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Review Article

Calcium, the Control of Smooth Muscle Function and Bronchial Hyperreactivity

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The Ca^{2+} requirements for excitation-contraction coupling in smooth muscle may be satisfied from both intracellular and extracellular sources, the relative extent of use of which is both tissue- and stimulant-dependent. Extracellular Ca^{2+} is apparently mobilized through two separate pathways, receptor operated (ROC) and potential dependent (PDC) Ca^{2+} channels. The latter process is sensitive to the Ca^{2+} -channel antagonists, a heterogeneous group of compounds including verapamil, nifedipine and diltiazem. Ca^{2+} mobilization in respiratory smooth muscle is reviewed. The available evidence for this multiple stimulant-sensitive system indicates that both intra- and extracellular sources of Ca^{2+} are used. Data from bovine, canine and guinea pig tracheal muscle indicate, from studies of Ca^{2+} -dependence of response and Ca^{2+} channel antagonist sensitivity, that the extent of use of extracellular Ca^{2+} lies in the order $\text{K}^+ > \text{histamine} \geq 5\text{-hydroxytryptamine} > \text{acetylcholine}$. The bronchodilator activity of the Ca^{2+} channel antagonists is noted. Bronchial hyperreactivity is characterized by an increased sensitivity to a variety of stimulants including cold air, exercise, histamine and acetylcholine. The possible origins of this defect are noted. It is suggested that a defect in Ca^{2+} mobilization or in the receptor - Ca^{2+} mobilization coupling process at the level of the smooth muscle may constitute an important underlying cause of bronchial hyperreactivity. Potential analogies to reactivity changes seen in hypertensive vascular smooth muscle are noted.

Key words: bronchodilation; bronchial hyperreactivity; Ca^{2+} ; Ca^{2+} antagonists; calcium channels; diltiazem; excitation-contraction coupling; nifedipine; smooth muscle; verapamil.

Accepted for publication 24 September 1982

The role of Ca^{2+} in the initiation and maintenance of contractile activity was discovered by Sidney Ringer 100 years ago. It is now realized that Ca^{2+} is a messenger species of vital importance to the generation of cellular excitability and function. This messenger role is made possible by the highly asymmetric distribution of Ca^{2+} across the plasma membrane and by the existence of specific cellular mechanisms for Ca^{2+} entry and exit, mobilization and sequestration (Fig. 1).

The mechanical activity of smooth muscle, in common with other muscle types and motile systems, is controlled by the concentration of free (ionized) intracellular Ca^{2+} (1). Subsequent to an initiating membrane signal the intra-

cellular Ca^{2+} concentration rises from a basal level of $\leq 10^{-7}$ M to approximately 5×10^{-6} – 10^{-5} M. It is further assumed for smooth muscle that, in common with skeletal muscle, muscle shortening and contractile activity arise from a sliding filament mechanism involving the thick myosin and the thin actin filaments and an associated increase in the myosin ATPase activity (2). Although skeletal and smooth muscle have many fundamental properties in common, they differ in certain very important respects, particularly the regulatory mechanism(s) by which Ca^{2+} sensitivity is conferred on the contractile proteins and the source(s) of the activator Ca^{2+} .

For smooth muscle there is considerable evi-

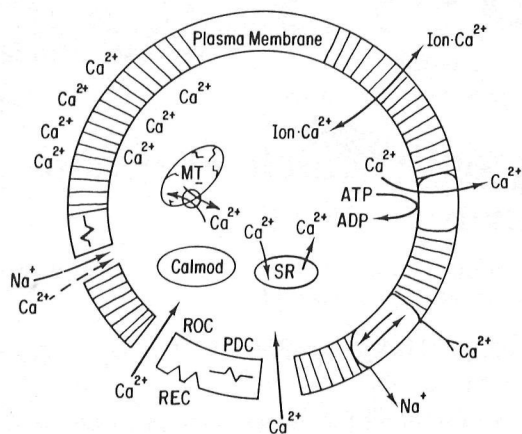


Fig. 1. Diagrammatic representation of cellular Ca^{2+} regulation. Intracellular Ca^{2+} , maintained in the resting state at $<10^{-7}$ M ionized Ca^{2+} , is shown bound to the internal membrane surface and sequestered within sarcoplasmic reticulum (SR) and mitochondria (MI) via active uptake processes. The intracellular Ca^{2+} binding protein (Ca^{2+} -receptor) is shown as calmodulin (Calmod). Calmodulin may be membrane- or cytosol-associated. Ca^{2+} exit can occur through a plasma membrane ATPase-drive Ca^{2+} pump and by a $\text{Na}^+:\text{Ca}^{2+}$ exchange mechanism. The latter can operate in both directions, and with high intracellular Na^+ can serve as an entry mechanism for Ca^{2+} . Additional entry routes for Ca^{2+} are shown as the receptor-operated (ROC) and potential-dependent (PDC) Ca^{2+} channels and (as a minor component) through the fast Na^+ channel. A nonphysiologic bypass mechanism is shown as ionophore-mediated Ca^{2+} entry (Ion = ionophore, A23187, ionomycin).

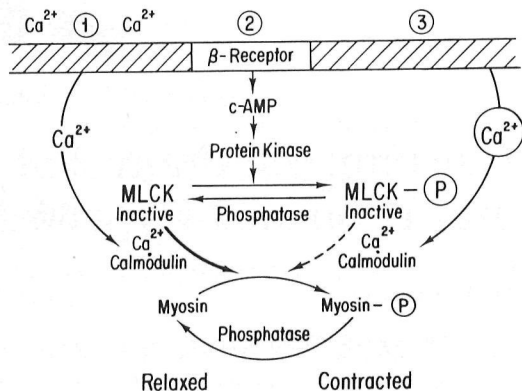


Fig. 2. Ca^{2+} -Induced events in smooth muscle contraction. Ca^{2+} (mobilized from either extracellular (①) or intracellular (③) sources) binds to calmodulin to activate myosin light chain kinase (MLCK) and initiate phosphorylation of myosin (Myosin - (P)) which leads to contraction. Activation of the cAMP dependent protein kinase, by a β -receptor activation process (②), leads to phosphorylation of MLCK (MLCK - (P)). Phosphorylated MLCK has a lower affinity for Ca^{2+} -calmodulin and is less active than MLCK in provoking myosin phosphorylation.

The pathway outlined in Fig. 2 for the control of smooth muscle activity is widely, but not universally, accepted. An alternative explanation (1) suggests that Ca^{2+} sensitivity is conferred not by calmodulin, but by leiotonin, a Ca^{2+} -binding protein with affinity for thin filaments and, furthermore, that myosin phosphorylation is not obligatorily coupled to actin-myosin interaction. However, regardless of the details of the activating mechanism the fundamental event in the contraction of smooth muscle is the increase in concentration of cytosolic activator Ca^{2+} .

Sources of Ca^{2+} in smooth muscle excitation-contraction coupling

Since Ca^{2+} is the regulator of smooth muscle tension development, elucidation of the sources of mobilized Ca^{2+} is of considerable importance to the definition of excitation-contraction coupling in smooth muscle. Unlike fast skeletal muscle, where an almost exclusively intracellular source of Ca^{2+} is mobilized, smooth muscle may employ both intracellular and extracellular sources of Ca^{2+} . The extent of use of these

dence that Ca^{2+} sensitivity is conferred by calmodulin, the ubiquitously distributed and structurally conserved Ca^{2+} binding protein, rather than the troponin C of skeletal muscle (3, 4). The function of calmodulin is to activate myosin light chain kinase (MLCK) as the activated Ca^{2+} -calmodulin-myosin kinase complex. Phosphorylation of myosin light chains permits the activation by actin of myosin ATPase activity. Smooth muscle relaxation is associated with dephosphorylation of myosin via a Ca^{2+} -independent phosphatase (Fig. 2). Myosin light chain kinase can be phosphorylated through the cAMP dependent protein kinase and in its phosphorylated state has a reduced affinity for calmodulin. Agents such as β -adrenergic agonists, which increase cAMP levels, can promote relaxation via this mechanism (Fig. 2).

sources depends upon the tissue, the stimulant and the component of response (6, 7). Furthermore, as indicated in Fig. 3, these sources are not necessarily independently activated.

There appear to be two primary pathways for Ca²⁺ entry, the so-called receptor-operated channels (ROC) and the potential-dependent channels (PDC), although it is not likely that either channel is totally selective for Ca²⁺ (6, 8). PDC are activated by stimulants, including K⁺ depolarization media, which produce membrane depolarization including action potential generation. The alternate ROC pathway is activated by stimulants in the absence of major evoked changes in membrane potential. Thus in a number of smooth muscles tension responses can be initiated in the absence of membrane potential change. This dual pathway provides one explanation of the many observations that stimulants can cause additional tension response when applied to K⁺-depolarized preparations (6) and provides a basis for the designation of pharmaco-mechanical and electro-mechanical modes of excitation-contraction coupling (9). In addition to extracellular sources (membrane-bound and free extracellular Ca²⁺), Ca²⁺

may be mobilized from intracellular sources including sarcoplasmic reticulum, despite its relative paucity in smooth muscle (6, 10). The process of intracellular Ca²⁺ release may involve a Ca²⁺ (or Na⁺) induced Ca²⁺ release process. These considerations indicate the complexities of Ca²⁺ mobilization within smooth muscle which are obviously of particular importance in respiratory smooth muscle, which consists of several levels of smooth muscle organization and which is responsive to a number of stimulants (Fig. 4).

Further distinction between these several routes of Ca²⁺ mobilization is provided by the group of agents referred to as Ca²⁺ channel blockers, Ca²⁺ entry blockers or slow channel blockers (11, 12, 13, 14). This chemically heterogeneous group of agents, which includes verapamil/D 600, nifedipine and diltiazem (Fig. 5), is of major clinical and pharmacologic significance. These agents appear to have considerable affinity and selectivity for the potential dependent Ca²⁺ channel, a property early reconized by Fleckenstein and his colleagues (11) who described the uncoupling effect of these agents on cardiac excitation-contraction coupling (suppression of plateau Ca²⁺ current). These agents are also potent inhibitors of excitation-contraction coupling in many smooth muscles which property, together with their cardiac activities, underlies their clinical and potential clinical uses, which currently include cardiac arrhythmias, angina and arterial hypertension. The relative activities of these agents in smooth muscle offer support to the previous comments on the routes of Ca²⁺ mobilization. K⁺-Induced responses are invariably very sensitive to the inhibitory activities of these agents, whereas considerable variations exist in the sensitivities of stimulant-evoked responses. Thus 5-hydroxytryptamine responses in a rabbit cerebral (basilar) artery are much more sensitive than the corresponding responses in a peripheral (saphenous artery). Within a single tissue significant differences in stimulant sensitivity to the Ca²⁺ channel antagonist may exist. In tracheal smooth muscle (see later discussion) responses to 5-hydroxytryptamine, histamine and acetylcholine show di-

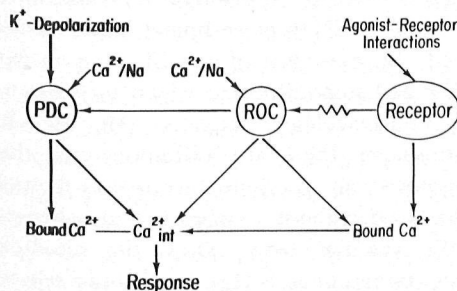


Fig. 3. Ca²⁺ mobilization in response to membrane stimuli. Shown are three primary routes of Ca²⁺ mobilization: Ca²⁺ entry through receptor operated channels (ROC) and potential dependent channels (PDC) and Ca²⁺ mobilization from bound intracellular stores. The entry mechanisms are probably not specific for Ca²⁺ and hence are designated Ca²⁺/Na⁺. The interaction of agonists with specific membrane receptors can lead to activation of ROC and to mobilization of intracellular Ca²⁺. Membrane potential changes from ROC activation can lead to activation of PDC. A K⁺ depolarizing stimulus leads to activation of PDC. Ca²⁺ entry mediated through either ROC or PDC may also lead to release of Ca²⁺ from internal stores (Ca²⁺/Na⁺-induced Ca²⁺ release).

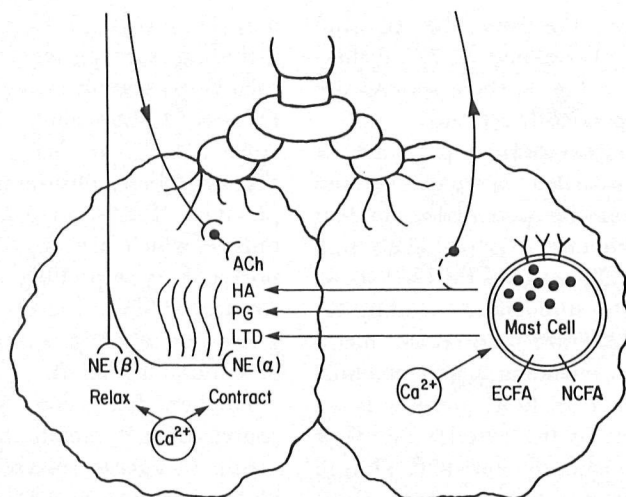


Fig. 4. The lung as a multisensitive system. Contraction of lung smooth muscle is achieved both by neurotransmitters including acetylcholine (ACh) and norepinephrine (NE) and by mediators, including histamine (HA), prostaglandins (PG), and leukotrienes (LTD), released from mast cells.

minishing sensitivity to these antagonists. Furthermore, and consistent with the scheme of Fig. 3, responses to low concentrations of stimulants may be more effectively antagonized (15), probably reflecting the relative extents of activation of PDC and ROC/intracellular stores by low and high concentrations of stimulants respectively. Results from isolated human tissues, though limited, appear to parallel those from animal tissues (16, 17, 18). It is thus clear that generalizations about the potency of the Ca^{2+}

channel antagonists in different smooth muscles are not easily made.

The Ca^{2+} channel antagonists are currently under intensive investigation to define further their clinical utility, mechanisms of action, and to develop new and more selective agents. Radioligand binding experiments with the nifedipine analog, [^3H]-nitrendipine, have demonstrated the existence of specific sites in both cardiac and smooth muscle which correlate well with pharmacologic activities (19, 20, 21). Furthermore, there are indications that these antagonists can discriminate not only between cardiac and smooth muscle, but also between specific vascular beds. Thus, the nifedipine analog, nimodipine, is reportedly more selective for cerebral vascular beds (22) and nisoldipine for the coronary vasculature (23). These findings are particularly exciting since they raise the possibility of antagonist selectivity for other smooth muscles including respiratory smooth muscle.

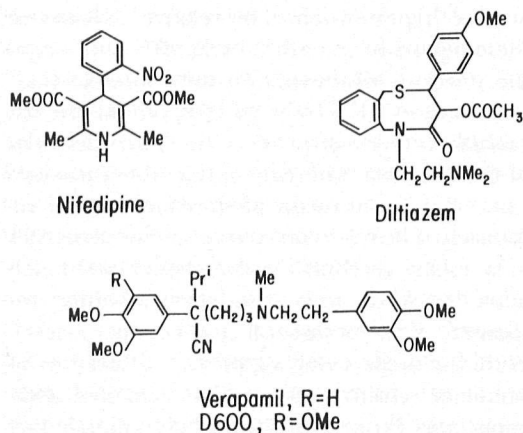


Fig. 5. Structural formulae of some Ca^{2+} -channel antagonists.

Excitation-contraction coupling in respiratory smooth muscle

The processes of excitation-contraction coupling in respiratory smooth muscle have not

been subject to systematic investigation. Notably absent is any serious comparative study of excitation-contraction coupling at the various levels (tracheal to alveolar) of respiratory smooth muscle organization. However, the data available, largely from canine, bovine and guinea-pig trachea, support the conclusions drawn in the previous section that Ca²⁺ mobilization processes do differ in the same tissue according to the stimulant.

Electrophysiologic studies in canine tracheal muscle show that contraction and membrane depolarization are only partially linked (24, 25, 26, 27). Thus, in contrast to K⁺-induced responses (electromechanical coupling) acetylcholine-induced contractile responses show a partial dissociation between mechanical and electrical events. At low concentrations acetylcholine (ACh) causes both contraction and depolarization, but at higher concentrations produces further contraction without depolarization (pharmacomechanical coupling). Furthermore, although 5-hydroxytryptamine (5-HT) behaves in a qualitatively similar manner to acetylcholine, it shows a quantitatively greater association of tension and electrical responses (25, 27). The order of use of the potential-dependent Ca²⁺ activation mechanism is thus, K⁺ > 5-HT > ACh.

The conclusions drawn for canine tracheal smooth muscle are supported generally by studies of Ca_{EXT}²⁺-dependence of response and by the effects of the Ca²⁺ channel antagonists. Ca_{EXT}²⁺-depletion experiments show that both intra- and extracellular Ca²⁺ sources are used. In bovine and guinea pig trachea responses to K⁺ and histamine (Hist.) are more dependent on Ca_{EXT}²⁺ than are responses to acetylcholine (28, 29, 30). In both guinea pig and canine trachea the responses to low concentrations of acetylcholine are more sensitive to Ca_{EXT}²⁺-depletion than are the responses to high acetylcholine concentrations. This suggests that Ca²⁺ influx is more important at low concentrations and accords with electrophysiologic studies indicating potential dependent tension development at low acetylcholine concentrations. The Ca_{EXT}²⁺-depletion experiments are supported by the actions of the Ca²⁺ channel antagonists. Quite gener-

ally, sensitivity of response parallels sensitivity to Ca_{EXT}²⁺-depletion, that is, K⁺ > 5-HT ≥ Hist > ACh (25, 26, 29). In canine trachea responses to K⁺ are completely blocked by verapamil and D 600, responses to acetylcholine are very resistant while responses to 5-hydroxytryptamine are of intermediate sensitivity (25). Of particular interest is the finding that responses to low concentrations of acetylcholine are more sensitive to the inhibitory effects of verapamil than are high concentrations of acetylcholine (29). This is completely consistent with the thesis that low concentrations of acetylcholine mobilize Ca²⁺ through potential dependent Ca²⁺ channels (electromechanical coupling) whilst high concentrations probably mobilize Ca²⁺ from intracellular stores (pharmacomechanical coupling). The collective data for Ca²⁺ mobilization in tracheal smooth muscle excitation-contraction coupling is schematically represented in Fig. 6. The importance of these considerations is that in a multisensitive tissue such as the lung (Fig. 4) patterns of Ca²⁺ mobilization will be dependent both upon the nature of the stimulant and its concentration. There is an almost complete lack of comparative data for other levels of respiratory tissue. However, nifedipine has been reported to inhibit in Ca²⁺-dependent manner both intrinsic tone and histamine- and carbachol-induced responses in guinea pig trachea and parenchyma, parenchymal tissue being more sensitive than tracheal tissue (31).

The data with the Ca²⁺ channel antagonists are of considerable interest to any role that

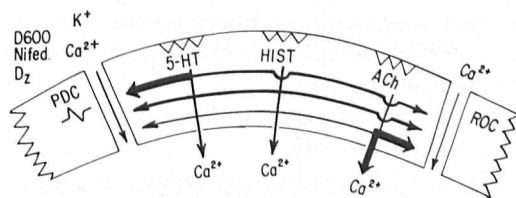


Fig. 6. Schematic representation of Ca²⁺ mobilization in tracheal smooth muscle in response to 5-hydroxytryptamine (5HT), histamine (HIST) and acetylcholine (ACh). The relative extent of mobilization of extracellular Ca²⁺ through ROC and PDC and from intracellular sources is depicted by the thickness of the arrows. Thus, 5-HT is shown as mobilizing extracellular Ca²⁺ largely through the D 600, nifedipine (Nifed) and diltiazem (Dz) sensitive PDC, whilst ACh mobilizes a dominantly intracellular source.

these agents may have in the alleviation of airway constriction. Some reports of the clinical applications of these agents have appeared (32, 33, 34, 35, 36), and although it is probably too early to judge their potential use, the use of selective Ca^{2+} -channel antagonists could be an attractive alternative to current bronchodilator therapy.

Ca^{2+} and bronchial hyperreactivity

The association of bronchial hyperreactivity and the asthmatic state is well established and the augmented responsiveness of respiratory smooth muscle of the susceptible individual to a wide variety of stimuli including cold air, exercise, acetylcholine, histamine and other bronchoconstrictor stimuli has been widely described (*inter alia*, 37, 38, 39). Appropriately designed reactivity tests can be used as a classification criterion of the asthmatic state (40). However, the underlying cause or causes of the enhanced reactivity of respiratory smooth muscle remain to be defined.

In principle, bronchial hyperreactivity may be associated with one or more defects in the control of respiratory smooth muscle. These potential defects include:

Physical changes:

1. Decreased airway caliber.
2. Smooth muscle hypertrophy.
3. Epithelial cell damage.

Physiologic and pharmacologic changes:

1. Increased parasympathetic activity.
2. Increased sympathetic (α -receptor mediated) activity.
3. Decreased sympathetic (β -receptor mediated) activity.
4. Decreased nonadrenergic inhibitory activity.

The relative importance and contribution of these several changes have been the subject of critical discussion elsewhere (38), to which the reader is referred. It is worth emphasising, that any postulated mechanism(s) of hyperreactivity must accommodate the fundamental observation that reactivity to a wide variety of stimuli is

increased. While this could be consistent with the existence of a group of specific defects (receptor changes, etc.) all of which are manifest as enhanced bronchial responsiveness, it is perhaps more likely that a single underlying defect in the pathway coupling stimulus to response (Fig. 7) should be considered.

Because Ca^{2+} plays a fundamental role in excitation-response relationships at the stimulus-secretion and excitation-contraction coupling levels (mast cell mediator release, mucous gland secretion, vagus nerve activity, smooth muscle contractility (41), alterations in Ca^{2+} regulation may be fundamentally and directly linked to bronchial hyperreactivity.

There is currently little direct evidence to support this speculation of a role for Ca^{2+} in the etiology or expression of bronchial hyperreactivity (but see refs. 45 and 46). However, since bronchial hyperreactivity is expressed at the level of the respiratory smooth muscle in response to both direct and indirect stimuli, a defect in Ca^{2+} handling by the smooth muscle cell is plausible. Further definition of Ca^{2+} mobilization mechanisms and pathways is urgently needed. There is however, at least a potential analogy between hyperreactivity and the reactivity changes associated with vascular smooth muscle in clinical and experimental hypertension (*inter alia*, 42, 43, 44). In hypertension a variety of reactivity changes in vascular beds and isolated smooth muscles have been docu-

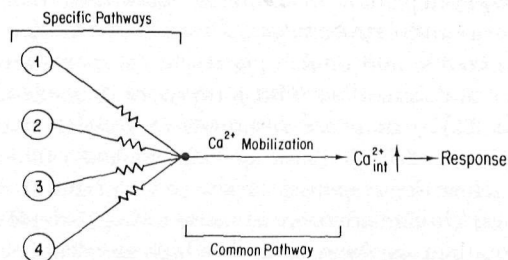


Fig. 7. In a multisensitive system the excitation-contraction coupling pathway may be composed of both specific and common steps. In this representation specific stimuli 1, 2, 3, and 4 (receptor activated events) lead to a common stage of Ca^{2+} mobilization. A single defect in the common pathway will impair responses to all stimuli, whereas distinct defects in each of the specific pathways will be necessary to produce the same impairment.

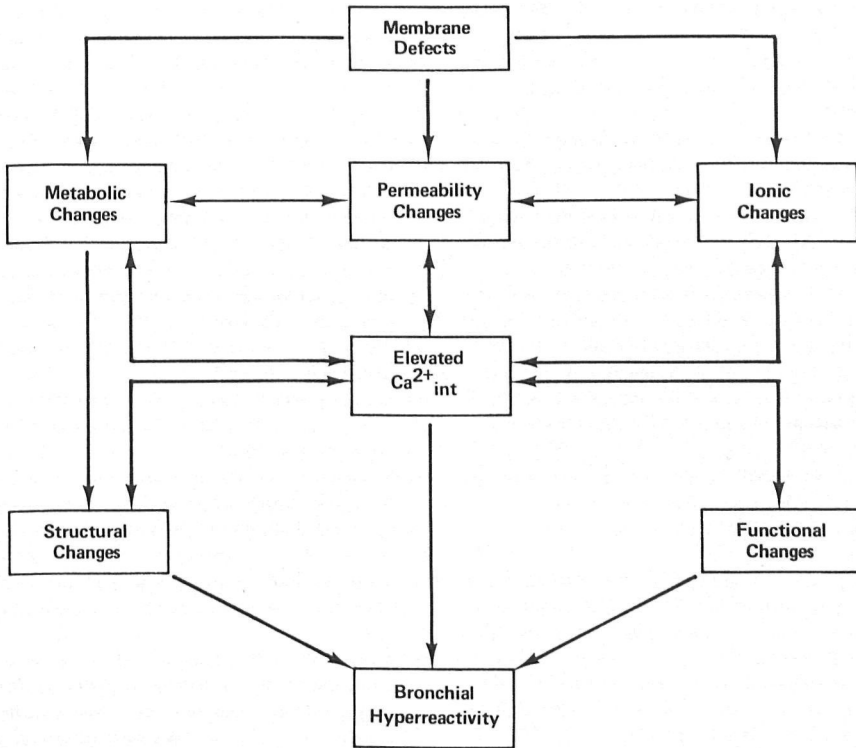


Fig. 8. Schematic representation of events in bronchial hyperreactivity. Shown are potential linkages between an elevated intracellular Ca²⁺ and metabolic, permeability, ionic, structural and functional changes. The initiating membrane defect(s) is not known but it is suggested that the consequences of this initial defect may become self-reinforcing because of several interrelationships depicted.

mented which are consistent with a basic defect in vascular smooth muscle Ca²⁺ handling. These changes include: increased sensitivity to Ca²⁺, increased ionic (Na⁺, K⁺) permeability, reduced relaxation rate following stimulus removal, reduced Ca²⁺ transport activity, and altered sensitivity to Ca²⁺ channel antagonists.

If changes in the relationship between stimulus strength and the concentration of free intracellular Ca²⁺ do underlie alterations in smooth muscle reactivity then it is important to note that, regardless of origin, these changes may also be associated with other changes in smooth muscle properties (Fig. 8) documented to occur in respiratory smooth muscle. Thus elevated intracellular Ca²⁺ may produce metabolic changes, including alterations in phospholipid metabolism via the Ca²⁺ sensitive phospholipase A₂ (47, 48), structural changes including smooth muscle hypertrophy (49, 50) and func-

tional changes, including changes in receptor population(s) (51) because of alterations in receptor-effector (Ca²⁺) coupling.

ACKNOWLEDGEMENTS

Preparation of this paper was assisted by grants from the National Institutes of Health (HL 16003 and AI 14198).

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