Calcium ions and respiratory smooth muscle function

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Introduction

There is normally a very low *resting* level of intracellular free calcium. This is true of smooth muscle cells as well as other excitable cells. It has been speculated that cells originally extruded calcium so that they could use phosphate as a basic energy currency. It is the informational content of this elevated intracellular calcium which is mobilised following a cell stimulus. Elevated cellular calcium may come from intracellular sources or it may arise from a variety of extracellular and membrane-bound sources. The target of the elevated intracellular calcium concentrations is a protein or a group of proteins which have substantial structural homology, referred to as the calcium-binding or calcium-modulated proteins. Calmodulin is the most important example, by virtue of its regulatory role in controlling a very large number of calcium-dependent processes (e.g. Cheung, 1980).

To discuss calcium ion function in smooth muscle, we must examine (1) the sources of calcium; (2) the pathways linking mobilisation of these diverse sources, i.e., the electrical, chemical or biochemical events which link the calcium source and the incoming signal, and (3) the events which occur when calcium is elevated in the cell interior; i.e., the sequence of events which leads to contraction or relaxation, in the case of smooth muscle, or to secretion, in the case of secretory cells such as mast cells.

Some general rules of calcium mobilisation are shown schematically in Figure 1. In principle we can talk about several discrete sources of Ca^{2+} , a model which may be extrapolated to a number of other cell types. Ca^{2+} mobilisation may be discussed in terms of (a) intracellular sources, (b) extracellular sources, or (c) mobilisation of both.

Intracellular sources

These sources are probably not homogeneous; in smooth muscle the intracellular sources may represent, for example, the sarcoplasmic reticulum (SR) which is present in smooth muscle, although not as well organised as it is in other muscle systems. There is a rough proportionality between the volume of what is believed to represent SR and the ability of smooth muscle to keep contracting in the absence of extracellular calcium (Devine *et al.*, 1972). Thus calcium may be mobilised from SR, from endoplasmic reticulum structures, from invaginations of the cell membrane (which are particularly prominent in a number of smooth muscle cell systems) as well as membrane-bound calcium i.e. calcium bound to the internal surface of the plasmalemma (Figure 1).

Extracellular sources

Similarly, we should distinguish between extracellular calcium which is free in the extracellular medium, and that which is extracellular but bound to or associated with specific membrane components. It may be speculated that calcium is associated with specific receptors present on the plasmalemmal surface, and that this calcium is mobilised following specific membrane stimuli. Even extracellular calcium may therefore be an heterogeneous source of mobilisation. The pathways through which extracellular calcium is mobilised are themselves also probably heterogeneous. It is speculated for a variety of smooth muscle types that the following types of pathways of calcium influx from the extracellular medium exist:

Figure 1 Diagrammatic representation of cellular calcium movements. Calcium influx can occur through ion channels, including the fast sodium channel (a minor component) and through the potential dependent (PDC) and receptor operated (ROC) calcium channels. Additionally, calcium influx can occur as one component of a plasmalemmal sodium-calcium exchange process that can operate in either direction. Calcium pumping is represented by a plasmalemmal calcium-ATPase, but calcium sequestration (and release) can take place at several intracellular sites, including mitochondria (MI), sarcoplasmic reticulum (SR), and the internal surface of the plasma membrane. A nonphysiological calcium entry process is represented by ionophore transport (Ion-Ca^{2+}). (Calmod = Calmodulin). For further details see text. Reproduced with permission from Triggle (1983).

(a) A 'leak' pathway, which occurs due to inward diffusion because of the electrochemical gradient; we may also include here calcium entering through sodium channels.

(b) Receptor-operated channels (ROC) (Figure 2). These channels are activated by membrane stimuli and are associated with receptor types. What is not clear from this definition is whether we are talking about channels which are an integral component of specific receptor types, or whether there are 'pools' of these so-called channels which may be stimulated by groups of receptors. It is possible that we have a mixture of both types of channel. These ROC permit the mobilisation of calcium; it is often suggested that this is in the absence of direct electrical signals in the membrane, although it should be pointed out that good electrophysiological data is lacking for most types of smooth muscle.

(c) Voltage or potential-dependent channels (PDC) (Figure 2). These are the channels which are hypothesised to be opened by depolarising stimuli. One can arrive at these stimuli by a variety of mechanisms—by artificial depolarising stimuli such as current injection or K^+ -depolarising solutions, or by the activation of specific receptor processes, e.g. the combination of ace-

tylcholine with muscarinic receptors in some smooth muscles leads to a membrane depolarisation and presumably to the activation of the voltage-dependent channels. These channels are sensitive primarily to the compounds known as calcium antagonists.

Finally, the possibility of Ca^{2+} -induced Ca^{2+} release in smooth muscle should be considered whereby an initial mobilisation of calcium (be it from an intracellular or extracellular source) can mobilise a larger pool of calcium to sustain the excitation/contraction coupling process (Saida & Van Breemen, 1983).

Clearly then in smooth muscle we are talking about a relatively complex situation. Indeed it was once remarked by Emile Bozler that 'the only constant thing about smooth muscle is its variability'. So the lessons we learn from one system need not necessarily apply to those of another system. Work from intestinal smooth muscle cannot necessarily be extrapolated to findings from vascular smooth muscle beds; the calcium mobilisation mechanisms in one system are not necessarily those which are dominantly employed in another system (Bolton, 1979; Cauvin *et al.*, 1983).

Calcium changes have been measured in a

Figure 2 The process of calcium mobilisation. Two discrete calcium channels, the potential-dependent (PDC) and the receptor-operated channel (ROC) are shown. Membrane depolarisation (e.g. K⁺ stimulation) activates the PDC population, which can also be activated through receptor-agonist interactions. Additionally, the receptor activation process can lead to the release of intracellular calcium. Calcium influx, whether mediated by PDC or ROC activation, can lead to release of intracellular calcium by a process of calcium-induced calcium release. PDC are extremely sensitive to calcium antagonist drugs such as verapamil (VP), nifedipine (Nif) or diltiazem (DZ).

variety of smooth muscle systems. As Hesketh (1985) mentioned, one of the difficulties in measuring intracellular calcium changes in smooth muscle systems is the enormous amount of rapidly exchangeable calcium found not only bound to the cell but also present in the interstitial spaces. A number of special techniques involve trying to get rid of contributions from extracellular calcium by the use of quenching solutions, lanthanum, calcium substitutes and the like. Ca²⁺

uptake into smooth muscle cells can be measured using such techniques. A selection of such literature data is shown in Table 1, taken for systems where contractions and calcium uptake are sensitive to the Ca²⁺ channel antagonists. This data also reveals that dose-response curves for inhibition by Ca²⁺ channel antagonists of Ca²⁺ uptake and contractions are essentially superimposable. This gives some confidence that associated events (⁴⁵Ca influx and contraction) are being measured, and that both are sensitive to this group of antagonists.

Figure 3 shows some of the possible intermediate events which occur between the initial arrival of the membrane stimulus—be it a depolarising event, an ischaemic insult or specific receptor stimulation. The biochemistry linking the initial stimulus to calcium mobilisation needs to be analysed. There are no shortages of candidates for pathways but we have little evidence which specifically and unequivocally links these proposed intermediate events to smooth muscle function. These intermediate events are not likely to be unique to smooth muscle. Firstly, the phosphatidylethanolamine-choline pathway (PE + PC) in which PE is methylated by two enzymes and, switched vectorially across the membrane, is believed to be involved in a number of calcium mobilisation processes (Hirata & Axelrod, 1980). Secondly there is the pathway of polyphosphatidyl inositol (PPI) metabolism and the messenger role of intracellular inositol phosphates (see Hesketh, 1985). Then there is the role of specific protein kinases; cyclic AMP dependent protein kinase, calcium-calmodulin dependent kinase, protein kinase C, which may be involved in the phosphorylation of membrane constituents linked to the passage of ions (Cohen, 1982) and, the more recently discussed pathway

Table 1 Comparison of 50% inhibiting concentrations (IC₅₀) of a number of calcium antagonists on contraction or calcium influx (⁴⁵Ca) of various tissues. YC93 = nifedipine; VP = verapamil; FZ = flunarizine. Data assembled from literature sources (for review see Triggle & Swamy, 1983).

System	Antagonist	IC ₅₀ (M)	
		Contraction	⁴⁵ Ca
Rabbit aorta, K ⁺	YC.93	1.9 × 10 ⁻⁹	1.0 × 10 ⁻⁹
	VP	1.7 × 10 ⁻⁷	6.8 × 10 ⁻⁸
	D600	2.0 × 10 ⁻⁷	1.5 × 10 ⁻⁷
Guinea pig ileum, ACh	Nifed	2.0 × 10 ⁻⁸	3.5 × 10 ⁻⁸
	D600	1.0 × 10 ⁻⁷	8.0 × 10 ⁻⁷
Rat aorta, K ⁺ , NA	FZ	2.2 × 10 ⁻⁷	1.8 × 10 ⁻⁷
	FZ	5.0 × 10 ⁻⁷	7.0 × 10 ⁻⁷
Rat superior medius, NA	FZ	2.4 × 10 ⁻⁸	5.0 × 10 ⁻⁸

Figure 3 Possible biochemical pathways linking receptor (REC) stimulation to calcium mobilisation. PE phosphatidyl ethanolamine, PC phosphatidyl choline, PPI polyphosphatidyl inositol, DG diacyl glycerol, PMT phospholipid methyltransferase.

involving ornithine decarboxylase and the products of this, the polyamines, putrescine, spermine and spermidine which may also be involved in calcium mobilisation (Koenig *et al.*, 1983). Thus there are several candidate pathways and their definition is important to the understanding of smooth muscle physiology, as well as to other calcium mobilising systems. In addition, the interrelationship between these pathways is important.

Calcium entry into a system may also provoke a variety of membrane-associated events which go on to modulate the smooth muscle responses. When we direct an initial signal at the membrane (e.g. noradrenaline, acetylcholine, bradykinin etc.), a chain of events is initiated that end up as the phenomena of smooth muscle contraction or relaxation but which may be greatly modified by the products of, for example, phospholipid metabolism (Figure 4). Some of these pathways may be involved in calcium gating mechanisms, and in addition may generate products of the arachidonic acid cycle which themselves modify the smooth muscle response (Mitchell, 1984). If the biochemical events themselves are not directly involved in calcium gating, entry or mobilisation of calcium by other mechanisms can switch on these processes, and these processes (the products of arachidonic acid metabolism) then go on to modify the smooth muscle response. We should also recognise that in a number of systems we have multiple sensitive processes. Thus, a number of smooth muscles are sensitive to a large number of stimuli. Figure 5 is a highly schematic summary of events that go on in the respiratory system, which is clearly an example of a multiple sensitive system. Here we are concerned not only with agents that act directly on smooth muscle, but also with agents which act by mobilising a variety of mediators which act on the smooth muscle (Figure 5). In any discussion of smooth muscle function, it is

Figure 4 Representation of receptor-mediated changes in phospholipid metabolism. Left, Phospholipid methylation sequence in which phosphatidylserine (PS) is converted to phosphatidylcholine (PC) via phosphatidylethanolamine (PE) and methylating enzymes I and II phospholipid methyltransferase, (PMT). Right, Phosphatidylinositol cycle. Both systems can provide substrate for arachidonic acid for the cyclo-oxygenase (CO) and lipoxygenase (LO) pathways. In addition activation of protein kinase C ($DG-Ca^{2+}-PL$ activated) may occur.

necessary to consider both the linkage of the initial stimuli to calcium mobilisation and the ways in which secondary stimuli, such as products of phospholipid metabolism, may modulate the initial stimuli, and the intervention of a variety of mediator processes. All these factors contribute to the final response of smooth muscle contraction and/or relaxation. These are important processes not only in the physiological state but also in the pathological state.

Finally, we should be concerned with the ways in which calcium metabolism may be linked to a variety of hyperreactive states including vascular and bronchial hyperreactivity. There may exist important similarities between the two states.

Figure 5 The lung as a multisensitive system. Contraction of lung smooth muscle is achieved both by neurotransmitters including acetylcholine (ACh) and noradrenaline (NA) and by mediators, including histamine (HA), prostaglandins (PG), and leukotrienes (LTD), released from mast cells. Reproduced with permission from Triggie (1983).

Thus (Figure 6), membrane defects, including changes in calcium binding, changes in calcium permeability, changes in ion transport, and metabolic changes (due to the incursion of additional ions and the intervention of phospholipid processes) lead to an elevation of intracellular calcium under what would normally be resting level of stimuli, and hence to the phenomenon of hyperreactive states. It is important to try and understand some of these processes since they will lead to a better understanding of (a) physiology and (b) pathology of airways smooth muscle. The lessons we learn from other systems can be applied to smooth muscle and *vice versa*.

The above discussion of Ca^{2+} mobilisation in smooth muscle has been deliberately general. In the context of Ca^{2+} channel antagonist actions in respiratory smooth muscle it is clear that the usefulness of these agents will depend upon which pathways of Ca^{2+} mobilisation operate in response to physiological and pathological stimuli. The nature of these stimuli and their operational levels in respiratory smooth muscle

Figure 6 Schematic representation of events in bronchial hyperreactivity. Shown are potential linkages between an elevated intracellular Ca^{2+} and metabolic, permeability, ionic, structural and functional changes. The initiating membrane defect(s) is not known but it is suggested that the consequences of this initial defect may become self-reinforcing because of the several interrelationships depicted. Reproduced with permission from Triggie (1983).

Figure 7 Comparison of the activities of calcium antagonists on rat mesenteric arteries and guinea pig trachea. Dilt = diltiazem; Nitr = nitrendipine; Nisol = nisoldipine; Nimod = nimodipine. Other compounds are aryl-substituted nifedipine analogues (2NO₂ = nifedipine). Data of Yousif & Triggle.

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Discussion

Barnes How much is known about calcium removal from the cell and/or sequestration? This may be a potential area for pharmacological intervention.

needs to be determined. In this connection the data in Figure 7 may be of some interest. A comparison of the activities of a series of 10 Ca²⁺ channel antagonists in guinea pig tracheal smooth muscle and rat mesenteric arteries to a common stimulus, 80 mM K⁺, shows that the vascular smooth muscle is significantly more sensitive to this series of compounds than is respiratory smooth muscle (Yousif & Triggle, unpublished observations). The basis for this apparent selectivity against respiratory smooth muscle remains to be determined, but such considerations are useful if we are able to modify selectively and successfully Ca²⁺ homeostasis in a specific smooth muscle type.

Triggle There is evidence now using plasmalemmal fractions for an ATP-driven process which is presumably involved. My interest would be more in how the duration of the calcium