change in Ca^{2+} binding to a transmitter molecule, a Ca^{2+}-binding signaling protein. This corresponds to the schematic reaction

$$Ca^{2+} + M \underset{k_-}{\overset{k_+}{\rightleftharpoons}} Ca^{2+}\text{-}M$$

where M is the signaling molecule, and k_+ and k_- are the kinetic constants of the reaction. Accordingly, any change in $[Ca^{2+}]_i$ induced by an increase or decrease in the total amount of Ca^{2+} in the cytosol will induce a change in the concentration of the Ca^{2+}-M complex. Hence, variations in $[Ca^{2+}]_i$ can be viewed as carrying a message. However, this does not mean that the temporal pattern of the Ca^{2+}-M complex, i.e., the message, is similar to that of the Ca^{2+} signal. This is true only if k_+ and k_- are such that the reaction can be considered as instantaneous. Additionally, it should be noted that a given Ca^{2+} signal can carry distinct messages, not only in the sense that it activates different cell systems but also by the fact that the temporal sensitivity of the signaling molecules of the different recipient systems may differ.

1.3 Deciphering the Code

A signal is an encoded message. Deciphering the code entails determining how the cell converts the spatiotemporal change in $[Ca^{2+}]_i$ into a given change in the contractile status of the ASMCs. More precisely, the identification of the mechanisms responsible for the changes in $[Ca^{2+}]_i$ upon ASMC stimulation corresponds to the identification of the encoding mechanisms used by the Ca^{2+} homeodynamic system to convert the stimulation induced by the agonist into a Ca^{2+} signal, and the identification of the mechanisms responsible for the Ca^{2+}-contraction coupling means decrypting the code used by the cell to carry contractile information.

About the decryption of the Ca^{2+} code, it should be noted that the choice in the parameters used to describe the Ca^{2+} signal determines the underlying hypotheses on the nature of the coding system. Indeed, decryption of the code is done by establishing correlations between the contractile response and parameters of the Ca^{2+} signal. Since correlation cannot be established with unstudied parameters, exclusion of parameters should be based on explicit and relevant hypotheses on the possible encoding significance of such parameters. What may appear as a signal for the investigator may not be a message for the cell. For example, several studies use average $[Ca^{2+}]_i$ recording on tissue containing several ASMCs. This average $[Ca^{2+}]_i$ response can provide information on cell physiology. However, this $[Ca^{2+}]_i$ response is the average of the $[Ca^{2+}]_i$ signal of each ASMC but cannot be considered as a signal, even as a signal of whole tissue contraction, because it carries no information for each ASMC contractile apparatus. In addition, the area of the Ca^{2+} response, i.e., the integral of the $[Ca^{2+}]_i$ curve, in some studies called the Ca^{2+} index [1], gives no information on the dynamics of the Ca^{2+} response. Hence, considering it as a parameter of the Ca^{2+} signal for the cell requires the underlying hypothesis that the information carried by the Ca^{2+} signal is independent of the dynamics of the signal. Change in the Ca^{2+} index can be viewed as a sign that the Ca^{2+} signal is altered but not as a signal of a message in itself if the contractile apparatus is sensitive to the dynamics of $[Ca^{2+}]_i$ variations.

How can we decipher the contractile code? Description of the signal is not the same thing as understanding the message. Identification of the quantitative relationship between the parameters of the Ca^{2+} signal is a way of deciphering the contractile code. However, identification of correlation does not mean identification of the causal relationship between Ca^{2+} and contraction. Mathematical modeling is a useful tool for investigating the causal relationships between the pattern of the Ca^{2+} signal and that of the subsequent contraction, which is the decryption of the contractile code.

1.4 Different Temporal Aspects of Ca^{2+} Signal

The temporal dimension is included in the concept of a Ca^{2+} signal itself. The temporal aspect of a Ca^{2+} signal appears at different stages of the stimulation-response pathway. First, insofar as $[Ca^{2+}]_i$ change is an intracellular message

induced by the stimulation of the cell by an extracellular agonist, and is hence a consequence of the activation of a network of intracellular mechanisms that determine the pattern of $[Ca^{2+}]_i$ variations, the temporal dimension of these interactions is critical for the shape of the Ca^{2+} signal and, hence, its informative content. This represents the encoding of the Ca^{2+} signal. Second, in as much as $[Ca^{2+}]_i$ variations represent a signal for the contractile machinery, in the sense that they determine the contractile behavior of ASMCs, both in amplitude and time, the time-dependent parameters that describe the Ca^{2+} signal should be considered information on contraction, i.e., information that can be detected by the contractile system. This represents the decoding of the Ca^{2+} signal, which depends on the kinetics of the functional interactions in the contractile system.

Hence, the temporal aspects of the Ca^{2+} signal include several distinct issues: (1) the temporal aspects of the Ca^{2+} signal pattern itself; (2) upstream of the Ca^{2+} signal, the temporal aspects of Ca^{2+} signal encoding, i.e., the dynamics of the mechanisms involved in the Ca^{2+} response to cell stimulation; and (3) downstream of the Ca^{2+} signal, the dynamics of the decoding machinery associated with the contractile apparatus that determines the pattern of the contractile response to Ca^{2+} signaling.

2 Overview of ASMC Ca^{2+} Handling

2.1 General Scheme: Bow-Tie Architecture

Free cytosolic Ca^{2+} concentration is the consequence of the balance between the ON mechanisms that tend to increase $[Ca^{2+}]_i$ and the OFF mechanisms that tend to decrease it. Signaling pathways that induce a Ca^{2+} signal can be viewed as acting on the ON/OFF balance and, by doing so, inducing a spatiotemporal change in $[Ca^{2+}]_i$ corresponding to the encoding of the Ca^{2+} signal. This Ca^{2+} signal can be "read" by the contractile machinery, and the resulting change in contraction corresponds to the decoding of the Ca^{2+} signal. This decoding process can be viewed also as a combination of ON mechanisms that tend to increase contraction for a given Ca^{2+} signal and OFF mechanisms that tend to decrease contraction. The Ca^{2+} signal is hence at the node of encoding and decoding processes, acting upstream and downstream of the Ca^{2+} signal, respectively, on which cell stimulation acts, and excitation-contraction coupling can be analyzed in the conceptual framework of this general scheme [2]. Such a scheme, called a bow-tie architecture, initially used to analyze engineered systems, is also useful for analyzing the information network that commands cell behavior such as excitation-contraction coupling [3–6]. This scheme is presented in Fig. 1a. According to this scheme, the structure involved in Ca^{2+} homeodynamics is the ON and OFF encoding mechanisms. They are summarized in Fig. 1b.

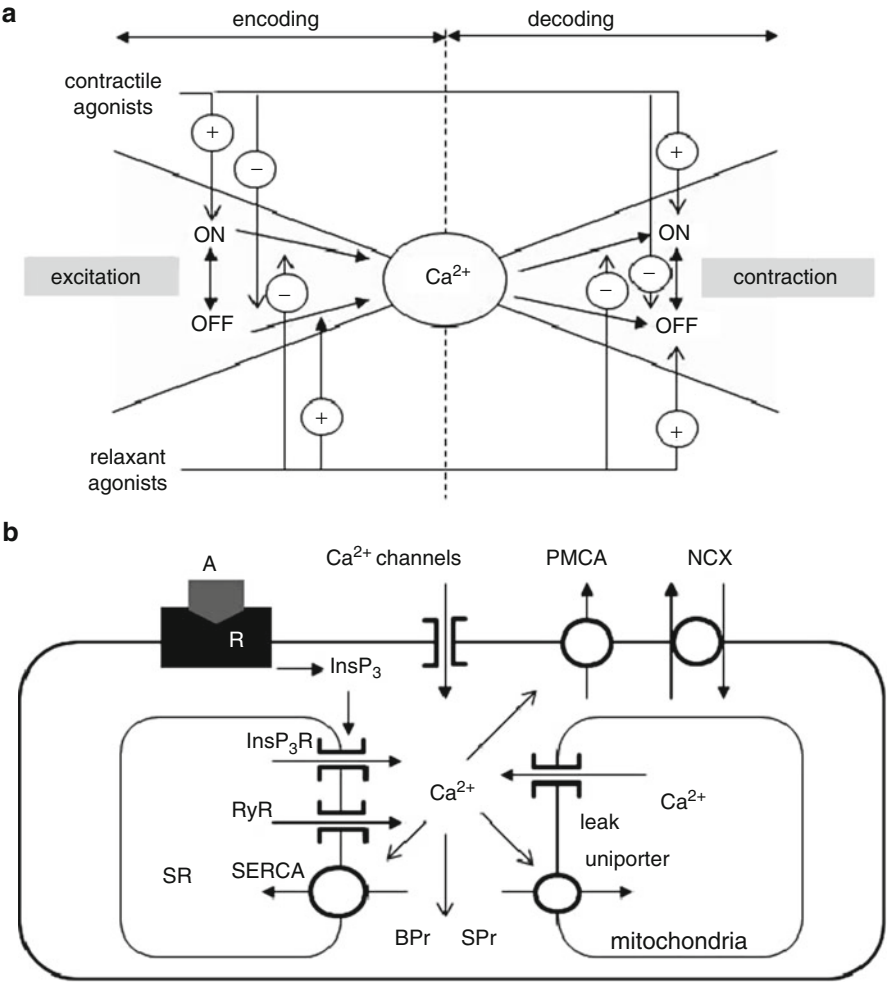

Fig. 1 General mechanisms of Ca^{2+} handling in ASMCs. (**a**) Representation of bow-tie architecture applied to ASMC excitation-contraction coupling. The Ca^{2+} signal pattern is determined by the temporal balance between ON and OFF mechanisms. The Ca^{2+} signal is also decoded by ON and OFF mechanisms on which depends the contraction. Contractant and relaxant agonists may act on the ON and OFF mechanisms of both encoding and decoding of the Ca^{2+} signal. (**b**) General scheme of main ON and OFF mechanisms of Ca^{2+} handling. The main ON mechanisms are (1) Ca^{2+} release from the SR through $InsP_3$ receptors ($InsP_3R$) activated by agonists (*A*) acting on membrane receptors coupled to PLC (*R*) or through ryanodine receptors (RyRs) and (2) extracellular Ca^{2+} influx through several Ca^{2+} channels (VOC, ROC, and SOC). The main OFF mechanisms are (1) Ca^{2+} extrusion through PMCA and NCX, (2) Ca^{2+} pumping into the SR by SERCA, (3) Ca^{2+} uptake by mitochondrial Ca^{2+} uniporter, and (4) Ca^{2+} binding to fast kinetics–low affinity cytosolic proteins (SPr) and slow kinetics–high affinity cytosolic proteins (BPr)

2.2 Encoding Mechanisms of Ca^{2+} Signal

2.2.1 ON Mechanisms

The ON mechanisms, by which $[Ca^{2+}]_i$ is increased upon ASMC stimulation, are basically Ca^{2+} release in the cytosol from the sarcoplasmic reticulum (SR) and extracellular Ca^{2+} influx through the plasma membrane. The major contractile agonists, such as acetylcholine and histamine, act primarily via Inositol 1,4,5-trisphosphate ($InsP_3$) production and Ca^{2+} release from the cytosol via $InsP_3$ receptors ($InsP_3R$) located in the sarcolemma [7–11]. Ca^{2+} release from the SR can also be due to the opening of the ryanodine receptors (RyR), which can be activated by $[Ca^{2+}]_i$ increase, the so-called Ca^{2+}-induced Ca^{2+} release (CICR), and cyclic ADP-ribose [12–14]. External Ca^{2+} influx can occur via different types of Ca^{2+} channel. L-type voltage-operated Ca^{2+} channels (VOCs) are present in ASMCs and are activated by membrane depolarization [15, 16]. Receptor-operated channels (ROCs) are activated by binding of the agonist on membrane receptors [17]. Both ROCs and VOCs can be physiologically involved in the Ca^{2+} response to airway smooth muscle stimulation [18]. For example, extracellular adenosine triphosphate (ATP) acts directly on P2X receptors, whose opening induces Ca^{2+} and Na^+ influx, which in turn depolarizes the plasma membrane, hence indirectly activating VOCs and subsequent additional Ca^{2+} influx [19]. Store-operated Ca^{2+} channels (SOCs) are activated by the emptying of intracellular Ca^{2+} stores [20, 21]. Though SOCs have been detected in ASMCs and contribute to general Ca^{2+} homeodynamics, their direct contribution to the physiological Ca^{2+} signal upon cell stimulation remains unclear [21–23].

2.2.2 OFF Mechanisms

A decrease in $[Ca^{2+}]_i$ results from Ca^{2+} extrusion out of the cell, Ca^{2+} uptake by intracellular organelles, or Ca^{2+} buffering by cytosolic Ca^{2+}-binding proteins. Ca^{2+} extrusion against its electrochemical gradient in ASMCs is mainly due to the activity of the plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^+-Ca^{2+} exchanger (NCX), though the contribution of the latter to the Ca^{2+} signal is debated [24], contributing to either Ca^{2+} extrusion but also, in reverse mode, to Ca^{2+} influx [25–28]. Ca^{2+} storage in intracellular compartments is due to Ca^{2+} pumping into the SR by sarco-/endoplasmic Ca^{2+}-ATPase (SERCA) [24, 29] and to Ca^{2+} uptake into the mitochondria by the mitochondrial Ca^{2+} uniporter [2, 30, 31]. Another mechanism that should not be underestimated is free Ca^{2+} buffering by cytosolic proteins [32]. Two main types of Ca^{2+}-binding proteins have been identified: fast kinetics–low affinity Ca^{2+}-binding proteins, so-called signaling proteins, and slow kinetics–high affinity Ca^{2+}-binding proteins, so-called buffering proteins. Actually,

these two types of Ca^{2+}-binding sites can coexist on the same protein [6]. Though the Ca^{2+} bound to these proteins remains in the cytosol, these Ca^{2+}-binding proteins can be functionally viewed as a cell compartment of Ca^{2+} sequestration since they remove free Ca^{2+} from the cytosol [2, 31].

3 Temporal Characterization of Ca^{2+} Signal in ASMCs

3.1 Overview of Biological Material and Methods

For the last two decades, a large number of studies have been published on the characterization of the cytosolic Ca^{2+} response to several agonists in ASMCs. The heterogeneity of these studies concerns the biological material used for these investigations, the type and mode of cell stimulation, and the methodology used for $[Ca^{2+}]_i$ recordings. Investigations were performed on ASMCs for different species, mainly rat [2, 8, 9, 19, 33–36], mouse [7, 33, 37–43], guinea-pig [25, 29, 44, 45], pig [12–14, 21, 22, 46, 47], horse [48, 49], cow [21, 50, 51], dog [24, 52–54], and human ASMCs [55–58], and interspecies differences have been demonstrated. Experiments were performed on either cultured cells or freshly isolated cells, and in some cases single cells in situ, for example in lung slides. These different conditions may be responsible for differences in the Ca^{2+} response pattern since it has been shown in rat tracheal cells that Ca^{2+} homeodynamics can be critically altered by cell culture, even for a short period (48 h) [19], and in situ recordings maintain cell-to-cell interactions that are disrupted in isolated myocytes. In most of the studies cited earlier, $[Ca^{2+}]_i$ recordings were done using intracellular Ca^{2+} probes such as indo-1 or fura-2 or similar fluorescent dyes, for which $[Ca^{2+}]_i$ variation is estimated by changes in fluorescence intensity or intensity ratio. These molecules allow real-time measurement of $[Ca^{2+}]_i$ variations, but, insofar as they are Ca^{2+}-binding molecules, their presence may alter the amount of free Ca^{2+} in the cytosol and, hence, the pattern of the Ca^{2+} response. Some studies have used an alternative electrophysiological method based on whole-cell patch clamp recording of Ca^{2+}-activated Cl^- currents (I_{ClCa}) [11, 59, 60]. Comparison of the Ca^{2+} signal profile obtained with both techniques indicates that fluorescent probes, at the usual concentrations, have no detectable effect on the $[Ca^{2+}]_i$ response pattern. For example, in freshly isolated rat trachea myocytes, similar $[Ca^{2+}]_i$ response patterns were obtained using indo-1 fluorescent probe or electrophysiological recording of I_{ClCa} [60].

Some differences may be due to the location along the airway tree. Studies have been performed either on tracheal or intrapulmonary bronchial myocytes, but few studies have compared the responses of these different cells in similar conditions. Since the histological organization of airways differs between trachea, extralobular, and intralobular bronchi, differences in ASMC $[Ca^{2+}]_i$ response depending on their location cannot be excluded. In rat airways, differences in contraction patterns and

stimulation-contraction mechanisms have been evidenced in response to purinergic stimulation [19]. In mouse airways, a difference in Ca^{2+} signaling has been evidenced depending on bronchial generations [40].

3.2 General Pattern of $[Ca^{2+}]_i$ Response

Taken together, notwithstanding the differences observed in the Ca^{2+} signal depending on species, agonists, location along the airway tree, and recording methods, the results obtained from the literature show that a general Ca^{2+} pattern can be identified. Examples of original Ca^{2+} traces of ASMCs from different species in response to different agonists are presented in Fig. 2. Recordings on single cells show that the response is characterized by an initial fast and transient $[Ca^{2+}]_i$ rise. The first $[Ca^{2+}]_i$ peak can be followed either by a progressive decay to a more or less steady-state value below the maximum of the $[Ca^{2+}]_i$ peak but above the resting value, the so-called plateau, or by a succession of transient $[Ca^{2+}]_i$ peaks, the so-called Ca^{2+} oscillations. These Ca^{2+} oscillations can be superimposed on the plateau phase, i.e., $[Ca^{2+}]_i$ does not return to baseline between two oscillations. Fluorescent probes give an estimate of the variations in $[Ca^{2+}]_i$, but the absolute $[Ca^{2+}]_i$ value requires calibration, which was not done in all studies that investigated the Ca^{2+} response. When performed, the calculations give a resting $[Ca^{2+}]_i$ of around 100–200 nM, and the amplitude of the Ca^{2+} peak usually ranges up to 1 μM above the resting value. The time-to-peak, i.e., the time needed to reach the maximum $[Ca^{2+}]_i$, is usually not given, indicating that the authors pay more attention to the amplitude of the peak than to its timing. However, considering the few studies that have measured this value [2], and the estimation that can be made from the original traces shown in the others, the top of the Ca^{2+} peak is obtained within a few seconds.

The dynamics of $[Ca^{2+}]_i$ decay has been quantified by some studies [2, 24, 29]. They show that this decay follows a more or less exponential profile, the decay being faster when the stimulation is stopped before the decay begins, but the general pattern remains similar whether cell stimulation goes on or is stopped.

In the case of nonoscillatory response, the slow decay reaches a plateau, which is part of the Ca^{2+} signal, that is above the resting value. However, several studies have focused on the peak and on the oscillations, paying little attention to the plateau. Its significance in terms of contraction response is discussed later. Usually this plateau, when observed, persists as long as the stimulation continues and drops within seconds to baseline when it stops. Observations of original traces show that the plateau is not strictly constant over time and exhibits irregular variations. This can be attributable to stochastic variations in Ca^{2+} release and uptake, whose consequences for the overall Ca^{2+} signal is exacerbated by a clustering of SR Ca^{2+} channels [61]. In some cases, it slowly decreases even upon stimulation, for example upon ATP stimulation in rat tracheal myocytes [19]. In other cases, the Ca^{2+} signal may persist for several seconds after the end of the stimulation, as

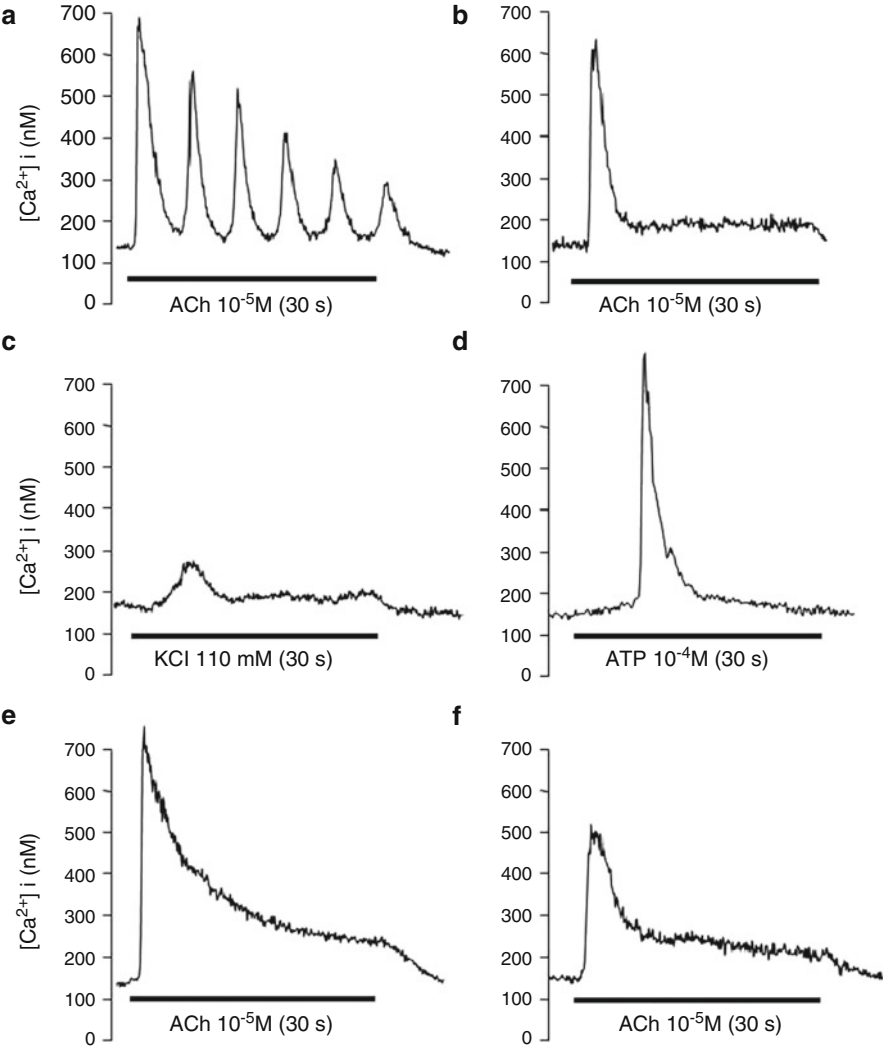

Fig. 2 Original typical traces of Ca^{2+} signals in freshly isolated ASMCs from several species: oscillatory (**a**) and nonoscillatory (**b**) Ca^{2+} response of rat tracheal myocyte to 10^{-5} M ACh. (**c**) Ca^{2+} response of rat tracheal myocyte to 110 mM KCl. (**d**) Ca^{2+} response of rat tracheal myocytes to 10^{-4} M ATP. (**e**) Ca^{2+} response of horse bronchial myocyte to 10^{-5} M ACh. (**f**) Ca^{2+} response of human bronchial myocyte to 10^{-5} M ACh. Cells were obtained from fresh tissues by enzymatic dissociation followed by mechanical dispersion and used within 1 day. $[Ca^{2+}]_i$ was measured by microspectrofluorimetry using the fluorescent dye Indo 1 (for the methods, see [19])

noticed in the same cell type with endothelin-1 [8]. In the case of oscillatory responses, the oscillations may be damped, with a progressive decay in their amplitude, or sustained as long as stimulation persists. Oscillatory responses have been evidenced in ASMCs from several species, such as mouse [42], guinea pig [29], pig [47, 62], and rat (with nonoscillatory responses as well) [9], whereas nonoscillatory responses have been observed in cow [50] and dog ASMCs [24]. In human airways, nonoscillatory Ca^{2+} responses have been described by some studies on isolated ASMCs [55, 57], whereas a recent in situ study describes an oscillatory Ca^{2+} signal [58].

The frequency of oscillations depends on species, agonists, and agonist concentration. In physiological conditions, oscillation frequency ranges between 2 and 3 per minute and 25–30 oscillations per minute. Some pathological conditions like exposure to oxidizing pollutants increase oscillation frequency [36]. Usually, oscillation frequency correlates, when recorded, with the intensity of contraction, and it is usually acknowledged that oscillation frequency encodes for the amplitude of contraction [34, 36–39, 42, 58, 63]. The mechanisms responsible for Ca^{2+} oscillations in ASMCs and their significance in contraction encoding are discussed later in the chapter.

3.3 Mechanisms Responsible for Ca^{2+} Peak and Plateau

3.3.1 ON Mechanisms

As shown earlier, the first step for the Ca^{2+} peak and plateau phases, for a large majority of agonists, is a fast Ca^{2+} increase followed by progressive decay that can usually be fitted by an exponential equation. This Ca^{2+} peak is observed with a quite similar profile whatever the source of Ca^{2+}, i.e., extracellular Ca^{2+} influx or Ca^{2+} release from the SR [42, 64]. In both cases, the elevation of $[Ca^{2+}]_i$ is due to a sudden increase in the amount of Ca^{2+} in the cytosol caused by the opening of Ca^{2+} channels, either located in the plasmalemma or in the sarcolemma. This influx is rapid because, when opened, these channels allow exergonic Ca^{2+} movement and the electrochemical gradient highly favors cytosolic Ca^{2+} influx. Mathematical modeling of Ca^{2+} influx through voltage-operated Ca^{2+} channels and $InsP_3R$ or RyR, based on experimental characterization of these channels, predicts a fast increase in $[Ca^{2+}]_i$, as observed in Pulmonary artery smooth muscle cell (PASMC) studies [2, 31, 65]. In the case of $InsP_3$-induced Ca^{2+} release, the kinetics of the Ca^{2+} response depend on the kinetics of the enzymatic reactions of $InsP_3$ production catalyzed by PLC. Hence, at the molecular level, the mechanisms responsible for $[Ca^{2+}]_i$ increase due to $InsP_3R$ stimulation are slower than that due to VOC activation. The velocity of $[Ca^{2+}]_i$ increase, however, depends not only on the kinetics of the molecular reactions that govern the opening and closure of Ca^{2+} channels but also on the ratio between the transmembrane fluxes and the volume of the cytosol. Experiments show that the kinetics of the Ca^{2+} peak induced

by $InsP_3$-mediated agonist are almost similar to direct $InsP_3$ stimulation in both cases in ASMCs, indicating that, in this cell type, the enzymatic kinetics of $InsP_3$ production are not a limiting factor for $[Ca^{2+}]_i$ increase velocity [8].

3.3.2 OFF Mechanisms

The Ca^{2+} peak involves not only a rise in $[Ca^{2+}]_i$ but also the decrease in $[Ca^{2+}]_i$ that follows. Then, the OFF mechanisms responsible for cytosolic Ca^{2+} clearance are as important as the ON mechanisms responsible for $[Ca^{2+}]_i$ increase. Experimental results on the respective roles of SR Ca^{2+} uptake, mitochondria, and cytosolic buffering capacity are presented in Fig. 3. Intuitively, Ca^{2+} decay is linked to the closure, at least partial, of Ca^{2+} channels and subsequent activation of mechanisms that remove Ca^{2+} from the cytosol, in particular Ca^{2+} pumping into the SR. Studies of the dynamics of Ca^{2+} handling in ASMCs have proven this intuitive representation to be largely wrong.

First, as far as the stimulation persists, the Ca^{2+} channels remain open, at least in the first step of the stimulation. The end of the Ca^{2+} increase and the beginning of the Ca^{2+} decrease do not correspond to the closure of the Ca^{2+} channels. It has been established, for example, that the $[Ca^{2+}]_i$ peak, including the decay phase, induced by caffeine stimulation is not due to fast opening and closure of RyRs but to persistent RyR opening associated with simultaneous mechanisms of cytosolic Ca^{2+} clearance [2]. In the case of RyR closure, the exponential decay is faster than when RyRs remain open, but the profile is similar in both cases, as shown in Fig. 3a. Both experimental and theoretical studies have shown that the profile of the $[Ca^{2+}]_i$ peak is a consequence of the balance between ON and OFF mechanisms [2, 24, 29, 31, 66]. In this balance, the kinetics of the buffering capacity of OFF mechanisms are a critical determinant of the Ca^{2+} peak profile.

Role of Ca^{2+} Pumping in SR by SERCA

In the buffering system, Ca^{2+} pumping back into the cytosol is usually considered the most important aspect [67]. This is actually true in the sense that the SR as a Ca^{2+} store is important for cell physiology, but Ca^{2+} pumping back by SERCA is not the major determinant of the Ca^{2+} decay. Actually, if Ca^{2+} efflux from the cytosol is activated, then Ca^{2+} pumping back by SERCA cannot in principle account for $[Ca^{2+}]_i$ decreases since as soon as Ca^{2+} is pumped back into the cytosol it leaks through the opened Ca^{2+} channels. Accumulation of Ca^{2+} would require that the velocity of Ca^{2+} pumping against the concentration gradient would be higher than that of a passive Ca^{2+} leak, which is not the case [66]. Even when the Ca^{2+} channels are closed during the decay phase, as occurs for short (1 s) stimulations, inhibition of SERCA does not significantly modify the dynamics of the $[Ca^{2+}]_i$ clearance, as shown in Fig. 3b.

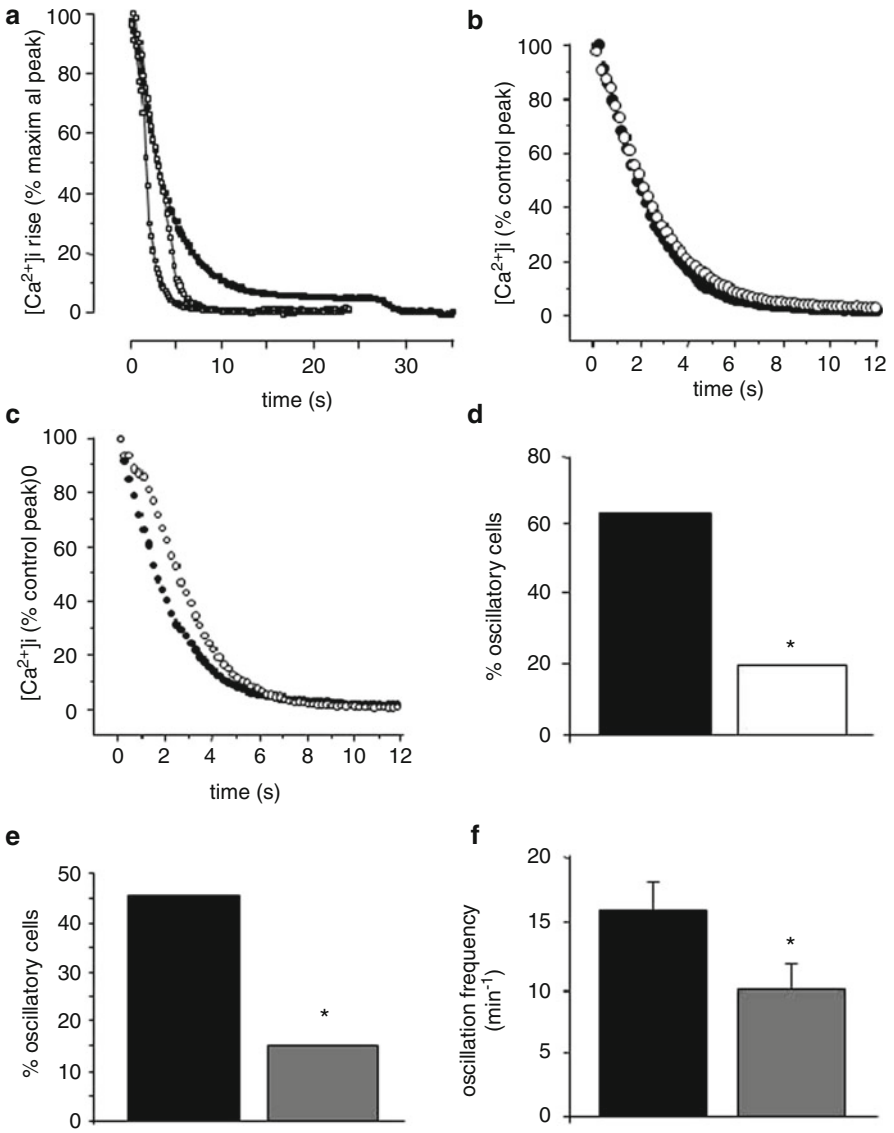

Fig. 3 Influence of OFF mechanisms on kinetics of Ca^{2+} signal. (**a**) Kinetics of $[Ca^{2+}]_i$ decay following Ca^{2+} peak induced by 5 mM caffeine stimulation for 1 s (*left trace*, *light gray squares*), 5 s (*dark gray squares*, *middle trace*), and 30 s (*right trace*, *black square*). (**b**) Influence of SERCA inhibition by cyclopiazonic acid (CPA) on the kinetics of $[Ca^{2+}]_i$ decay following 5 mM caffeine stimulation (1 s). *Black circles*: control; *open circles*: 10^{-5} M CPA. (**c, d**) Influence of mitochondrial Ca^{2+} uptake inhibition by 5.10^{-6} M FCCP on (**c**) the kinetics of $[Ca^{2+}]_i$ decay following 5 mM caffeine stimulation (1 s) (*black circles*: control; *open circles*: FCCP) and (**d**) the percentage of oscillating Ca^{2+} responses to 10^{-5} M ACh stimulation. (**e, f**) Influence of increased cytosolic Ca^{2+} buffering by 5.10^{-5} M BAPTA-AM on (**e**) the percentage of oscillating Ca^{2+} responses and (**f**) the frequency of Ca^{2+} oscillations to 10^{-5} M ACh stimulation. Cells were obtained from fresh tissues by enzymatic dissociation followed by mechanical dispersion and used within 1 day. $[Ca^{2+}]_i$ was measured by microspectrofluorimetry using the fluorescent dye Indo 1 (for the methods, see Roux and Marhl [2]). $*P < 0.05$ (Student's *t*-test)

Role of Ca^{2+} Uptake in Mitochondria by Ca^{2+} Uniporter

It has been shown that SERCA is neither the only nor the fastest mechanism of free cytosolic Ca^{2+} clearance. When $[Ca^{2+}]_i$ increases, Ca^{2+} uptake into mitochondria by the mitochondrial Ca^{2+} uniporter plays a more important role than Ca^{2+} uptake by SERCA, even when SR Ca^{2+} channels are closed, because Ca^{2+} uptake by mitochondria occurs faster than that by the SR [30, 68–70]. As shown experimentally, for short caffeine stimulations, inhibition of SERCA does not significantly modify the slope of the $[Ca^{2+}]_i$ decay, whereas inhibition of the mitochondrial uniporter by Trifluorocarbonylcyanide phenylhydrazone (FCCP) does, as illustrated in Fig. 3c. Mitochondria seem also to be involved in the occurrence of oscillations since FCCP decreases the percentage of oscillatory response to ACh stimulation in freshly isolated rat tracheal myocytes (personal data) (Fig. 3d).

Role of Ca^{2+} Buffering by Cytosolic Ca^{2+}-Binding Proteins

Though significant, the effect of mitochondrial inhibition on the shape of the $[Ca^{2+}]_i$ decay following caffeine stimulation is modest, and theoretical analysis has shown that the shape of the $[Ca^{2+}]_i$ decay, as well as the amplitude of the Ca^{2+} peak, critically depends on Ca^{2+} binding to cytosolic proteins capable of buffering free cytosolic Ca^{2+} [2, 65, 66]. Though few experimental studies have been dedicated to investigating the role of cytosolic buffering capacity on the pattern of the Ca^{2+} signal, manipulation of the buffering capacity of the cytosol by BAPTA, a Ca^{2+} chelator, shows that an increase in the buffering capacity of the cytosol can alter Ca^{2+} signaling [71]. As shown in Fig. 3e, f, increased cytosolic Ca^{2+} buffering capacity by the Ca^{2+} chelator BAPTA decreases both the percentage of oscillating cells and the frequency of oscillations in response to cholinergic stimulation (personal data).

3.3.3 Integrated View of Ca^{2+} Handling upon ASMC Stimulation

Hence, besides $[Ca^{2+}]_i$ decay following an initial $[Ca^{2+}]_i$ increase, multiple Ca^{2+} buffering mechanisms act simultaneously, but not instantaneously. According to the theoretical analysis of Ca^{2+} handling, a fast $[Ca^{2+}]_i$ increase is followed by free cytosolic Ca^{2+} buffering by cytosolic proteins and Ca^{2+} uptake by the mitochondria prior to Ca^{2+} uptake in the cytosol, even in the case of cell stimulation and Ca^{2+} release into the cytosol [66]. This has several consequences for our understanding of Ca^{2+} signaling.

First, the temporal aspects of the multiple mechanisms involved in the profile of the Ca^{2+} signal are critical determinants of its timing and amplitude. Indeed, the amplitude of the Ca^{2+} peak depends on the buffering capacity of the so-called signaling proteins (fast kinetics Ca^{2+}-binding proteins). The sequential description of the mechanisms of the Ca^{2+} signal according to which Ca^{2+} is *first* released in the

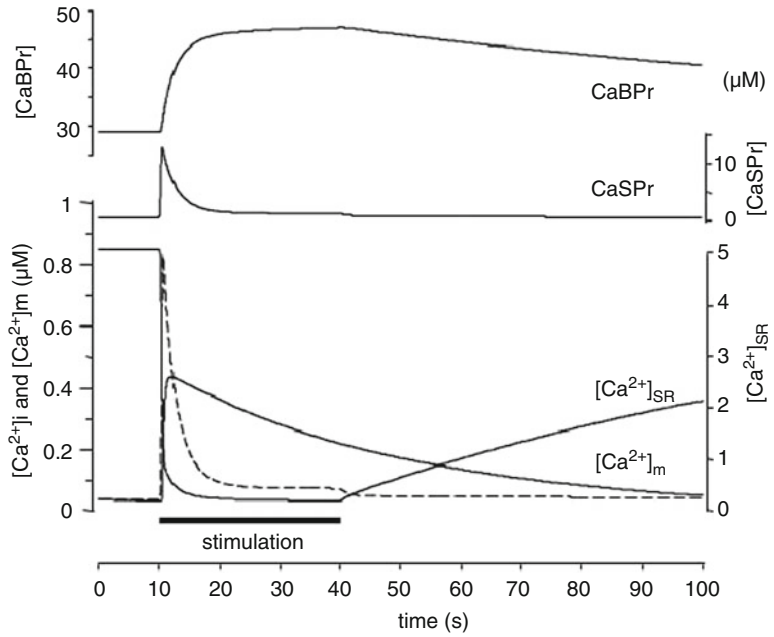

Fig. 4 Model prediction of Ca^{2+} movements upon 30 s caffeine stimulation. Simulated application of caffeine began at time 10 s. *Dashed line*: $[Ca^{2+}]_i$; *full lines*: concentrations of free Ca^{2+} in SR ($[Ca^{2+}]_{SR}$), in mitochondria ($[Ca^{2+}]_m$), and bound to signaling (CaSPr) and buffering (CaBPr) proteins. Though $[Ca^{2+}]_i$ returns to baseline at the end of caffeine stimulation (time 40 s), a return to resting Ca^{2+} concentration in the various cell compartments requires much more time

cytosol, *then* buffered by cytosolic protein, *later* taken up by the mitochondria, and *eventually* pumped back into the SR is only a metaphorical way to express the fact that these mechanisms have different kinetics that should not hide the fact that they occur simultaneously. Cell stimulation disrupts the dynamic equilibrium responsible for the $[Ca^{2+}]_i$ resting value, and the Ca^{2+} peak is the result of the subsequent transitory nonequilibrium state. The Ca^{2+} plateau is the consequence of the new dynamics of Ca^{2+} handling when the new equilibrium state is reached. Fast kinetics mechanisms are critical for the peak shape, whereas the plateau value is dependent on the relative buffering capacity of each cell compartment, even if the kinetics of these buffering reactions are slow. It should be noted that even if the plateau value seems slightly different from the resting $[Ca^{2+}]_i$ value, the equilibrium state may be quite different in both cases, with great differences in the amount of Ca^{2+} in the various intracellular compartments at rest versus upon cell stimulation.

The second consequence is that, although $[Ca^{2+}]_i$ values return to baseline within seconds after the end of cell stimulation, this does not mean that the cell is already back to its initial Ca^{2+} homeodynamic state. Theoretical studies, in correlation with experiments, show that complete Ca^{2+} refilling of the SR requires several minutes [2], as illustrated in Fig. 4. As a consequence, two successive cell stimulations

triggering Ca^{2+} release from the SR will not result in similar Ca^{2+} response as long as Ca^{2+} handling is not back at its initial state, and the time needed to recover to this initial state corresponds to a relative refractory period. The delay needed for the return to resting equilibrium, which is determined by the temporal characteristics of the mechanisms of cytosolic Ca^{2+} clearance, has a quite opposite consequence for cell homeostasis depending on the source of Ca^{2+}. In the case of Ca^{2+} release from internal Ca^{2+} stores, repeated stimulations result in progressive depletion of the SR and subsequent decrease in stimulus responsiveness, whereas in the case of external Ca^{2+} influx, they would result in progressive Ca^{2+} overloading if the mechanisms of Ca^{2+} extrusion were saturated [2, 72].

In conclusion, it should be emphasized that the peak and the plateau involve similar mechanisms responsible for Ca^{2+} homeodynamics. The difference between the peak and the plateau profiles corresponds to two different phases of the transition between two equilibrium states of the Ca^{2+} handling, at rest and upon stimulation. The peak is a consequence of the transient nonequilibrium phase of the activated state, whereas the plateau corresponds to the state of the system when equilibrium is reached. When stimulation ends, return to the initial resting state is longer than the return of $[Ca^{2+}]_i$ to baseline, due to silent Ca^{2+} fluxes between the Ca^{2+} buffering elements that occur at constant free cytosolic Ca^{2+} concentration.

3.4 Mechanisms of Ca^{2+} Oscillations

As indicated earlier, Ca^{2+} oscillations have been reported in a variety of ASMCs, though it should not be forgotten that in some species, including humans, the Ca^{2+} signal can be a nonoscillatory one. In ASMCs, Ca^{2+} oscillations have been mainly observed in response to contractile agonists acting on G-protein-coupled receptors and $InsP_3$ production, such as ACh, 5-HT, endotheline-1, and ATP [9, 11, 13, 19, 42, 43, 73]. Additionally, experiments using direct stimulation by $InsP_3$ have shown that nonoscillatory $InsP_3$ concentration was able to generate oscillatory Ca^{2+} responses [8]. However, Ca^{2+} oscillations have also been noticed in response to stimulation acting via external Ca^{2+} influx [42]. Figure 5a, b compares Ca^{2+} oscillations induced by cholinergic stimulation and by direct $InsP_3$ exposure.

Basically, Ca^{2+} oscillations can be explained by asynchronous coupling between ON and OFF mechanisms. Since oscillations are observed in response to $InsP_3$, much attention has been paid to the kinetic properties of $InsP_3R$. Several isoforms of $InsP_3R$ have been identified, types 1, 2, and 3. These isoforms are sensitive to both cytosolic $InsP_3$ and Ca^{2+}. Both types 2 and 3 exhibit a sigmoid-shaped sensitivity curve to Ca^{2+} concentration, whereas type 1 has a bell-shaped sensitivity curve: for low $[Ca^{2+}]_i$, sensitivity to Ca^{2+} increases with $[Ca^{2+}]_i$, whereas for high $[Ca^{2+}]_i$, an increase in $[Ca^{2+}]_i$ decreases $InsP_3P$ opening [74–77]. Moreover, this loss in sensitivity for high $[Ca^{2+}]_i$ is delayed. $InsP_3R$ by itself seems to be an asynchronous ON/OFF switch capable of generating oscillations, an idea supported by the fact that $InsP_3R$ inhibition abolishes Ca^{2+} oscillations [7]. Several theoretical models

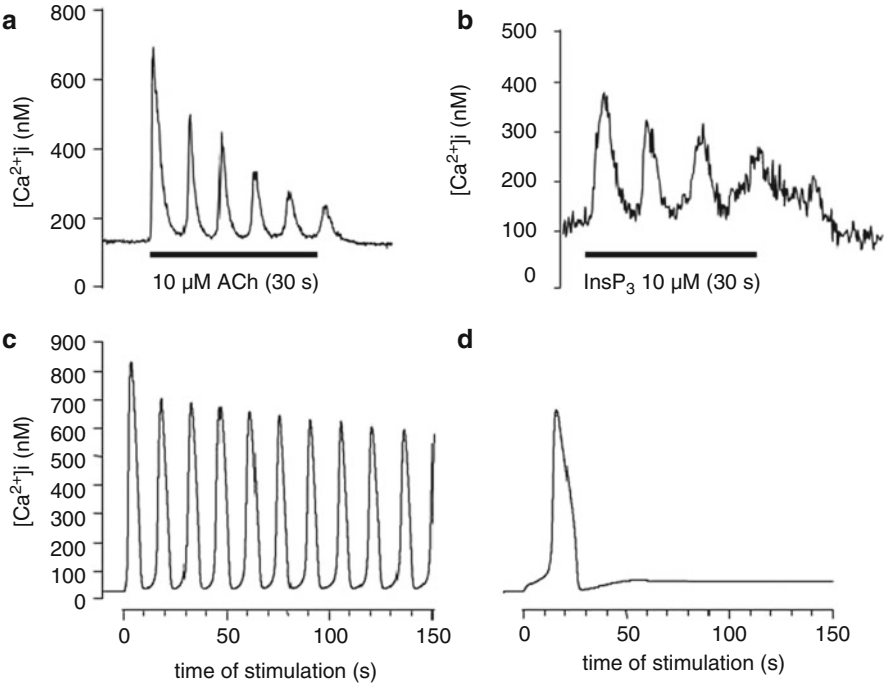

Fig. 5 Ca^{2+} oscillations. Original traces of Ca^{2+} response to ACh stimulation (**a**) and $InsP_3$ stimulation (**b**). Cells were obtained from fresh tissues by enzymatic dissociation followed by mechanical dispersion and used within 1 day. $[Ca^{2+}]_i$ was measured by microspectrofluorimetry using fluorescent dye indo-1. $InsP_3$ was applied on β-escine-permeabilized cells [8]. (**c**, **d**) Model prediction to 1 µM $InsP_3$ stimulation for different $InsP_3R$ isoform ratio. (**c**) 75 % IP_3R subtype 1 and 25 % subtype 3; (**d**) 25 % IP_3R subtype 1 and 75 % subtype 3. The model used is that of Haberichter et al. [65]

have been developed to explain how $InsP_3R$ can be a molecular oscillator [78]. However, the mechanisms of Ca^{2+} oscillations have appeared to be more complex and could not be attributed to the properties of the $InsP_3R$ type 1 alone. First, experimental studies have shown that type 1 $InsP_3R$ expression can be associated with nonoscillatory Ca^{2+} signals [57]. On the other end, studies in vascular SMCs showed intercellular variation in $InsP_3R$ isoform expression and that $InsP_3R$ alone cannot generate Ca^{2+} oscillations, which requires a mixture of type 1 and type 2 isoform expression in the same cell [79]. In rat ASMCs, an immunofluorescence study suggested that such a heterogeneous expression is observed, with 100 % of ASMCs expressing type 1 isoform, 27 % type 2, and 40 % type 3 (personal data). Theoretical modeling of the $InsP_3$-induced Ca^{2+} response indicates that both oscillatory and nonoscillatory Ca^{2+} signals can be predicted in cells expressing a mixture of type 1 and type 3 $InsP_3R$, depending on the total amount of $InsP_3R$ and the type1/type3 ratio, and the presence of cytosolic Ca^{2+}-binding proteins [65]. The role of the isoform ratio in the occurrence of Ca^{2+} oscillations is illustrated in Fig. 5c, d.

RyRs have also been shown to contribute to ACh-induced as well as KCl-induced Ca^{2+} oscillations [12, 13, 72].

It appears, then, that Ca^{2+} oscillations in ASMCs are not due to the intrinsic property of one specific molecular oscillator whose presence determines their occurrence. Rather, the occurrence of Ca^{2+} oscillations is an emerging property of the machinery of Ca^{2+} handling, and oscillations may appear when asynchronous ON/OFF mechanisms are simultaneously active. As shown by theoretical investigations, one cell may occupy different possible positions in the phase space, at one moment being in an oscillatory phase space and at another moment in a nonoscillatory space phase, depending on the intensity of cell stimulation [65, 72]. In a cell population, interindividual variations in $InsP_3R$ expression and in the ratio of isoform expression generate the distribution of the cells in the different phases of the space, so that heterogeneous cell behavior, oscillatory and nonoscillatory Ca^{2+} signals, may be the consequence of a homogeneous cell type associated with inherent cell-to-cell variations [65].

Oscillatory Ca^{2+} responses have been reported in some cases in response to KCl stimulation [42]. In the case of forced membrane depolarization, the mechanisms responsible for the Ca^{2+} signal, i.e., Ca^{2+} influx through VOCC and CICR through RyRs, are different from that activated by agonists acting on G-coupled membrane receptors like muscarinic receptors. This emphasizes the fact that oscillations may occur as a consequence of the activation of distinct pathways. However, in the case of KCl, Ca^{2+} oscillations require simultaneous involvement of ON/OFF mechanisms, unlike with $InsP_3$. Oscillations have also been reported in vascular SMCs, and theoretical modeling, including voltage-dependent Ca^{2+} influx and RyR activation, accounts for this oscillatory profile [80]. Though these models have been developed for vascular SMCs, the mechanisms they describe also account for what is observed in ASMCs, as was confirmed by recent modeling of KCl-induced oscillations in ASMCs [72].

Taken together, these results indicate that the pattern of the Ca^{2+} signal is critically determined by the kinetic constants of each element involved in ASMC Ca^{2+} handling. Perturbation of the functional properties of any of these structures may lead to loss of the oscillatory response, which does not mean that this structure is *the* element that determines the Ca^{2+} profile, but it is one element, among others, where functional properties contribute to shape the Ca^{2+} signal.

4 Decoding of Ca^{2+} Signals

4.1 Contractile System

How is a message carried by the Ca^{2+} signal? In other words, how does the Ca^{2+} signal determine the behavior of the contractile apparatus? What is, in the time-dependent variations of $[Ca^{2+}]_i$ that occur upon cell stimulation, information for the

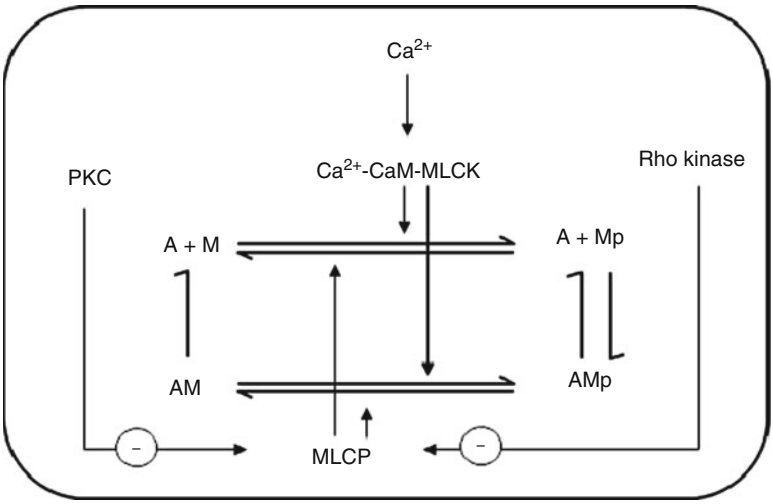

Fig. 6 General mechanisms of Ca^{2+}-contraction coupling in ASMCs. Ca^{2+} binds to calmodulin (*CaM*) and MLCK. The Ca^{2+}-CaM-MLCK complex is the active form of MLCK, which catalyzes MLC_{20} (*M*) phosphorylation. Phosphorylated MLC_{20} (*Mp*) binds to actin (*A*) to form the actin-myosin bridge (*AMp*) that can later be dephosphorylated (*AM*). Dephosphorylation is achieved by MLCP, whose activity is modulated by several kinases, mainly PKC and RhoK

contractile machinery, and what type of information is it? The object of this part of the chapter is not to detail the contractile apparatus and its regulation pathways but to present an overview of the "semantics" of the Ca^{2+} signal in terms of contraction. Basically, a rise in $[Ca^{2+}]_i$ activates the contractile apparatus by the following pathways, summarized in Fig. 6. Ca^{2+} binds to the cytosolic protein calmodulin (CaM), which can bind to the myosin light chain kinase (MLCK), and the Ca^{2+}-CaM-MLCK complex is the active form of MLCK. When activated, MLCK phosphorylates the 20 kDa regulatory myosin light chain (MLC_{20}), and this phosphorylation is required for the formation of the actin-myosin cross bridge and, hence, contraction. Dephosphorylation of MLC_{20} is ensured by myosin light chain phosphatase (MLCP). Both MLCK and MLCP activity can be up- and downregulated by several kinase- and phosphatase-dependent pathways [81]. The canonical way that controls contraction in ASMC is the Ca^{2+}-CaM-MLCK pathway [34, 82]. The other regulatory mechanisms are grouped into the so-called concept of regulation of Ca^{2+} sensitivity since they modify the contractile response for a given Ca^{2+} signal. Though useful, this distinction between the Ca^{2+}-induced contraction pathway on the one hand and the regulation of Ca^{2+} sensitivity on the other hand is somewhat artificial since regulation of Ca^{2+} sensitivity can itself be Ca^{2+}-dependent [83].

4.2 Contractile Significance of Ca^{2+} Signal

The semantics of the Ca^{2+} signal have long been investigated by correlating contraction with the Ca^{2+} signal parameters, mainly the Ca^{2+} peak, the plateau, and, when present, the oscillations [9, 19, 35–37, 42, 64]. Regarding the contraction itself, several distinct parameters are used to describe it. Usually, the intensity of the steady-state response is used to characterize the contractile response, measured by either isometric recording [19, 34, 36, 45, 51, 83–85] or changes in the transsectional area of the bronchial lumen [33, 40, 43], but other parameters are also used to describe the temporal aspects of the contractile response, such as shortening velocity in isotonic measurements [86, 87] or half-time maximal contraction (the time needed to achieve 50 % of the maximal contraction) in isotonic recording [34, 83, 86, 87].

Peak, plateau, and oscillation frequency all vary with the intensity of agonist stimulation, as does the amplitude of contraction. It is hence difficult to determine experimentally the specific message carried by each Ca^{2+} signal parameter because of the difficulty of varying one parameter independently of the others. Theoretical modeling is a way to investigate the specific significance of the distinct parameters of the Ca^{2+} signal since it is possible, in contrast to experimentation, to vary them independently [34, 83, 87–89].

4.2.1 Contractile Message of Initial Ca^{2+} Peak

Realistic computational modeling of Ca^{2+}-contraction coupling, including both the canonical MLCK pathway and MLCP regulation by RhoK and PKC, indicates that the amplitude of the Ca^{2+} peak determines the velocity of the early phase of the contraction [34, 83]. This may explain why ASM contraction develops slower for low than for high levels of agonist concentration. However, according to the model, the contractile apparatus is much less sensitive to a transient $[Ca^{2+}]_i$ rise than to a sustained one, and the peak does not determine the amplitude of contraction. This is basically due to the inertia of the MLCK-dependent activation of the contractile apparatus compared with the velocity of the Ca^{2+} peak, both in its increase and decrease, so that during the Ca^{2+} peak, the MLC phosphorylation rate decreases before phosphorylated MLC has reached its maximal value.

4.2.2 Contractile Message of Ca^{2+} Plateau

The amplitude of the plateau is, according to the model, the major determinant of the amplitude of contraction [34, 83]. Since the plateau is a stable $[Ca^{2+}]_i$ value, the system can reach its equilibrium. Usually, the amplitude of the plateau is considered small compared with the maximal peak value. However, both theoretical and experimental studies indicate that the level of phosphorylated MLC is not

linearly related to the steady-state $[Ca^{2+}]_i$ value, and this explains the fact that the amplitude of the plateau is a critical determinant of the amplitude of the contraction [34].

4.2.3 Contractile Message of Ca^{2+} Oscillations

Experimental studies have demonstrated statistical correlations between the frequency of Ca^{2+} oscillations and the amplitude of contraction, suggesting that the Ca^{2+} oscillation frequency determines the amplitude of contraction [9, 19, 33, 35, 39, 43, 63]. However, such correlations do not establish an unequivocal causal relationship since, as stated earlier, other parameters simultaneously correlate with contraction intensity [9, 35]. Moreover, the meaning of frequency encoding should be clarified. Indeed, Ca^{2+} oscillations induce an average $[Ca^{2+}]_i$ increase that may induce contraction whether this increase is oscillating or not. Thus, if we consider oscillations as carrying a specific message, this means that the contractile apparatus is specifically sensitive to the dynamics of Ca^{2+} oscillations. In other words, the contractile system is oscillation-frequency-sensitive only if the contractile response to an oscillatory signal differs from that of a nonoscillatory signal with an identical average $[Ca^{2+}]_i$ value, i.e., for which the surface area under an oscillating trace is equal to that under a nonoscillating trace. It is hard, if not impossible, to design an experimental protocol to compare these two conditions. Additionally, since airway smooth muscle contraction is the response at the tissue level, the question arises as to whether the observed nonoscillatory contractile response at the tissue level is the consequence of the summation of asynchronous oscillatory responses at the cellular level or results from the integration at the cellular level of an oscillatory Ca^{2+} message into a nonoscillatory contractile response. For both questions, mathematical modeling is a useful tool to test these hypotheses. Computational modeling with realistic kinetic constants indicates that oscillation frequency within the range observed in experiments (5–30 oscillations min^{-1}) generates a nonoscillatory contractile response [34, 83]. Hence, it seems that the oscillatory message is integrated at the cellular level by the contractile machinery. This integration occurs at different steps of Ca^{2+}-contraction coupling, illustrated in Fig. 7. Model prediction indicates that the temporal pattern of MLCK activity is oscillating and follows more or less that of the Ca^{2+} signal, though MLCK activity decay might be slightly slower than Ca^{2+} decay (Fig. 7b). In contrast, the temporal pattern of MLC phosphorylation, though oscillatory, exhibits smoothed oscillations, the level of phosphorylated MLC remaining high between two oscillations (Fig. 7c). This is due to the inertia of the system, i.e., physiological MLCP activity is not sufficient to trigger an immediate drop in phosphorylated MLC when MLCK activity decreases between two Ca^{2+} oscillations. The second, and most important, step of the integration of the Ca^{2+} signal is the formation of actin-myosin bridges. Computational modeling predicts that the amount of actin-myosin bridges, which corresponds to isometric contraction, remains high between two Ca^{2+} peaks even if the amount of phosphorylated MLC tends to decrease. This is due to the fact that actin-myosin

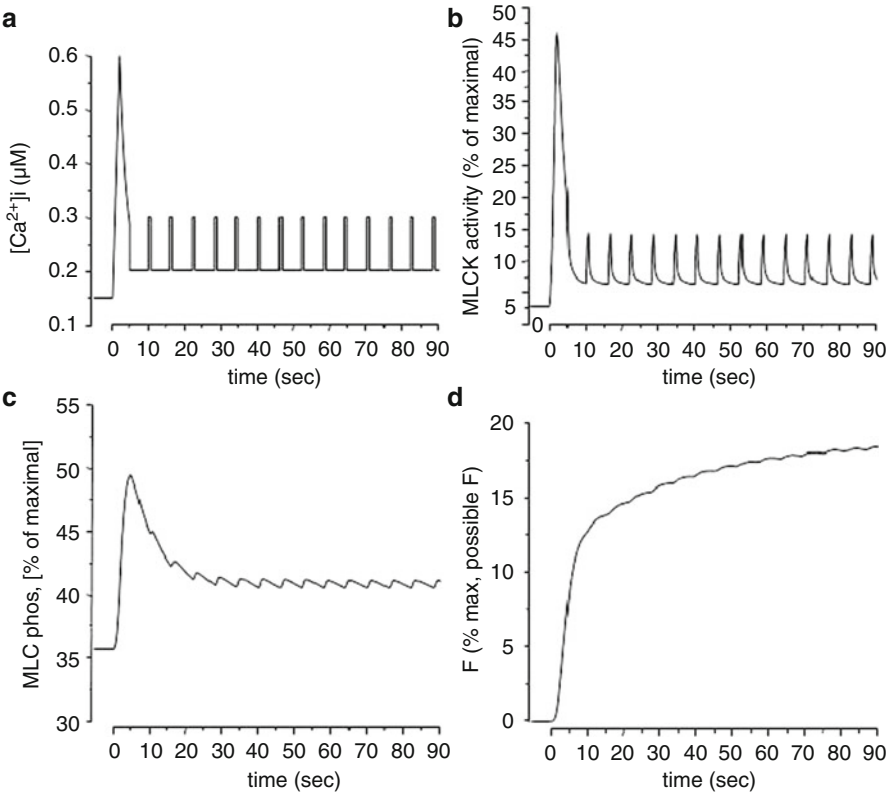

Fig. 7 Temporal integration of oscillatory Ca^{2+} signal. Model prediction of MLCK activation (**b**), MLC phosphorylation (**c**), and contraction (**d**) upon oscillating Ca^{2+} signal (**a**), according to the model of Mbikou et al. [83]

binding can be maintained even if MLC is dephosphorylated because of the low kinetic constant for the rupture of the dephosphorylated myosin-actin bridge, the so-called latch bridge. When the next Ca^{2+} peak and its subsequent rise in MLC phosphorylation occur before a significant drop in the actin-myosin bridge, which depends on the balance between oscillation frequency and the kinetics of actin-myosin bridge cycling, actin-myosin bridges tend to accumulate until an equilibrium is reached, and this corresponds to a slow additional increase in contraction (Fig. 7d). Changing the oscillation frequency within the physiological range in the model predicts an oscillation-frequency-dependent increase in contraction. It can hence be concluded that the oscillations actually encode for the intensity of ASM contraction since ASMC modeling predicts that the contractile system is specifically sensitive to oscillation frequency independent of the average $[Ca^{2+}]_i$ value. However, if we compare, as shown in Fig. 8, the sensitivity of the contractile machinery to the peak value (or transient $[Ca^{2+}]_i$ increase), the plateau value (or the average $[Ca^{2+}]_i$ value), and the oscillation frequency, the model prediction

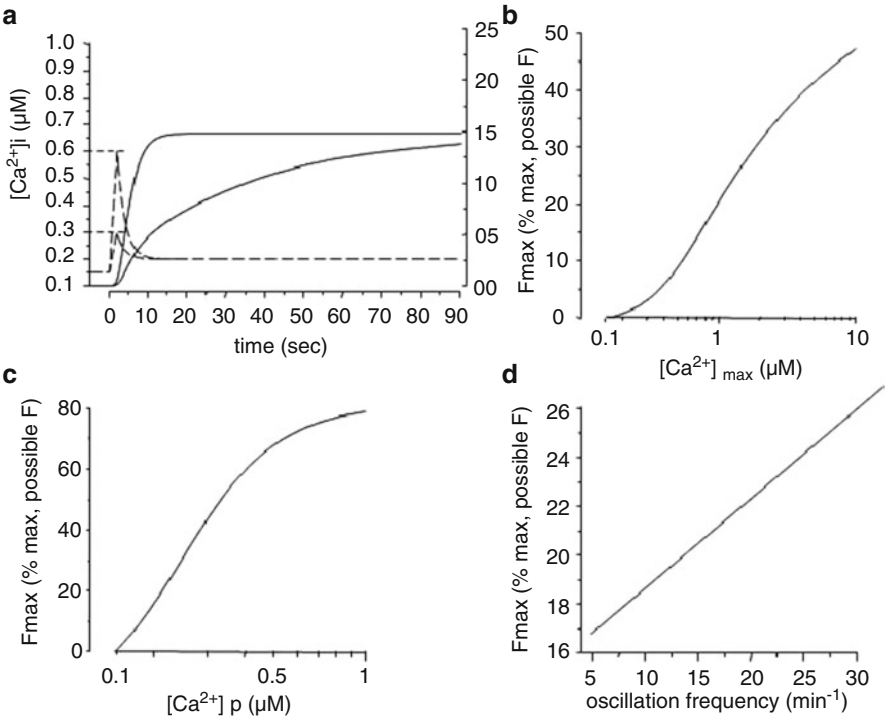

Fig. 8 Contraction encoding by peak, plateau, and frequency of oscillations. Model predictions are done using the model of Mbikou et al. [83]. (**a**) Predicted time course of force for two peak amplitudes (0.3 and 0.6 μM) for fixed resting $[Ca^{2+}]_i$ and plateau. (**b**) Predicted relationship between amplitude of transient $[Ca^{2+}]_i$ increase and force. (**c**) Predicted relationship between amplitude of sustained $[Ca^{2+}]_i$ and force. (**d**) Predicted relationship between frequency of oscillations and force. The simulated Ca^{2+} signal used is similar to that of Fig. 7, with variation in oscillation frequency

is that the contractile apparatus will be much more sensitive to the plateau value than to the frequency of Ca^{2+} oscillation per se. Hence, the importance of the plateau phase or, in the case of oscillatory Ca^{2+} signals, of the average $[Ca^{2+}]_i$ value over time independently of Ca^{2+} oscillations should not be underestimated. The sensitivity to a transient $[Ca^{2+}]_i$ rise is quite low, as shown in Fig. 8b, which explains the fact that, for a standard biphasic Ca^{2+} response, the amplitude of the Ca^{2+} peak does not determine the amplitude of the contraction, which depends on the plateau, but not its velocity (Fig. 8a).

It is tempting to speculate about the advantage of frequency encoding versus amplitude encoding in cell physiology. For example, it has been hypothesized that oscillation encoding may be more precise than amplitude encoding, hence ensuring more accurate functional response to cell stimulation, especially for low agonist concentrations [90]. Though this may be true in principle, one should be cautious, however, when speculating about the putative advantage of oscillatory versus

nonoscillatory encoding, and at least in ASMCs, there is no clear evidence that oscillatory signaling may be advantageous. The main argument against such a speculation is that, in various species, ASMCs exhibit nonoscillatory Ca^{2+} signals. Additionally, analysis of cell-to-cell variability of the Ca^{2+} signal has shown, as a general property, high interindividual variations in the Ca^{2+} response to the same stimulation [91]. The Ca^{2+} signal appears, then, to be highly noisy, and it is hard to imagine the specific evolutionary advantage of highly precise decoding of a highly noisy signal. Robustness to the noise of the signal would, in principle, be more advantageous.

Functional analysis shows that Ca^{2+} oscillations can appear as an emerging property of the system responsible for intracellular Ca^{2+} handing and that these oscillations have functional consequences for the contractile apparatus. Whether the occurrence of an oscillating Ca^{2+} signal is a byproduct of the functional constraint of the system controlling Ca^{2+} homeodynamics [92] or a consequence of a selective pressure that has designed this system for the production of frequency-encoded Ca^{2+} signal is purely speculative and has no real support.

5 Conclusion

In conclusion, the temporal aspects of Ca^{2+} signals are inherent in the concept of Ca^{2+} signal, which is, by definition, a spatiotemporal change in $[Ca^{2+}]_i$. Temporal variations upon contractile stimulation exhibit a general pattern characterized by a transient Ca^{2+} peak followed either by a steady-state phase or Ca^{2+} oscillations. This pattern is the consequence of simultaneous, but not instantaneous, interactions between all the elements involved in Ca^{2+} handling, both ON and OFF mechanisms. Among the latter, Ca^{2+} pumping back into the SR is not the only important mechanism, and Ca^{2+} uptake by mitochondria and Ca^{2+} binding to cytosolic proteins are critical determinants of the Ca^{2+} signal. The transient peak is the consequence of a transitory nonequilibrium phase between the resting and the activated states of Ca^{2+} homeodynamics. If an equilibrium is reached, then $[Ca^{2+}]_i$ stabilizes with respect to the plateau, whereas if asynchronous interactions between ON and OFF occur, Ca^{2+} oscillations appear. These different parameters of the Ca^{2+} signal are contractile messages. According to theoretical analysis, the amplitude of the Ca^{2+} peak encodes for the velocity of contraction, whereas the plateau determines the amplitude of the contraction. When they exist, oscillatory signals seem to be integrated at the cell level, leading to nonoscillatory contractions due to the temporal inertia of the MLC dephosphorylation process and dephosphorylated myosin-actin bridges. Oscillation frequency seems to encode specifically for the amplitude of contraction, but the contractile system is less sensitive to Ca^{2+} oscillation frequency per se than to the average Ca^{2+} value over time, whether or not the Ca^{2+} signal is oscillatory.

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