Cytosolic Calcium Oscillations in Smooth Muscle Cells

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In a variety of smooth muscle cells, agonists activating membrane receptors induce oscillations in the cytoplasmic Ca\(^{2+}\) concentration via an inositol trisphosphate-activated mechanism. Ca\(^{2+}\) oscillations participate in the control of cell membrane potential and the tone of smooth muscle. There is evidence that alterations in Ca\(^{2+}\) oscillations modulate smooth muscle responsiveness.

An increase in the cytoplasmic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) is generally considered the major event of the activation process of the contractile apparatus in smooth muscle cells. However, the kinetic, the pattern of the [Ca\(^{2+}\)]\(_i\), increase, and its temporal correlation with the contractile response are not yet clearly understood. Techniques such as enzymatic isolation of smooth muscle cells and microspectrofluorimetry (using fluorescent indicators such as indo 1, fura 2, and fluo 3), allowing the measurement of the [Ca\(^{2+}\)]\(_i\), in single cells, sometimes combined with electrophysiological experiments (patch clamp), are now widely used and have brought about new and important information in the field of cellular Ca\(^{2+}\) signaling. Studies from our laboratory as well as from others have revealed that agonists controlling smooth muscle tone via activation of membrane receptors induce a complex temporal [Ca\(^{2+}\)]\(_i\), response in single cells from some smooth muscles (e.g., vascular, airways, intestine). This response is composed of a series of cyclic increases in the [Ca\(^{2+}\)]\(_i\), so-called Ca\(^{2+}\) oscillations. The pattern of these oscillations depends on several factors, including the type of tissue/species, the phenotype of the cells, e.g., fresh vs. cultured cells, and the agonist considered.

Characteristics of agonist-induced Ca\(^{2+}\) oscillations

As a general feature, Ca\(^{2+}\) oscillations are produced in single, freshly isolated smooth muscle cells in response to agonists interacting with seven transmembrane domain, G-coupled surface membrane receptors, such as acetylcholine (ACh), angiotensin II (All), endothelin-1 (ET-1), extracellular ATP and UTP, noradrenaline, vasopressin, serotonin, and so forth. When agonists are applied by means of superfusion or microinjection near the cell, the [Ca\(^{2+}\)]\(_i\), rises, after a delay of 5–10 s, from a resting value of ~100 nM to a peak value of 400–800 nM (2, 11, 13). This first increase is transient and followed by successive peaks of constant duration. The frequency varies from 4–6 to 25–30/min, according to the type of cell and/or the agonist concentration (see below). In some cases, the [Ca\(^{2+}\)]\(_i\), returns to its resting value between each increase (2, 5, 11), whereas in others, [Ca\(^{2+}\)]\(_i\), remains above its resting value between each increase (6) (Fig. 1, A and B). These two patterns correspond to the so-called baseline spiking and sinusoidal types of oscillations, respectively. In these smooth muscles exhibiting Ca\(^{2+}\) oscillations, the average percentage of oscillating cells is ~50–80% under identical experimental conditions. In contrast, we and others have consistently failed to observe oscillations in smooth muscles such as uterus, saphenous, and portal veins (Fig. 1, E and F). The reason for this difference remains unclear but may be related to the presence of different subtypes of protein receptor/channel in the membrane of intracellular Ca\(^{2+}\) stores [i.e., subtypes of inositol 1,4,5-trisphosphate (IP\(_3\)) receptor] and, consequently, to a different modulation of these subtypes by the [Ca\(^{2+}\)]\(_i\), (see below).

The concentration of agonist is the main factor that modulates the pattern of Ca\(^{2+}\) oscillations in smooth muscle cells. However, this modulation depends on both the type of tissue and the agonist considered. In some cases (e.g., rat and porcine tracheal muscle, human uterine arteries, rat aorta, and so forth), the frequency, but not the amplitude, of oscillations increases with the concentration of agonist, the maximal frequency usually being observed in response to the same concentration as that producing the maximal mechanical response (6, 13). In other cases (e.g., cultured cells from canine pulmonary arteries), both the amplitude and the frequency of Ca\(^{2+}\) oscillations increase with the concentration (5). Finally, in some other smooth muscles (e.g., rat pulmonary artery), the overall pattern of oscillations appears to be mainly independent of the agonist concentration, but the percentage of cells exhibiting Ca\(^{2+}\) oscillations in response to agonist stimulation does depend on the agonist concentration (2, 6). In this latter case, the combination of the number of responding cells with the amplitude of the first [Ca\(^{2+}\)]\(_i\), peak also reveals a relationship between the concentration of agonist and the [Ca\(^{2+}\)]\(_i\), response (6).

Cellular mechanisms of agonist-induced Ca\(^{2+}\) oscillations

In a variety of smooth muscle cells, Ca\(^{2+}\) oscillations are modified neither by the presence of organic Ca\(^{2+}\)-channel blockers (verapamil, nifedipine), nor by La\(^{3+}\) or removal of extracellular Ca\(^{2+}\) (Fig. 2) (2, 6, 13). Furthermore, in these types of cells, Ca\(^{2+}\) oscillations are maintained when the membrane potential is altered by K\(^+\)-rich solutions (12). In some other smooth muscle cells, Ca\(^{2+}\) oscillations progressively disappear when external Ca\(^{2+}\) is removed but are maintained in the presence of Ca\(^{2+}\) blockers (5). Nevertheless, in all of the above-mentioned cells,
agonists fail to induce Ca$$^{2+}$$ oscillations when they are pre-treated with specific blockers of the sarcoplasmic reticulum Ca$$^{2+}$$-ATPase pump, thapsigargin (TG), or cyclopiazonic acid (CPA). This result suggests that the main Ca$$^{2+}$$ compartment involved in Ca$$^{2+}$$ oscillations, or at least in the triggering of Ca$$^{2+}$$ oscillations, is an intracellular store (mainly the sarcoplasmic reticulum) (Fig. 2). Hence, agonist-induced Ca$$^{2+}$$ oscillations appear to be underlaid by a cytosolic Ca$$^{2+}$$ oscillator.

Agonist-induced Ca$$^{2+}$$ oscillations are inhibited by 1) neomycin or U73122 (2, 5, 6), potent inhibitors of the phosphoinositide phospholipase C (PI-PLC), and 2) phorbol ester (2, 6), specific activators of protein kinase C (PKC). These results indicate that the IP$_3$ pathway is implicated in the mechanism of Ca$$^{2+}$$ oscillations for the following reasons. On the one hand, agonists acting on $G_{q/11}$-coupled receptors such as AII and ET-1 increase both [Ca$$^{2+}$$]$_i$ and IP$_3$ concentration. On the other hand, direct activation of PKC by phorbol esters produces a negative feedback modulation of PI-PLC and thus a concomitant inhibition of agonist-stimulated IP$_3$ generation. Moreover, the delay (5–7 s) between the beginning of agonist ejection and the development of the first rise in [Ca$$^{2+}$$]$_i$ observed in smooth muscle cells (2, 6) is in agreement with the rapid rise (5–20 s) in IP$_3$ concentration produced by the same agonists in cultured arterial myocytes. This finding further supports the hypothesis that the IP$_3$ pathway is implicated in the mechanism of Ca$$^{2+}$$ oscillations. Finally, direct confirmation of this hypothesis has been obtained by introducing low-molecular weight heparin, a specific inhibitor of IP$_3$ receptor/channel (RIP$_3$), in the intracellular space.
miiui via the patch pipette) to show that it blocks agonist-
induced [Ca\(_{\text{2+}}\)] oscillations (3, 10). Whereas the above-
described results undoubtedly indicate that the IP\(_3\) pathway is
implicated in the mechanism of Ca\(_{\text{2+}}\) oscillations, the origin of
the cyclic character of [Ca\(^{2+}\)] variation requires further discus-
sion since it could be due to either a cyclic discontinuous
production of IP\(_3\) (receptor-controlled oscillator) or a cyclic
opening and closure of the RIP\(_3\) (second messenger-controlled
oscillator). So far, no direct evidence for a cyclic agonist-medi-
ated production of IP\(_3\) has been reported in smooth muscle. In
contrast, the regulation of RIP\(_3\) in smooth muscle is complex
and involves positive and negative feedback controls by
cytosolic and/or luminal Ca\(^{2+}\) (7). Ca\(^{2+}\) acts as a cofactor at the
site of RIP\(_3\). At a low [Ca\(^{2+}\)] (<300 nM), Ca\(^{2+}\) potentiates the
effect of IP\(_3\), whereas, at higher [Ca\(^{2+}\)] (>300 nM), Ca\(^{2+}\) antagonizes
the IP\(_3\) effect (7). This biphasic regulation of the RIP\(_3\),
combined with the activity of the sarcoplasmic and plasmalemmal Ca\(^{2+}\)
pumps, can explain the cyclic nature of the agonist-
induced [Ca\(^{2+}\)] increase. The amplitude of each [Ca\(^{2+}\)] spike may
represent the balance between Ca\(^{2+}\) release, the loss of
Ca\(^{2+}\) from the cell, and the sequestration of Ca\(^{2+}\) into intracel-
ular stores. The termination of the spike would occur when the
release process is inactivated, allowing the extrusion of Ca\(^{2+}\)
from the cell and the sequestration of Ca\(^{2+}\) into intracel-
ular stores (Fig. 3). There is the following additional evidence in
favor of a main role of RIP\(_3\) itself in the cyclic variation of the
[Ca\(^{2+}\)]. First, in some smooth muscle cells, oscillations occur
only when agonists are applied at a concentration high enough
to induce a first Ca\(^{2+}\) transient that reaches a value higher than
300 nM, the threshold [Ca\(^{2+}\)] value that antagonizes RIP\(_3\) (13).
Second, in vascular as in airway smooth muscle cells, Ca\(^{2+}\)
oscillations can be generated by exogenously applied IP\(_3\) in
permeabilized cells, i.e., at constant cytosolic IP\(_3\) concentra-
tion. Third, substances that release intracellular stored Ca\(^{2+}\) in
smooth muscles via a non-IP\(_3\)-dependent pathway, such as caf-
eine, ryanodine, or procaine, always induce a transient or
monotonic increase in [Ca\(^{2+}\)], that is never followed by oscil-
lations (2, 6). Such transient [Ca\(^{2+}\)] increase is modified by nei-
ther PI-PLC inhibitors nor phorbol esters but is blocked by
tetracaine or ruthenium red (2, 6), two potent inhibitors of the

**FIGURE 2.** Sources of Ca\(^{2+}\) implicated in agonist-induced Ca\(^{2+}\) oscillations in rat pulmonary arterial myocytes. Ca\(^{2+}\) oscillations induced by AII (A, a) or ATP (B, a) are not altered by removal of extracellular Ca\(^{2+}\) (A, b and B, b). In contrast, pretreatment of cells with thapsigargin (TG) for 10–15 min inhibits oscillations (A, c and B, c), suggesting that main source of Ca\(^{2+}\) involved in oscillations is intracellular source (probably sarcoplasmic reticulum).
Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) mechanism. It thus clearly appears that smooth muscle cells exhibit two Ca\textsuperscript{2+}-release systems located at the sarcoplasmic reticulum membrane site. One is the RIP\textsubscript{3} underlying Ca\textsuperscript{2+} oscillations through the IP\textsubscript{3}-induced Ca\textsuperscript{2+} release (IICR) mechanism, the other is the ryanodine receptor/channel protein (RyR) responsible for the CICR mechanism, which is potentiated by caffeine (Fig. 3).

Since RIP\textsubscript{3} clearly appears to be a key structure involved in agonist-induced Ca\textsuperscript{2+} oscillations, the lack of such oscillations in some smooth muscles, despite their ability to produce IP\textsubscript{3} increase in response to agonists, deserves further comment. In smooth muscle, as in nonmuscle cells, RIP\textsubscript{3} is encoded by three different genes, resulting in three isoforms, type 1-, type 2-, and type 3-RIP\textsubscript{3}, respectively. Recent studies performed on RIP\textsubscript{3} reconstituted in lipid bilayer have revealed important functional differences between the three isoforms. Interestingly, type 1-RIP\textsubscript{3} is the only isoform that exhibits the biphasic regulation by cytosolic Ca\textsuperscript{2+} (4). It is thus tempting to speculate that type 1-RIP\textsubscript{3} is the actual molecular basis of Ca\textsuperscript{2+} oscillations and that this pattern of Ca\textsuperscript{2+} signaling in smooth muscle cells is related to the relative expression of the three different RIP\textsubscript{3} isoforms in these cells. This hypothesis requires further investigation.

Since IICR and CICR are both sensitive to cytosolic Ca\textsuperscript{2+}, release of Ca\textsuperscript{2+} through one Ca\textsuperscript{2+} channel may influence the activity of the other and vice versa. Evidence for this cross talk has been obtained in porcine tracheal smooth muscle, because 1) application of ryanodine or ruthenium red during the course of ACh-induced Ca\textsuperscript{2+} oscillations inhibits these oscillations (8, 12), and 2) under these same experimental conditions, application of heparin decreases the frequency but does not abolish Ca\textsuperscript{2+} oscillations (8). These results are in favor of the involvement of both RIP\textsubscript{3} and RyR in ACh-induced Ca\textsuperscript{2+} oscillations in these cells. Such is not the case in pulmonary arterial myocytes, in which Ca\textsuperscript{2+} oscillations are not modified in the presence of CICR inhibitors (6).

Under appropriate experimental conditions, sustained Ca\textsuperscript{2+} oscillations have been observed for as long as 30 min with little change in the amplitude of the successive peaks (5). This finding suggests that the progressive loss of Ca\textsuperscript{2+} from the intracellular store related, for example, to the efflux of Ca\textsuperscript{2+} via the sarcolemmal Ca\textsuperscript{2+}-ATPase is compensated for by an influx of extracellular Ca\textsuperscript{2+}. This influx could occur via different pathways, including voltage-dependent Ca\textsuperscript{2+} channels or capacitative influx (5, 12). The relative contribution of these pathways may explain some discrepancies between studies regarding the role of the extracellular Ca\textsuperscript{2+} source in Ca\textsuperscript{2+} oscillations.

Alternatively, Ca\textsuperscript{2+} oscillations observed in some cell lines, such as A\textsubscript{R}5, disappear in Ca\textsuperscript{2+}-free solution or in the presence of Ca\textsuperscript{2+}-channel blockers but are maintained in the presence of TG. These latter oscillations are due to spontaneous variation of the membrane potential value and correspond to a membrane oscillator. Interestingly, it has been shown in the guinea pig ileal smooth muscle that the cholinergic agonist carbachol (CCh) activates one or the other of these oscillators, depending on its concentration. At a low concentration (0.2 µM), CCh evokes Ca\textsuperscript{2+} oscillations that are dependent on extracellular Ca\textsuperscript{2+} and associated with action potential discharge, whereas at a high concentration (2 µM), CCh evokes Ca\textsuperscript{2+} oscillations related to Ca\textsuperscript{2+} release from internal stores (9).

FIGURE 3. Mechanism of agonist-induced Ca\textsuperscript{2+} oscillations in smooth muscle cells. Agonists bind to specific G protein-coupled membrane receptors and activate phospholipase C (PLC), which hydrolyses phosphatidylinositol 4,5-biphosphate (PIP\textsubscript{2}) in diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP\textsubscript{3}) (1). IP\textsubscript{3} diffuses in cytosol and binds to specific receptor/channels (RIP\textsubscript{3}) at site of sarcoplasmic reticulum (RS) membrane (2). Cyclic opening of this channel due to its biphasic regulation by cytosolic Ca\textsuperscript{2+} induces oscillations both in [Ca\textsuperscript{2+}]\textsubscript{i} and in transmembrane chloride current (I\textsubscript{Cl}) (3). Activation of I\textsubscript{Cl} leads to membrane depolarization and opening of voltage-operated Ca\textsuperscript{2+} channels (4). Resulting Ca\textsuperscript{2+} influx, together with Ca\textsuperscript{2+} oscillations, participate in contractile response (5). In some smooth muscle cells, ryanodine receptor/channel (RyR) could also be involved in Ca\textsuperscript{2+} oscillations and contraction. (This possibility is indicated on the scheme with the sign ?.) Generally, in smooth muscle cells, a constant concentration of agonist induces a constant production of IP\textsubscript{3}, which induces cyclic variation of [Ca\textsuperscript{2+}]\textsubscript{i}. Ca\textsuperscript{2+} oscillations, together with Ca\textsuperscript{2+} influx through voltage-operated Ca\textsuperscript{2+} channels, contribute to a maintained contraction (see insets).
Role of agonist-induced Ca\(^{2+}\) oscillations

In smooth muscle, as in nonexcitable cells, the role of Ca\(^{2+}\) oscillations has not yet been clearly elucidated. As discussed above, in many types of smooth muscle, the oscillation frequency is sensitive to the agonist concentration. It is thus suggested that oscillations may represent a digitalization of the Ca\(^{2+}\) signal, allowing a frequency-dependent control of the cellular response. For example, in rat tracheal myocytes, the relationship between ACh concentration and Ca\(^{2+}\) oscillation frequency is similar to that between ACh concentration and the amplitude of contractile response (Fig. 4A) (13).

Ca\(^{2+}\) oscillations are also implicated in the control of the membrane potential through the activation of Ca\(^{2+}\)-activated ionic currents (chloride, potassium, or nonspecific cationic currents) (1, 15). For example, in swine tracheal and rat pulmonary arterial myocytes clamped at a value near the resting potential, agonists (ACh, All, ET\(_{1}\), and ATP) induce an oscillatory inward current, the pattern of which is similar to that of Ca\(^{2+}\) oscillations (Fig. 4B) (1, 3, 10, 15). This current is mainly independent of extracellular Ca\(^{2+}\) but disappears after treatment of cells with TG. Electrophysiological and pharmacological characteristics indicate that this oscillatory current is carried by Cl\(^{-}\) ions (1, 3, 10). Moreover, simultaneous measurement of membrane current (by means of the patch-clamp technique) and [Ca\(^{2+}\)]\(_{i}\) in the same single smooth muscle cell has revealed that 1) each peak of inward current corresponds to a peak of [Ca\(^{2+}\)]\(_{i}\), 2) there is a relationship between the amplitude of each [Ca\(^{2+}\)]\(_{i}\) peak and that of the corresponding membrane current, 3) heparin (added to the pipette solution) inhibits both the current and Ca\(^{2+}\) oscillations, and 4) niflumic acid, which inhibits the current, has no effect on Ca\(^{2+}\) oscillations (Fig. 4B) (3, 6). These data clearly demonstrate that oscillations in the

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**FIGURE 4.** Roles of agonist-induced Ca\(^{2+}\) oscillations in smooth muscle cells. A: in rat tracheal muscle, frequency of muscarinic agonist-induced Ca\(^{2+}\) oscillations (a) controls amplitude of contraction (b). Maximal amplitude of contraction is observed for highest frequency of oscillations. B: in rat pulmonary artery, Ca\(^{2+}\) oscillations control membrane potential value. Oscillations in [Ca\(^{2+}\)]\(_{i}\) induce oscillations in membrane current (a), both of which are inhibited by heparin, a specific antagonist of RIP\(_{3}\), introduced inside cell (b).
membrane current are induced by oscillations in [Ca\(^{2+}\)]. Depending on the electrophysiological conditions, the resulting Ca\(^{2+}\)-activated chloride current could participate in the membrane depolarization that secondarily opens voltage-gated Ca\(^{2+}\) channels, implicating the extracellular Ca\(^{2+}\) source in the agonist-induced contractile response (Fig. 3) (3, 6, 10).

Finally, oscillations may allow Ca\(^{2+}\) to function as a messenger while limiting the toxic effects of a sustained elevation of [Ca\(^{2+}\)]. Sustained elevation may desensitize Ca\(^{2+}\)-sensitive cellular response elements (contractile apparatus through the activation of the Ca\(^{2+}\)-calmodulin kinase II). Moreover, sustained elevation in cytosolic Ca\(^{2+}\) may increase energy loss due to stimulation of Ca\(^{2+}\)-activated ATP-dependent-enzymes (Ca\(^{2+}\) pumps).

**Conclusion**

In a variety of smooth muscle cells, it has now been clearly demonstrated that agonists produce cytosolic Ca\(^{2+}\) oscillations. In most of the cases, these oscillations are due to a cyclic release of Ca\(^{2+}\) from intracellular stores through the RIP\(_3\), as a consequence of the dual effect of cytosolic Ca\(^{2+}\) itself on the control of the channel. Although the understanding of the precise role of these oscillations requires further investigation, as a general rule oscillations may allow Ca\(^{2+}\) to function as a messenger while obviating the deleterious effect of sustained elevation of [Ca\(^{2+}\)]. Moreover, Ca\(^{2+}\) oscillations can contribute to a frequency-dependent control of smooth muscle cell response and to the value of membrane potential via the control of Ca\(^{2+}\)-dependent membrane ion channels.

There is also increasing evidence that pharmacological and/or pathophysiological factors modulate smooth muscle responsiveness via an alteration in Ca\(^{2+}\) oscillations. Nitric oxide (NO) and NO donors, β-adrenoceptor agonists, intravenous anesthetics, or benzodiazepines reduce the frequency of Ca\(^{2+}\) oscillations along with inducing relaxation, whereas gas pollutants do the opposite (14). These results obtained mostly in vascular or airway smooth muscles suggest that Ca\(^{2+}\) oscillations should be taken into account to better understand the pathophysiology and treatment of smooth muscle diseases such as hypertension, asthma, and remodeling of vascular or airway wall.

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**References**


