BRONCHIAL ASTHMA

Mechanisms and Therapeutics
Second Edition

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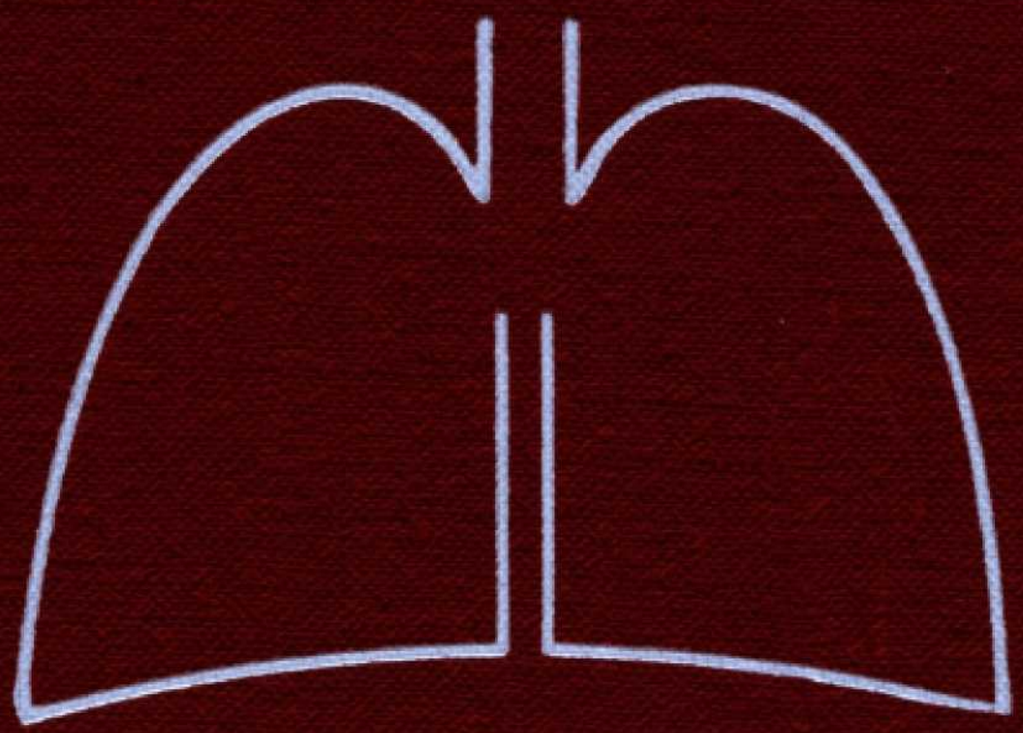

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65. CALCIUM AND ITS ROLE IN THE ASTHMA PROCESS

LINDA J. BARBERO, WAYNE H. ANDERSON, AND EARLE B. WEISS

In all living organisms calcium exerts a major regulatory role upon diverse physiologic processes. With respect to asthma its potential role may influence many interrelated processes such as smooth muscle tone and contraction, mucociliary function, mast cell mediator release, involvement in inflammatory processes, cellular permeability, neurotransmitter function, and a host of intracellular biochemical events. Its intracellular function is so vital that any drug or agent capable of regulating the entry of calcium into the cell can influence cellular events and metabolism as specific physiologic changes.

Since the actions of calcium are specific and its cellular effects subject to small changes in concentration, its ionic concentration is critically regulated: 10^{-3} M extracellularly and 10^{-7} M intracellularly. These narrow physiologic limits have imposed the evolution of complex systems to regulate extracellular and intracellular calcium concentrations. Extracellularly its function to stabilize membranes was shown by Ringer in 1890 when he dissociated tissue cells in a calcium-free medium and demonstrated that calcium was more effective than potassium and sodium in protecting aquatic organisms from swelling in distilled water [90]. Intracellular calcium is characterized by its affinity for forming organic compound complexes, notably with proteins: As the result of calcium-protein interaction, protein function is altered (e.g., activation to an enzyme). That calcium influences cell-to-cell adhesion is shown by numerous experiments where calcium removal disaggregates cells that can be reaggregated by the addition of calcium to the medium [5, 55]. This divalent cation also controls the cell membrane permeability of the physiologically important monovalent cations, sodium and potassium, in excitable tissue [14, 76, 98] and will also protect cells against lysis by osmotic and pH effects [73].

Calcium is well suited to mediate cell adhesion and permeability effects because as an alkaline earth metal it forms a stable metal chelate with ligands found in abundance on the cell surface (e.g., collagen, phospholipids, and mucopolysaccharides). This interaction reduces the net charge of the cell surface, resulting in stabilization of the cell. The charge, ionic radii, degree of hydration, and geometry of orbitals used in covalent bonding between a metal and a chelating group determine the stability of the resulting complex. Divalent cations (Ca^{++}, Mg^{++}) form stronger complexes than do the monovalent cations (Na^{+}, K^{+}), which have a lower charge density. Between the physiologically relevant divalent cations, the smaller ion (Mg^{++}) is more strongly hydrated than the larger ion (Ca^{++}), where the charge is dispersed over a greater surface area. To form a chelate complex, a metal ion must lose most of its hydration sphere. Thus the larger, less hydrated metal ion binds more strongly than the smaller, more hydrated one. The transition metal ions (Mn^{++}, Fe^{++}, Cu^{++}, Zn^{++}) supply d orbitals, which participate in covalent bonding and thus are better suited to occupy sites in proteins, where they bind to nitrogen and sulfur. Calcium and magnesium are best suited to complex with the numerous phosphate and carboxylate ions found on the cell surface, on cytoplasmic enzymes and contractile proteins, and in various organelles.

Calcium mediates cellular regulatory effects on enzyme activity, contractile proteins, and secretory processes. These activities are based on its chelation properties as well as on an intricate compartmentalization system related to the relative membrane permeabilities of different anions and cations. Although sodium and potassium occur in similar amounts in the crust of the earth, living cells accumulate potassium ions almost to the exclusion of sodium. For example, seawater is about 0.5 M in Na^{+} and 0.01 M in K^{+}. Within cells the sodium content is one-tenth the K^{+} content, yet the relationship is reversed in serum, which is 0.15 M in Na^{+} and 0.005 M in K^{+}. This ionic distribution results in a negative charge within the cell and gives rise to an electric membrane potential. That the maintenance of this membrane potential is of utmost physiologic importance is evidenced by the fact that a cell will expend 20 to 25 percent of its energy supply in order to maintain this ionic distribution [72]. It is this differential distribution of ions across the cell membrane that gives rise to action potentials in excitable cells and signals disruptions in cell membrane integrity in all cells.

In normal human serum the total calcium concentration is 2.5 mM; 0.8 mM is bound to proteins, 0.3 mM is in the form of complexes with inorganic and organic ions, and not more than 1.3 mM is free. In most cells the resting permeability to calcium is low, being one to three orders of magnitude less than for monovalent cations [60]. Free cytoplasmic calcium levels in resting cells are about 10^{-7} M. This low intracellular calcium ion concentration is maintained as a result of different homeostatic mechanisms, which include sequestration of free ion by binding to endoplasmic reticulum, mitochondria, and other organelles [32, 71], by extrusion of calcium to the extracellular space by Na^{+}-Ca^{++} exchange [111], or by active transport by a Ca^{++}-ATPase [97]. It has been hypothesized that the low level of free calcium derives from the ubiquitousness of inorganic phosphate in biologic systems. The hydrolysis of phosphate esters is the principal source of cellular energy, and a high concentration of intracellular phosphate must be maintained, which is incompatible with appreciable intracellular calcium levels. Elaborate regulatory controls have evolved to maintain the free cytoplasmic calcium levels and thus prevent calcium phosphate deposition within cells. Hence the evolution of cel-

lular organization has resulted in a special role for calcium as a messenger. Any change in the intracellular calcium concentration, upon stimulation by various agents, is an efficient means of transmitting information concerning a change in the cell's environment [70]. Depolarization during the action potential of skeletal muscle fibers is caused almost entirely by the rapid influx of Na^+, while in smooth muscle large quantities of calcium ions are involved. This influx of calcium ions enters the muscle fiber from the extracellular medium and in addition to other intracellular sources (e.g., sarcoplasmic reticulum) elicits smooth muscle contraction. That smooth muscle cells respond to chemical or electric stimulation by opening specific calcium ion channels through which calcium enters from the surrounding media is discussed in a later section. The resulting rise in the cytoplasmic calcium concentration from 10^{-7} M to 10^{-5} M couples the specific stimulus with secretion (stimulus-secretion coupling) or, as discussed, with contraction (excitation-contraction coupling). This is relevant to the asthmatic process in the release of histamine from mast cells by an antigenic stimulus or the contraction of the airway smooth muscle by, for example, agonist histamine receptor stimulation.

Many cell-surface receptors undergo a conformational change when they bind signaling molecules to the cell surface. This change produces an intracellular message, altering the activity of the target cell. The extracellular ligands, termed *first messengers*, thus activate intracellular second messengers. The two common mechanisms by which receptors on the cell surface generate intracellular signals are (1) activation of adenylate cyclase bound to membranes, which then increases cytosolic c-AMP, and (2) opening of membrane-bound gated Ca^{++} channels, permitting Ca^{++} entry to the cell. Many hormones (e.g., epinephrine) or local chemical mediators are biologically active by activating adenylate cyclase, each cell responding to an increase in c-AMP in its characteristic physiologic fashion. Similarly, there are increasing data that Ca^{++} acts as an important intracellular regulator, functioning as a second messenger for certain signaling extracellular molecules. Like c-AMP the concentration of *free* Ca^{++} in the cytosol is normally very low ($\leqslant 10^{-7}$ M), because most of this cation is bound to other molecules or sequestered in intracellular organelles. Since there appears to be a large gradient tending to flux Ca^{++} into the cell, the continuous and rapid removal of free Ca^{++} is necessary. These efflux or sequestering processes make possible the rapid increase and decrease in concentration of Ca^{++}, permitting cellular response to stimuli.

In muscle cells calcium has a special role by directly controlling the interaction of myosin cross bridges with actin. Of the three major types of muscle in vertebrates, the smooth muscles produce a slower and longer lasting contraction of such tissues as bronchi and blood vessels compared to cardiac or skeletal muscle contractures. Smooth muscle in contrast to skeletal muscle has no striations but consists instead of long, tapered cells with both thick and thin filaments oriented longitudinally. The actin and myosin are special to these tissues. For example, actin amino acid sequences are different from cardiac or skeletal muscle. Smooth muscle myosin, while resembling skeletal tissue, is physiologically different in that the level of its ATPase activity is tenfold lower than that of skeletal muscle and is subject to greater Ca^{++} regulation. In addition, smooth muscle myosin interacts with actin to cause contraction only when its light chains are phosphorylated. While smooth muscle actin and myosin cause contraction in a way fundamentally like skeletal muscle, these actin-myosin filaments are less highly ordered than skeletal muscle, and their movement is dependent upon a Ca^{++}-regulated myosin phosphorylation.

A general schema of smooth muscle activation is presented below:

$$\text{Calmodulin} + Ca^{++} + \text{inactive kinase} \rightarrow \text{active kinase} - Ca^{++} - \text{calmodulin};$$

$$\text{Active kinase} - Ca^{++} - \text{calmodulin} + \text{inactive myosin} \rightarrow \text{myosin-P (active)};$$

$$\text{ATP} \rightleftharpoons \text{ADP}$$

$$\text{Myosin-P (active)} \rightarrow \text{interaction with actin} \rightarrow \text{contraction.}$$

For smooth muscle cells the identity of the myoregulatory system is controversial but is well reviewed by Hartshorne [54]. The most widely accepted theory is that control is by a calcium-dependent phosphorylation of myosin light chains. Calmodulin, a recently discovered evolutionary ancient and ubiquitous protein, is described in many eukaryotic species and cell types [110]. This protein modulates the level of free intracellular calcium and activates important enzymes such as myosin light chain kinase (MLCK), adenylate cyclase, and cyclic 3′,5′-phosphodiesterase [16, 21, 28]. Myosin light chain kinases are inactive at resting muscle free calcium concentrations of 10^{-7} M [102]. Smooth muscle is triggered to contract by an influx of calcium following neurohormonal stimuli, and when the intracellular calcium concentration rises to 10^{-5}, MLCK is activated in vitro. This activation is hypothesized to arise from the binding of calmodulin to the kinase, which then phosphorylates myosin light chains and effects contraction. Myosin light chain kinase can be phosphorylated by cAMP-dependent protein kinase, which decreases its activity [1]. Conti and Adelstein [25] have shown this to be related to a decreased affinity of MLCK for calmodulin. This and other studies have resulted in an interesting model for calmodulin modulation of smooth muscle contraction, which also accounts for epinephrine relaxation of airway smooth muscles in, for example, acute asthma [2]. It should be noted that Ca^{++} binding to calmodulin

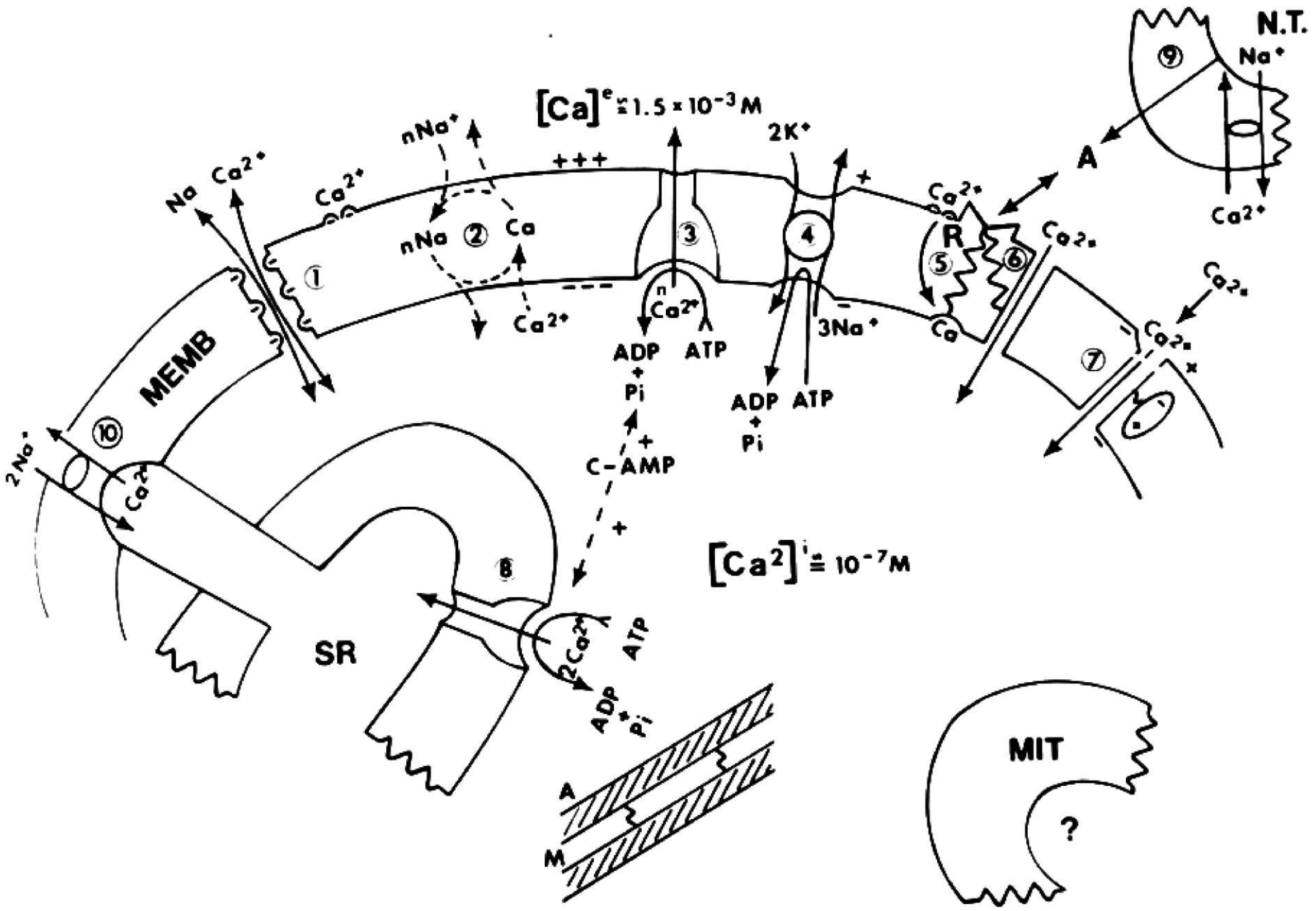


Figure 65-1. *Hypothetical Ca^{++} transport mechanisms involved in the regulation of cytoplasmic calcium that could be affected by sodium ions:* 1, *a pore lined with fixed negative sites that have affinity for both Na^{+} and Ca^{++};* 2, *the Na, Ca exchange carrier, which couples the inward movement of n Na^{+} to 1 Ca^{++} moving out of the cell* (n *refers to the number of Na^{+} ions bound by a hypothetical Na, Ca exchange carrier). Since evidence in the literature does not support its presence in smooth muscle, a* dashed line *is used;* 3, *an active Ca^{++} extrusion pump energized by ATP hydrolysis. No stoichiometric coefficients are as yet available for this pump;* 4, *Na, K-ATPase pump for this pump for active Na^{+} extrusion and active K^{+} accumulation;* 5 *and* 6, *a receptor complex for smooth muscle agonists. Activation of the receptor* (R) *leads to release of Ca^{++} bound on the inner surface of the membrane and opens a channel,* 6, *that allows Ca^{++} to enter from the extracellular space. The squiggles linking the receptor with the Ca^{++} binding sites and channel represent unknown energy transduction pathways. The channel may be the same as the one that refills the internal storage sites from Ca^{++} bound to the outer cell membrane surface,* 5; 7, *a voltage-dependent Ca^{++} channel;* 8, *the sarcoplasmic reticulum* (SR) *Ca-ATPase;* 9, *the neurohormonal release mechanism that involves Ca^{++} flux across the nerve terminal membrane;* 10, *a hypothetical special pathway for Ca^{++} between the SR and extracellular space. Its features are (1) greater Ca^{++} permeability of the SR membrane facing the plasmalemma, (2) restriction on lateral diffusion of Ca^{++} in the special cytoplasmic region between the SR and plasmalemma, and (3) a Na, Ca exchange mechanism that facilitates Ca^{++} transport across the plasmalemma. (Reprinted with permission from C. Van Breeman, P. Aaronson, and R. Loutzenhiser, Sodium-calcium interactions in mammalian smooth muscle.* Pharmacol. Rev. *30:167, 1979.)*

is relatively slow in activating smooth muscle contraction as contrasted with skeletal muscle. This slower myosin cross-bridge cycle causes the smooth muscle cells to contract slowly, enabling such muscles to maintain a constant tension with much greater efficiency; for example, to perform a given equivalent work task, hydrolysis of five- to tenfold less ATP is required than would be needed by a skeletal muscle cell.

In studying the disordered function of smooth muscle in diseases such as asthma or hypertension, one must discern if the muscle is functioning abnormally or has a faulty control mechanism. While force development by smooth muscle is directly regulated by the concentration of free myoplasmic calcium ions, extensive evidence incriminates many factors in this process, as, for example, the influence of intra- and extracellular Na^{+} concentrations upon myoplasmic free Ca^{++}. This ionic influence may be important in vascular smooth muscle in the pathogenesis of a disorder such as vascular hypertension.

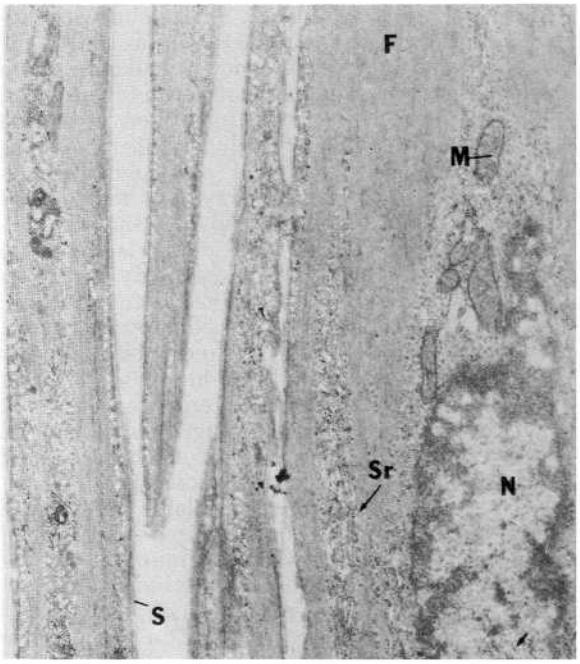
A summary of such calcium transport mechanisms in smooth muscle is depicted in Figure 65-1. The basic problem in smooth muscle cell phys-

iology and pharmacology is the mystery of myocontractile regulation in comparison to skeletal muscle (Fig. 65-2). Skeletal muscle is large, and each fiber is under direct control by innervation of a motor neuron axon. Skeletal muscles contract when presented an electric stimulus carried by calcium action potentials through voltage-dependent channels. In contrast, smooth muscles are smaller, some are not directly innervated by nerves, and they cannot propagate a stimulus over great distances. Thus, smooth muscle cells are 40 to 100 μ in length but only 2 to 5 μ in diameter compared to cardiac muscle cell diameter of 10 to 20 μ. Cardiac cells have numerous invaginations in the plasma membrane, called the *T tubule system*, which act as an extensive interface between the cytoplasm and the extracellular fluid. In addition, cardiac and skeletal muscle cells have extensive sarcoplasmic reticulum which store intracellular calcium, but these reticular membranes are considerably diminished in smooth muscle. In smooth muscles the contractile proteins themselves are not as highly organized into interdigitating arrays of filaments as found in cardiac muscle. Smooth muscle cells have been shown to possess voltage-dependent and receptor-oriented calcium channels [11, 53] (see also subsequent discussion). Calcium movements within muscle are difficult to study experimentally, because the resultant calcium levels that are measured are the sum of influx and redistribution within the cell as well as efflux homeostatic mechanisms. Analysis of smooth muscle contraction to K^+ depolarization, acetylcholine, histamine, or other mediators in vitro is often performed by measuring tension (isotonic or isometric), which has distinct components. An initial phasic component is thought to arise when calcium enters through voltage-dependent channels, releasing an intracellular store of calcium, since a phasic contraction can often be obtained in calcium-free medium [12]. A subsequent slower, sustained tonic contractile component is believed to arise by the entry of extracellular calcium, as this is abolished in Ca^{++}-free media [99]. Thus, in order to dissect the regulatory process of smooth muscle contraction, many questions must be answered concerning the source(s) of the trigger calcium [51, 62] and the relative contribution of different stimuli (voltage-dependent or receptor-mediated) [17] (Fig. 65-3). The regulatory processes that sense the increase or decrease in available intracellular Ca^{++} have been reviewed recently by Hartshorne [54].

The role of calcium in asthma is complicated because this ion plays a role in stimulus-secretion coupling as well as in muscle contraction (plus many other events!). Some chemical mediators of muscle contraction are released from the mast cell through immunologic and even nonimmunologic mechanisms. These complex processes and their relation to calcium are reviewed by Foreman [47] and are summarized in Figure 65-4. Mast cells release histamine and other mediators as slow-reacting substances of anaphylaxis (SRS-A); the latter have recently been identified to be of the lipopeptide class, leukotrienes (LT). The calcium dependency of preformed histamine release as well as the extracellular Ca^{++} dependency of leukotrienes on smooth muscle contraction have been reported [43, 49, 121]. Some leukotrienes have been found that are very active on, for example, peripheral airways while histamine and $PGF_{2\alpha}$ (prostaglandins) are more active in the large airways and trachea (see Chaps. 8 and 15). Experimentally, histamine release is maximal when 1 to 2 mM calcium is present in the bathing media [49], but interestingly, histamine can also be released by the calcium ionophore A23187 in the absence of extracellular calcium [65]. This suggests that a rise in the free cytoplasmic calcium is a sufficient condition for such histamine release. Histamine release in response to anti-IgE (antibodies to mast cell surface IgE) has been found to be enhanced by products of arachidonic acid metabolism in purified rat mast cells [104].

The sources of calcium available for smooth muscle contraction are, however, not fully defined and may vary with the animal source of the smooth muscle and the agonist utilized to induce contractions. For example, in potassium depolarization–induced contractures, extracellular Ca^{++} appears to be the main source, while caffeine activates largely intracellular stores [68, 83]. Similarly, Farley and Miles [42] have shown that excitation-contraction coupling for acetylcholine in dog trachealis varies with the drug dose, with low ($< 10^{-6}$ M) concentrations depending upon Ca^{++} influx associated with electromechanical coupling, while at high concentrations intracellular Ca^{++} is additionally operative.

Arachidonic acid is released from plasma membrane phospholipids by a specific phospholipase following the binding of certain agonists at their receptor site. The phospholipases, which liberate the arachidonate precursors, are also calcium dependent, with membrane phospholipids implicated in stimulus-secretion coupling at receptors that control cell-surface calcium gates [78]. Liberated arachidonate may take either of two pathways: the cyclooxygenase pathway, which generates prostaglandins (PG), or the lipoxygenase pathway, which generates leukotrienes. That an imbalance of arachidonic acid metabolism may contribute to airway hyperreactivity was suggested by Yen and Morris [125]. Prostaglandin effects on smooth muscle have been extensively studied, and it is hypothesized that they regulate the intrinsic tone of airway smooth muscle [24]. The effects may also be differential, depending on the particular PG (e.g., PGE as a dilator, $PGF_{2\alpha}$ as a constrictor) and the airway size. Inhibition of the cyclooxygenase pathway by acetylsalicylic acid or indomethacin shunts released arachidonate to lipoxygenase pathways, which may account for the effects of aspirin in some asthmatic patients [19]. Although the central role of calcium in immune degranulation of mast cells is well established, the lack of effect of antihistamine in the treatment of asthmatic patients [3] as well as the potency of LTs has turned research attention to the



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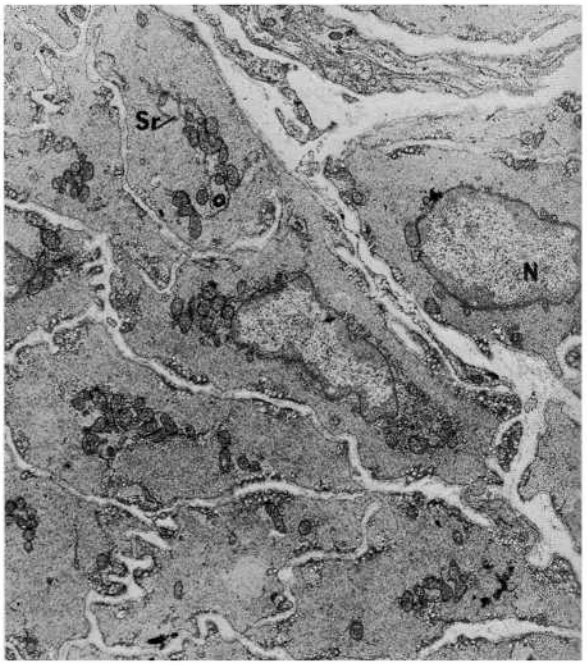

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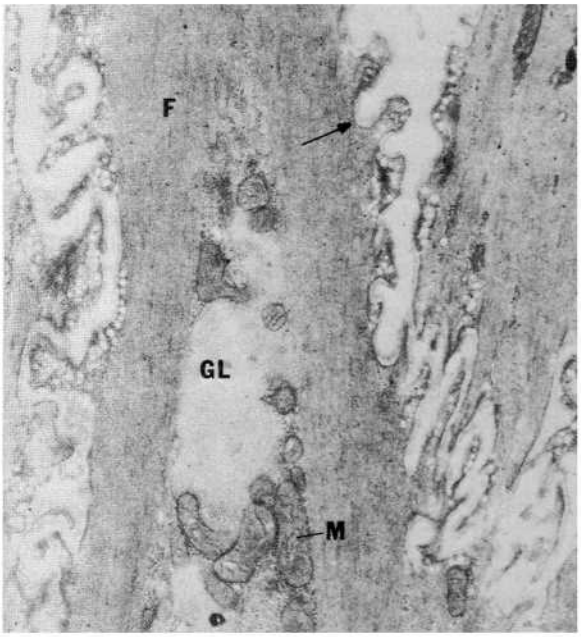

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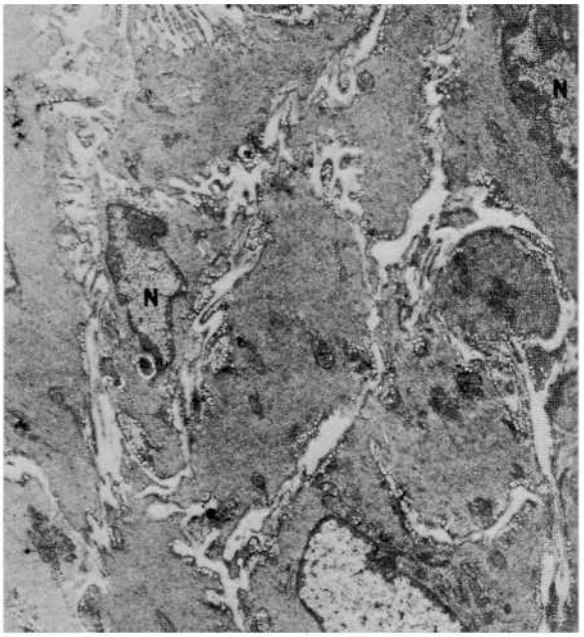

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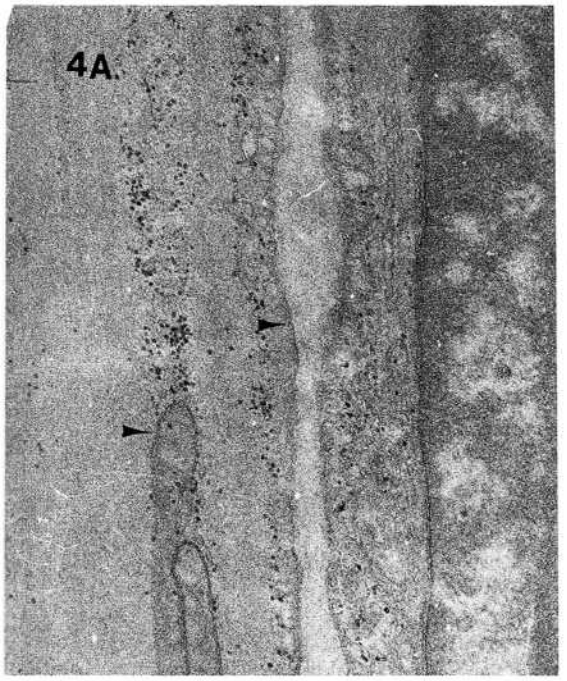


E

Figures 65-2A–E. *Electron micrographs of guinea pig trachealis. Fig. 65-2A–B: Resting muscle. A. Longitudinal section (×13,000 before 20% reduction). B. Transverse section (×65,000 before 20% reduction). Fig. 65-2C–D: Anaphylaxis-induced contraction. C. Longitudinal section. D. Transverse section; arrow indicates extensive membrane invaginations and evaginations. In Fig. 65-2E (resting) arrows indicate calcium oxidate precipitates. The normal trachealis muscle cell has several ultrastructural characteristics that are constant regardless of the relative length at which the tissue was fixed. There is a prominent, large, and single nucleus centrally located in the cell. Mitochondria are usually found grouped and intertwined with sacroplasmic reticulum (Sr) at the polar ends of the nucleus. The Sr, however, is relatively sparse. Occasionally, mitochondria can be found scattered throughout the cell. There is also an as yet unexplained interaction between the mitochondria and Sr at the cell surface with the caveolae. The caveolae line the cell surface (surface vesicles, pinocytotic vesicles, etc.) and are most often found as the projections that form the evaginations of the cells. Cell endings usually appear to be literally covered with these surface vesicles. Glycogen is often found scattered throughout the cell. Cell to cell junctions (cell contacts, gap junctions desmosomes, nexuses) are also readily observed. (S = sarcolemma membrane; F = myofilaments; M = mitochondria; Sr = sarcoplasmic reticulum; N = nucleus; GL = glycogen.) (Courtesy of J. Jacobs Ph.D.)*

arachidonic acid metabolites, specifically the leukotrienes [15].

Leukotrienes are released following immunologic challenge of mast cells and lymphocytes [96]. Slow-reacting substance of anaphylaxis has been demonstrated to consist of a mixture of leukotrienes C_4, D_4, and E_4 (LTC_4, LTD_4, and LTE_4) [95]. These products have been shown to be released following anaphylactic (immunogenic) challenge of human bronchus and guinea pig trachea and lung strips [29, 101]. A major characteristic of leukotrienes is their uniquely slow but potent and sustained contraction seen in a variety of smooth muscles. Several investigators have examined the role of extracellular calcium in the contraction produced by the LTs. Findlay and coworkers [44] have reported the presence of an SRS "receptor" in smooth muscle, which shares a pool of calcium channels in common with all smooth muscle agonists [44]. In guinea pig trachea Hedman and Andersson [56] have found SRS-A contractions to be completely dependent upon extracellular Ca ions; Weiss et al. [121] have described the extracellular dependency of LTC_4, LTD_4, and LTE_4. In guinea pig ileum the time course of LTD_4 contractions and the effect of the calcium channel antagonist D-600 on the various phases of the contractile response [43] suggest that LTD_4 activates the calcium channel mechanism of calcium translocation; the slowness of the LTD_4-induced contraction may be caused by the failure of this leukotriene to release cellular calcium.

Calcium Antagonists

It has long been appreciated that calcium is an integral component of cell function, and elaborate mechanisms have evolved to control the level of intracellular calcium, particularly with respect to muscle function and secretory processes. In a disorder such as asthma a variety of calcium-dependent events may coexist: bronchial muscle contraction, mast cell mediator release, vagally mediated reflexes, mucus secretion, mucociliary function, and inflammatory responses. Given the critical biologic role of calcium, it is clear that pharmacologic intervention may be of importance in asthma therapy. The potential for specifically (or nonspecifically) interfering with selective calcium-mediated cellular processes is as exciting as the role for drugs in probing basic asthma mechanisms. Table 65-1 indicates some possible actions of antiasthma drugs on intracellular calcium activity. Early studies of airway smooth muscle employing the local anesthetic lidocaine indicated that calcium antagonism to agonist or immune-induced contraction and/or anaphylactic histamine release provided another approach to asthma therapy [117, 118].

Fleckenstein and colleagues [46, 69] were pioneers in the use and definition of a class of organic compounds they described as *calcium antagonists*. In general, the term *calcium antagonists* could refer to any mechanism that affects calcium-activated cellular processes, including (1) the inhibition of

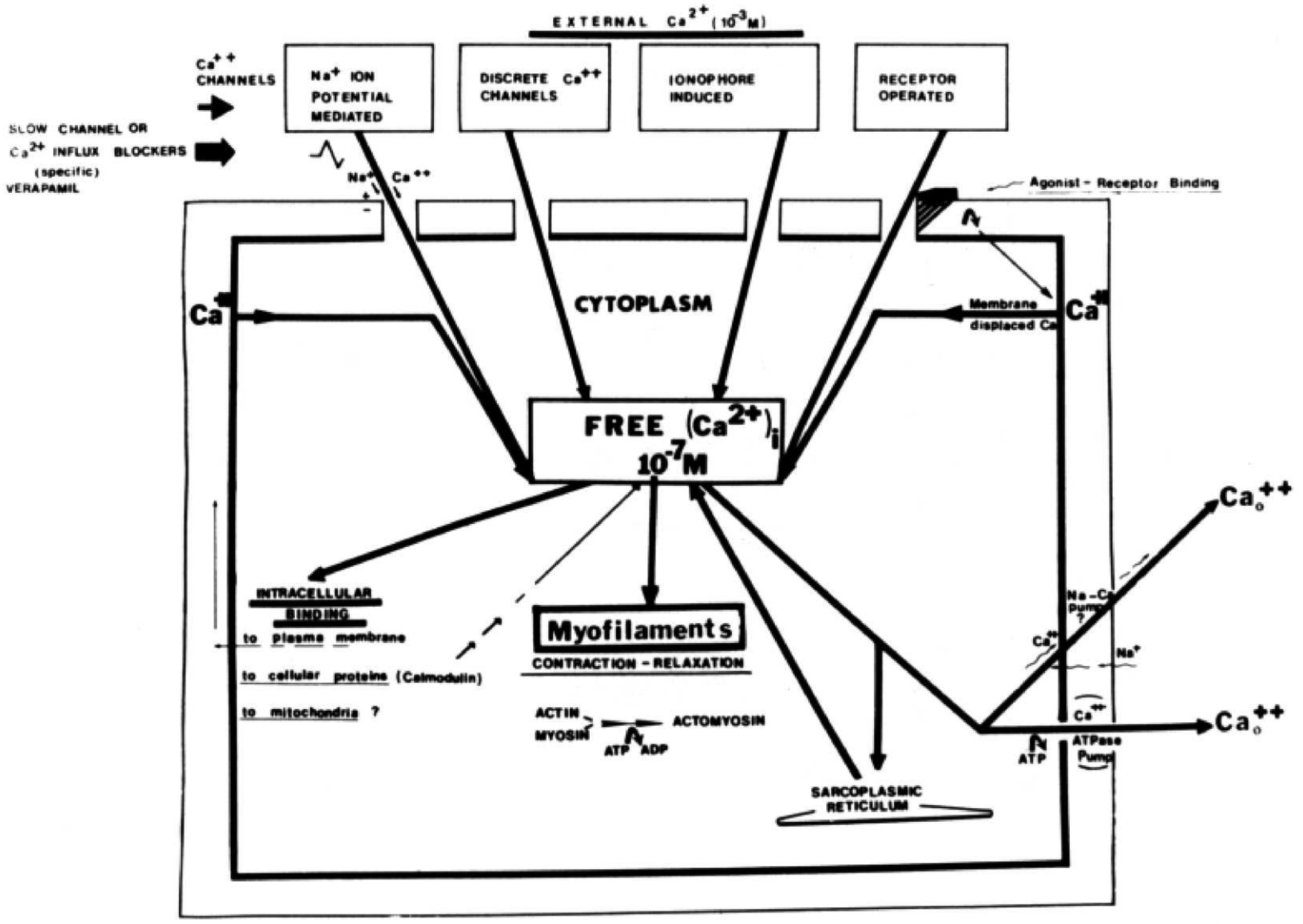


Figure 65-3. *Possible calcium sources in smooth muscle contraction. For muscles that do not generate an action potential, calcium enters the cell through receptor-operated ion channels, and subsequently calcium is released from sarcoplasmic reticulum or inner membrane displaced (hypothetical) or from mitochondria (postulated). Calcium entry in smooth muscles generating action potentials through ion potential–mediated channels is important at low concentrations of stimulant drugs. Within the cell release or binding to intracellular proteins including calmodulin or to membranes, etc., influences the level of free intracellular calcium; cytosolic free calcium increasing from 10^{-7} M to 10^{-5} M will activate contraction. Storage and exit sites are also depicted. Pharmacologic interaction with calcium is possible at any of the sites or mechanisms.*

calcium binding to various calcium-binding proteins such as calmodulin, (2) the lowering of intracellular calcium by stimulation of intracellular uptake or calcium-pumping mechanisms, (3) the inhibition of intracellular release, and (4) the inhibition of calcium channels. Two general calcium channels have been defined [11]: voltage dependent and receptor operated. Voltage-dependent channels are activated by membrane depolarization, whereas receptor-operated channels are activated by agonist-receptor occupation. In canine tracheal smooth muscle 5-hydroxytryptamine and potassium have been demonstrated to elicit contractions through voltage-dependent mechanisms, while acetylcholine activates voltage-independent, receptor-operated channels [23].

The inhibition of calcium influx through calcium channels is the proposed mechanism of organic compounds such as verapamil and nifedipine. There are at least four classes of organic calcium channel inhibitors (Fig. 65-5). They are the dihydropyridines (nifedipine), phenylalkylamines (verapamil), benzothiazepines (diltiazem), and diphenylalkylamines (prenylamine).

Despite the diversity of Ca^{++}-associated events in vivo, the predominant pharmacologic effect of the organic calcium channel inhibitors is on the cardiovascular system [22]. Some of these compounds are not new. Verapamil has been clinically available as an "adrenolytic" before its definition as a calcium channel inhibitor [113]. Therapeutically, these agents are useful in the treatment of angina pectoris, supraventricular tachycardia, hypertension, and hypertrophic cardiomyopathy (variant angina) and for protecting the ischemic myocardium during cardiac surgery [45, 126]. It is somewhat obvious that a calcium channel inhibitor might be used to treat bronchoconstriction as well as coronary artery spasm, since both are caused by contraction of smooth muscle. The following discussion deals with the biochemical pharmacology of calcium channel inhibitors and their therapeutic use in allergic diseases and asthma.

BIOCHEMICAL MECHANISM OF ACTION. Preparation of cardiac and smooth muscle membranes have demonstrated specific, saturable, stereospecific reversible binding of labeled calcium antagonists to membrane receptors [8, 64, 81]. In general, a single class of binding sites has been demonstrated by lin-

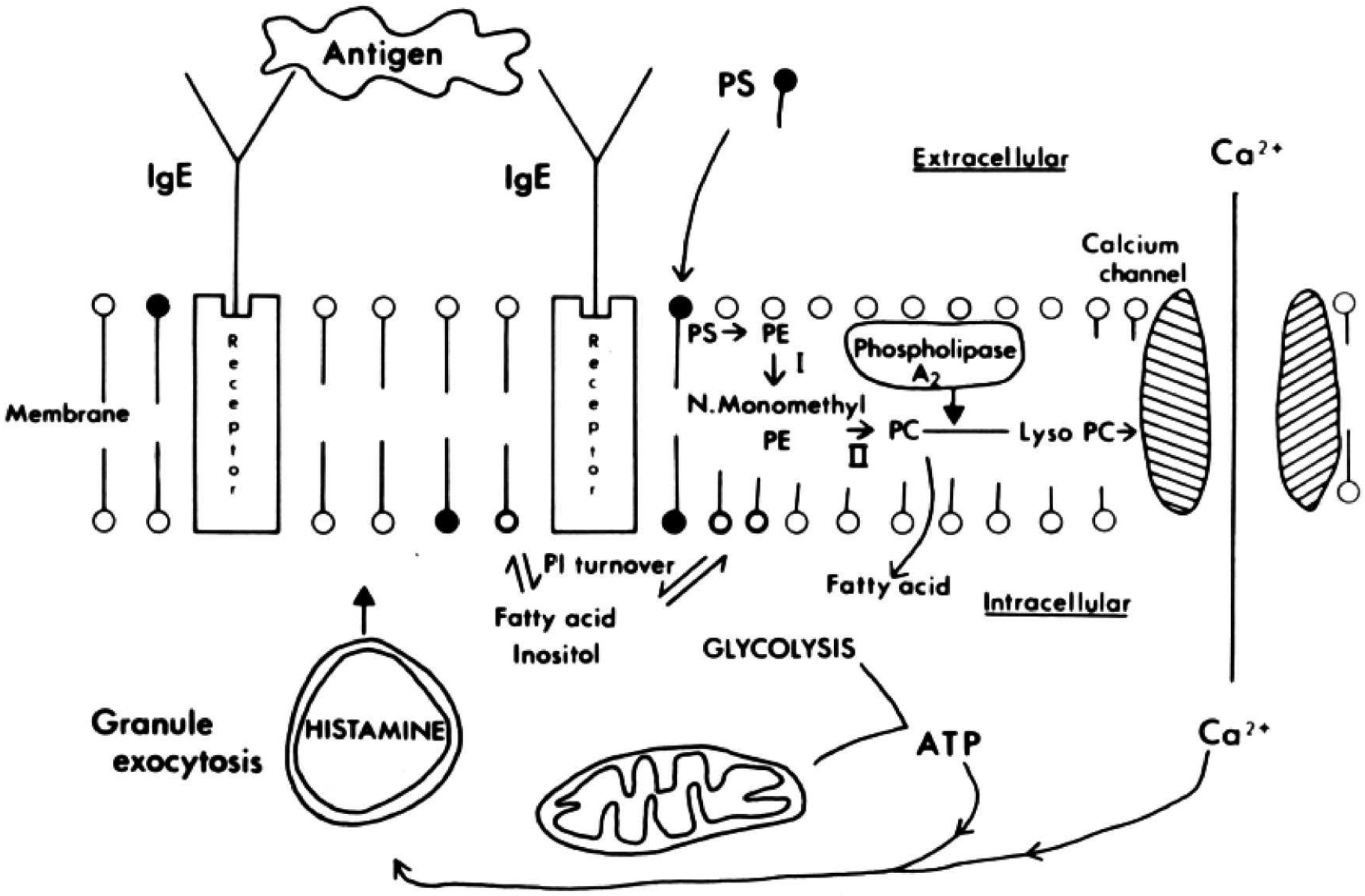


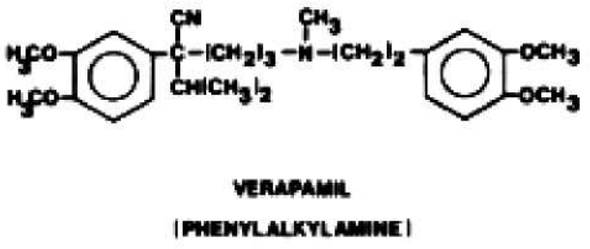
Figure 65-4. *Summary of the membrane events considered to be involved in activation of mast cells to secrete their histamine. Receptor cross-linking initiates a sequency of phospholipid methylations and the activation of a phospholipase, which together bring about the opening of calcium channels. Cyclic AMP (not shown) is believed to be responsible for inactivating the channel, possibly by a protein kinase–mediated phosphorylation. (I and II indicate the two methyltransferase enzymes.) (Reprinted with permission from J. C. Foreman, Receptor-secretion coupling in mast cells.* Trends Pharmacol. Sci. *1:460, 1980.)*

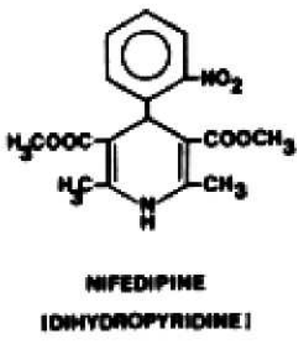
ear Scatchard analysis in a variety of tissue types including heart [8, 36], brain [36, 37], and vascular smooth muscle [33, 124]. The dissociation constant (Kd) for these receptors is similar for each of the tissues, but the number of binding sites differs considerably. For example, in rat mesenteric artery [108] the Kd for ^{3}H-nitrendipine is 0.1 nM with 18 fmoles of receptors per milligram of membrane (B_{max}) as compared with skeletal muscle transverse tubules, which have a Kd of 1.8 nM and B_{max} of 50,000 [50]. A greater similarity in Kd and B_{max} can be observed in similar tissue types (Table 65-2).

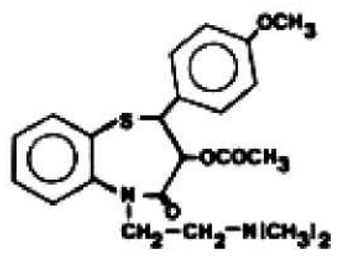
Binding of ^{3}H-nitrendipine is reduced by the trivalent inorganic calcium antagonist lanthanum, suggesting that binding of nitrendipine is closely associated with the calcium channel [36, 52]. Removing calcium with EDTA reduces binding to brain membrane preparations to 15 percent, without altering the Kd. Heart membrane preparations did not show a similar calcium lability [52].

Table 65-1. *Antiasthma Drugs and Possible Interaction with Calcium*

Drug (or Drug Class)	Mechanism
Cromolyn sodium	Inhibition of antigen-antibody–induced Ca^{++} influx
Calcium channel blocking agents (verapamil, nifedipine)	Inhibition of Ca^{++} channel influx
Methylxanthines	Inhibition of phosphodiesterase and increase cyclic AMP levels; this alters cystosol-free Ca^{++}
Corticosteroids	?Augment adenylate cyclase activity
Beta-adrenergic agonists	Increase cyclic AMP
Atropine	Inhibition of acetylcholine-induced Ca^{++} mobilization
Prostaglandin E_2	Increase in cyclic AMP
Local anesthetics (lidocaine)	Multiple potential (nonspecific) mechanisms including excitation, mediator release, and calcium binding







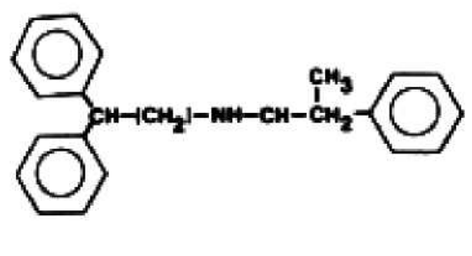


Figure 65-5. *Classes of organic calcium channel inhibitors (see text).*

Table 65-2. *High Affinity ^{3}H-Nitrendipine Binding Sites in Smooth Muscle*

Tissue	Kd (nM)	B_{max} (fmoles/mg)	Reference
Rat longitudinal ileum	0.26	25	(37)
Guinea pig longitudinal ileum	0.16	1,100	(10)
Canine thoracic aorta	0.31	20.3	(108)
Canine mesenteric artery	0.25	25.0	(108)
Rat mesenteric artery	0.10	18.0	(108)
Pig coronary artery	1.6	35.0	(33)
Bovine aorta	2.1	40–60	(124)

Kd = dissociation constant; B = fmoles receptors/mg membrane.

Inhibition of ^{3}H-nitrendipine binding by calcium channel inhibitors in the dihydropyridine class is consistent with their relative potencies on isolated tissue for inhibition of calcium-dependent contractions [10, 36, 81]. Neither the phenylalkylamine verapamil nor the benzothiazepine diltiazem could completely displace ^{3}H-nitrendipine binding (70% and 39% displacement, respectively) [33, 36]. The kinetics are consistent with allosteric inhibition of the dihydropyridine binding site as opposed to competitive inhibition by compounds in the dihydropyridine class. Diltiazem has been reported to stereospecifically increase ^{3}H-nitrendipine binding to cardiac membrane preparations [33] and to reverse the inhibition by verapamil [81].

Recently it was reported that compounds in the diphenylalkylamine class (prenylamine and lidoflazine) similarly decrease ^{3}H-nitrendipine binding through an allosteric interaction, and this site was the same binding site as that of the phenylalkylamines [81]. Calcium-dependent contractions of guinea pig ileum are inhibited by all the agents acting at the allosteric regulatory site. Murphy et al. [81] described a unitary model for calcium channels where the allosteric site is a bipartite receptor. Drugs binding to a single site demonstrate diltiazem effects, whereas agents such as verapamil, binding to both domains, allosterically inhibit but not stimulate ^{3}H-nitrendipine binding. Other agents such as the H_1 antihistamines dimethindene and chlorpheniramine, the muscarinic anticholinergic biperiden, and the neuroleptics mesoridazine and thioridazine have demonstrated diltiazem-like effects by increasing ^{3}H-nitrendipine binding. Pyrilamine, histamine, atropine, and promethazine did not affect binding.

Calcium channel inhibitors have also been shown to interact with other receptor binding sites. Verapamil, but not diltiazem or nifedipine, displaced ^{3}H-prazosin and ^{3}H-yohimbine from alpha$_1$ and alpha$_2$ receptors, respectively [6, 80]. The affinity of the alpha receptors was reduced by the calcium channel inhibitors without an apparent change in receptor number [6]. Concentrations required, however, are generally greater than for inhibition of calcium-dependent contractions or binding.

Binding of calcium channel inhibitors to membrane preparations suggests that the site of action is associated with plasma membrane calcium channels. Supporting this site of action, calcium channel inhibitors did not block calcium-induced contractions in smooth and cardiac tissue chemically skinned of the plasma membrane [46, 94, 103]. Also, $^{45}Ca^{++}$ uptake into smooth muscle preparations is blocked by calcium channel inhibitors [77, 112]. Results utilizing direct measurements of $^{45}Ca^{++}$ influx, however, are not conclusive. Church and Zsoter [22] and Boström et al. [13] were unable to detect inhibitory effects of diltiazem, nifedipine, or felodipine on $^{45}Ca^{++}$ influx utilizing the lanthanum method, whereas vanBreemen's laboratory [77, 112] using similar techniques, demonstrated that nifedipine blocked $^{45}Ca^{++}$ into rabbit aortic smooth muscle. It is difficult to evaluate one set of these studies versus another, since each utilized a different smooth muscle from different species.

Calcium channel inhibitors may also inhibit Ca^{++} release from intracellular stores. This was demonstrated indirectly utilizing isolated mesenteric artery preparations contracted to norepinephrine in calcium-free media. Under these conditions, where contractions were primarily dependent on intracellular Ca^{++} release, nifedipine inhibited the norepinephrine responses over the same concentration range as in normal calcium [116]. Another intracellular effect of calcium channel inhibitors is the proposed inhibition of the calcium-binding protein calmodulin [13, 66]. The critical question is whether these drugs can achieve intracellular concentrations high enough to inhibit calmodulin. At 10^{-5}M or less, there is little effect of felodipine, nifedipine, dil-

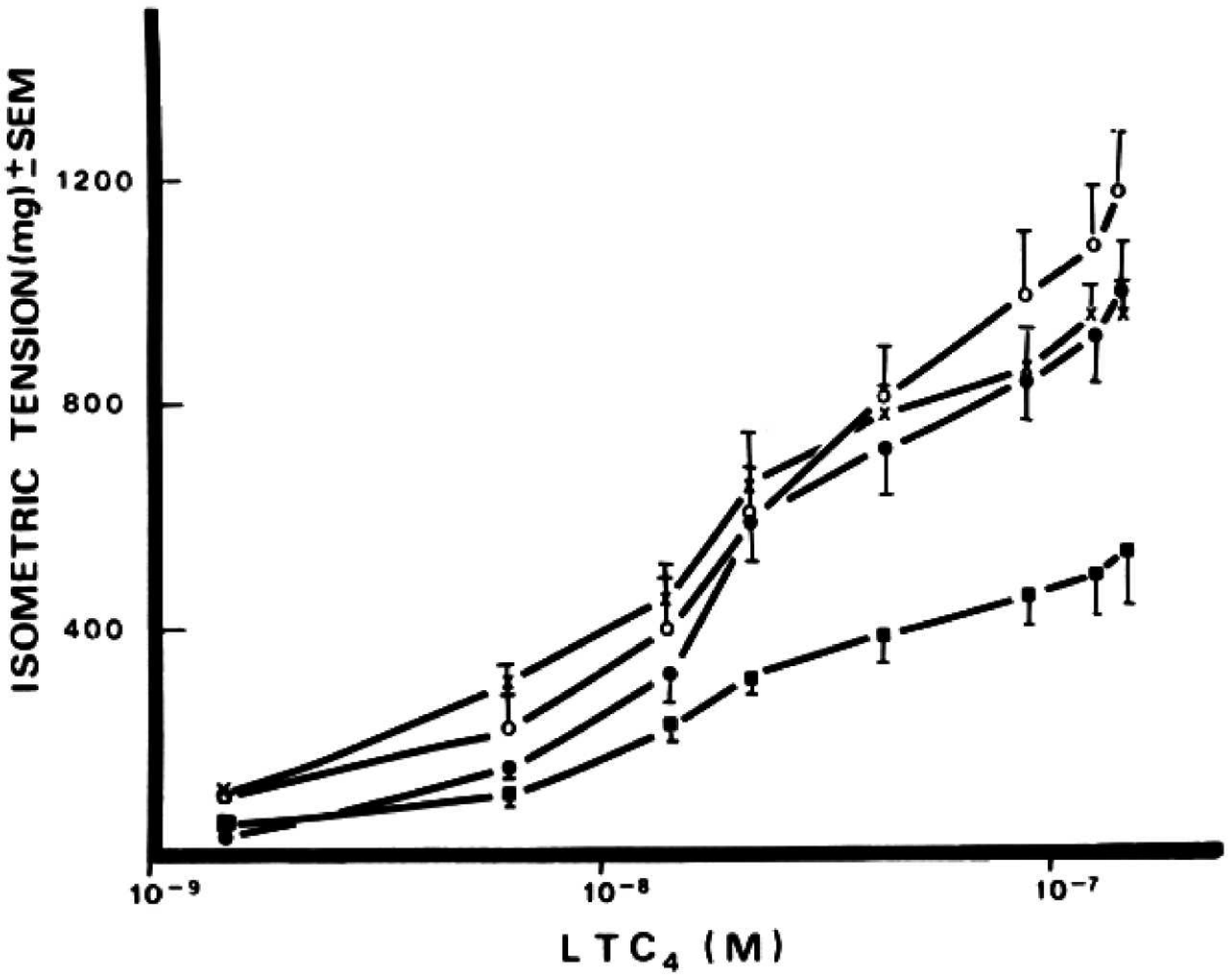


Figure 65-6. *Reversal of verapamil inhibition of LTC_4 with 5.0 mM $(Ca^{++})_E$. O = cumulative dose–isometric tension response to LTC_4 at 2.5 mM $(Ca^{++})_E$, control muscles; X = cumulative dose-response of LTC_4 in 5.0 mM $(Ca^{++})_E$, control muscles; ■ = effect of verapamil (1.1×10^{-4} M) inhibition of LTC_4 response at 2.5 mM $(Ca^{++})_E$; • = reversal of verapamil (1.1×10^{-4} M) inhibition at 5.0 mM $(Ca^{++})_E$. Mean tension at 1.6×10^{-7} M LTC_4 and 5.0 mM $(Ca^{++})_E$ with verapamil was not statistically different from control muscle tension at 2.5 mM or 5.0 mM $(Ca^{++})_E$ at this same LTC_4 concentration ($P = .3$). Each data point represents five experiments ± SE after 20 minutes of agonist incubation. Slope changes are visually apparent. (Reprinted with permission from E. B. Weiss and P. C. Mullick, Leukotriene effect in airways smooth muscle: Calcium dependency and verapamil inhibition. Prostaglandins Leukotrienes and Medicine 12:53, 1983.)*

tiazem, or verapamil on calmodulin-dependent activation of brain phosphodiesterase [30, 107]. When binding of ^{3}H-nitrendipine to calmodulin was directly determined, binding was found to be of low affinity and nonsaturable, suggesting that the interaction is pharmacologically insignificant [107]. Utilizing fluorescent hydrophobic probes, trifluoperazine and verapamil were found to interact with calmodulin at two different sites [39]. Yet even at relatively high concentrations (>2 μM), Ca^{++} antagonists are 2 to 5 times more potent on calmodulin-activated phosphodiesterase than on calmodulin-independent basal activity [39].

CALCIUM CHANNEL INHIBITORS AND AIRWAY SMOOTH MUSCLE. While the effects of calcium channel inhibitors on vascular tissue have been extensively studied, airway smooth muscle studies are conspicuously lacking, especially with respect to receptor binding. Good correlations have been established between binding studies and the inhibition of contractile responses in vascular tissue [33, 81, 108, 109] with Kd and IC_{50} values generally in the low nanomolar range, but binding studies have not been completed in airway smooth muscle. Effects on isolated airways indicate that inhibition of mechanical responses occur at doses 1,000 times greater than for vascular tissue. IC_{50} values for airway smooth muscle range from 10^{-6} to 10^{-4}M, with significant variations apparently dependent on tissue type and agonist used.

In canine tracheal smooth muscle it has been established that histamine mobilizes a loosely bound, verapamil-sensitive Ca^{++} pool, whereas acetylcholine mobilizes a tightly bound Ca^{++} pool [4, 42]. Acetylcholine contractions are sensitive to verapamil only at low acetylcholine concentrations, implying that the calcium pool mobilized is dependent on the acetylcholine concentration. The effective verapamil concentration was 10^{-5} to 10^{-4}M. Contractions induced with 5-hydroxytryptamine are also sensitive to verapamil and D600 [23].

Calcium channel inhibitors have been shown to inhibit histamine, leukotriene, and carbachol responses [20, 40, 121] and to decrease resting tension in isolated guinea pig tracheal smooth muscle and lung parenchymal strips [20, 40] (Fig. 65-6). Anaphylactic contractions of passively sensitized guinea pig trachea were also inhibited by verapamil, without a reduction in histamine release, which suggests that the effect of calcium channel inhibitors is on the smooth muscle and not the mast cell [119].

Contractile responses and spontaneous phasic tension of human airway tissue are inhibited by the calcium channel inhibitors verapamil (10^{-5}M and 10^{-4}M) and nifedipine (10^{-6}M) [35, 58]. Antigen-challenged human bronchial responses were also inhibited by 10^{-6}M nifedipine [35], but whether this was associated with direct smooth muscle antagonism or effects on mediator release was not determined.

It is readily apparent that calcium channel blockers exert their effects on airway tissue at higher concentrations than required for equivalent effects on vascular tissue. In rat trachea responses to potassium depolarization were attenuated by nifedipine at 10^{-6}M [20], which is one of the few instances where the potency of calcium channel inhibitors approaches that observed for non–airway smooth muscle. Since binding data, structure-activity relationships, and determination of stereoselectivity are missing from most of these studies, caution must accompany conclusions based solely on the ill-de-

fined mechanism of the action of calcium channel inhibitors in airway tissue. Qualitatively, however, these inhibitors parallel cardiovascular effects in airway smooth muscle, where they more effectively inhibit responses resulting from extracellular calcium mobilization through voltage-dependent channels. It is possible that airway smooth muscle and cardiovascular smooth muscle contain different receptors for calcium channel inhibitors, contributing to the apparent selectivity of these compounds for cardiovascular smooth and cardiac muscle.

CALCIUM CHANNEL INHIBITORS AND MEDIATOR RELEASE. In isolated mast cells it has been demonstrated that $^{45}Ca^{++}$ influx precedes mediator release [48, 63]. Even though the process of Ca^{++} mobilization is poorly understood in the antigen-challenged mast cell, the calcium antagonists nifedipine, D600, and verapamil have been demonstrated to reduce histamine release stimulated by antigen and Con-A [38, 105, 106]. Nifedipine but not D600 has been reported to inhibit histamine release induced by 48/80 and ionophore A23187 [105, 106]. Inhibition of histamine release correlates with a decrease in ^{45}Calcium uptake, even though a greater effect on ^{45}Calcium flux than mediator release was observed [106]. High concentrations (1–100 μM) were required, and the inhibition was not reversed by increasing extracellular calcium [38].

Histamine release from human neutrophils isolated from ragweed-sensitive patients was not inhibited by nifedipine, verapamil, or D600 [79], but nifedipine was effective at dose-dependently decreasing the synthesis of SRS-A and platelet-activating factor (PAF) [67]. Even though the mechanism of action has not been established, calcium channel inhibitors have been demonstrated to inhibit airway smooth muscle responses as well as the release and/or synthesis of many of the mast cell mediators.

The in vivo effectiveness of these compounds may thus be related to the inhibition of mediator release, direct smooth muscle antagonism, or combined effects. In guinea pigs [40] and dogs [74] in vivo, nifedipine has been demonstrated to attenuate bronchoconstrictor responses to exogenous histamine and $PGF_{2\alpha}$, consistent with direct effects of this calcium channel inhibitor on airway smooth muscle. Nifedipine was active by aerosol as well as intravenously. Against antigen challenge in *Ascaris*-sensitized dogs, aerosolized nifedipine failed to inhibit either resistance or compliance responses [75].

Table 65-3. *Summary of Clinical Studies Employing Calcium Channel Inhibitors*

Challenge	Calcium Channel Inhibitor	Dose/Conc., Route	Measured Parameter(s)	Pulmonary Function Effects		Cardiovascular Side Effects	Reference
				Base	Challenge		
Exercise	Nifedipine	20 mg subling.	Vmax50; VC; PEF; Vmax25	N.E.	+ +	±	(18)
Exercise	Verapamil	5 min 2.5 gm/L aerosol	FEV_1	N.E.	+	N.R.	(86)
Exercise	Verapamil	5 min 2.5 gm/L aerosol	FEV_1	N.E.	+ +	N.R.	(88)
Exercise	Nifedipine	20 mg subling.	PEF; FEV_1	N.E.	+	N.E.	(7)
Cold air	Nifedipine	20 mg subling.	FEV_1; SGaw	N.E.	+ + +	+	(57)
Deep inspiration	Nifedipine	10–20 mg subling.	FEV_1; Raw	N.E.	+ + +	+	(92)
Antigen	Nifedipine	20 mg subling.	FEV_1	N.E.	+ +	+	(58)
Histamine, methacholine by aerosol	Verapamil	5 min 2.5 gm/L aerosol	FEV_1	N.E.	N.E.	N.R.	(87)
Histamine	Nifedipine	20 mg subling.	FEV_1	N.E.	N.E.	N.E.	(89)
Histamine	Nifedipine	20 mg subling.	FEV_1	N.E.	±	N.E.	(123)
Circadian nocturnal variations	Nifedipine	10 mg subling.	FEV_1; SGaw	+ (lg airways)	N.S.	±.	(85)

N.E. = no effect; + = slight effects; + + = positive effects with considerable variation; + + + = very consistent effects; N.R. = not reported; N.S. = not studied; subling. = sublingual.

Aerosolized verapamil, in contrast to nifedipine, prevented both resistance and compliance changes. Intravenously, both nifedipine and verapamil effectively inhibited only large airway responses. In contrast to the effects of calcium channel inhibitors in canine models, calcium channel inhibitors appear to affect the mast cell in sensitized sheep. Responses to antigen, but not aerosolized histamine or carbachol, were attenuated by verapamil [93].

A number of clinical studies have been reported on the use of the calcium channel inhibitors nifedipine and verapamil in asthma (Table 65-3). In general, the calcium channel inhibitors were effective in attenuating bronchoconstriction induced by exercise [7, 18, 86, 88], cold air [57], and deep inspiration [92]. Considerable patient variability occurred, with some patients completely protected. The most consistent inhibition of bronchoconstriction occurred when deep inspiration and cold air were used as stimuli [57, 92]. Responses to antigen challenge were also significantly, but less consistently, attenuated by nifedipine [58].

When asthmatics were challenged directly with aerosolized histamine or methacholine, neither verapamil nor nifedipine were effective in preventing the bronchoconstriction [87, 89]. In one study [123] nifedipine gave some protection against aerosolized histamine, but these effects were marginal. While no effect of aerosolized verapamil was observed on the sensitivity of asthmatics to histamine, verapamil significantly increased the dose of histamine required to decrease the $FEV_{1.0}$ by 20 percent in control subjects [87]. Speculative interpretation might suggest that in asthmatics different calcium pools are mobilized by histamine or that calcium mobilization in asthmatics is subject to differing mechanisms of regulation.

In all but one study [85] neither verapamil nor nifedipine improved basal pulmonary functions. This may be explained by the selection of the patient populations. Generally basal pulmonary function was required to be greater than 75 percent of predicted values. In contrast, when nifedipine was evaluated in patients with circadian nocturnal bronchoconstriction, a significant improvement in pulmonary function was observed during periods of bronchoconstriction [85].

From these limited clinical studies it appears that the therapeutic effectiveness of the presently available calcium channel inhibitors is through the inhibition of mediator release, but effects on reflex vagal responses cannot be dismissed. Consistent with this mechanism of action is the observed reduction in plasma histamine levels by nifedipine following exercise-induced asthmatic episodes [7].

It is entirely possible that calcium channel inhibition and calcium antagonism in general may represent a new therapeutic approach to airway disease. It is obvious, however, that studies on airway smooth muscle analogous to the relatively well-defined studies on vascular smooth muscle are required. Based on a potency differential of calcium channel inhibitors on vascular versus airway smooth muscle, the apparent selectivity of the currently available calcium channel inhibitors is promising in that specificity (i.e., receptor differentiation) may exist. It therefore follows that the most potent and most selective cardiovascular agents currently available may be the least potent and effective in the lung.

Role of Calcium in Airways Reactivity

The possible role of an abnormality in calcium homeostasis in the pathogenesis of airways hyperreactivity was first reported in 1979 by Weiss and Viswanath [122]. The basis for this hypothesis was the finding of an increased sensitivity of resting isometric tension to extracellular calcium $(Ca^{++})_E$ following in vitro anaphylaxis in guinea pig trachealis. This experimental model exhibited an increase in resting isometric smooth muscle tension as assayed by cumulatively restoring extracellular calcium to muscles previously immunogenically contracted and then immersed in a calcium-free medium. This observation suggested the following:

Normal airway smooth muscle + anaphylaxis → acquired smooth muscle Ca^{++} defect (e.g., increased membrane permeability) → increased sensitivity to $(Ca^{++})_E$ → increased basal smooth muscle tone.

Conceptually an increase in free myoplasmic Ca^{++} could account for the reactivity of airway smooth muscle to a variety of nonspecific stimuli or agonists, a feature characteristic of asthmatic airways reactivity. The potential contribution of resting basal muscle tone to airways hyperreactivity has been raised. [9].

Since that time other observations lend support to this concept. Dhillon and Rodger [34] observed changes attributable to the utilization or binding of calcium following histamine interactions in calcium-free buffer in airways of ovalbumin-sensitized guinea pigs. Hedman and Andersson [56], assaying ^{45}Ca release from microsomal fractions of sensitized guinea pig lungs, reported a small but statistically significant difference in ^{45}Ca microsomal binding compared to control animals [56]. However, Creese and Bach [27] view such hypersensitivity of airway smooth muscle as tested in vitro (and at subphysiologic calcium concentrations) to be caused by an enhanced sensitivity to released or activated leukotrienes and not per se by a defect in calcium homeostasis. It is of interest that the experimental approach of Creese and Bach indicating leukotriene-induced hyperresponsiveness to other bronchoconstrictor agents required low (0.1 mM) extracellular calcium conditions for analysis.

More recently we have utilized the model of exposure of trachealis smooth muscle to a calcium-free medium to further examine this problem [120]. It was observed in vitro after an ovalbumin-induced anaphylactic contraction that such trachealis mus-

Table 65-4. *Trachealis Relaxation in O $(Ca^{++})_E$, Mean ± SEM*

Paired Condition	Number	Initial Mean Rate (mg isometric tension/sec)	P	Maximal Relaxation (mg at 15 min)	P
Normal control vs. postanaphylaxis	16	8.7 ± 2.1 14.6 ± 2.9	<.001	775 ± 83 1179 ± 84	<.001
Normal control + FPL 55712 (10^{-5} M) vs. anaphylaxis + FPL 55712 (10^{-5} M)	8	9.2 ± 1.1 8.1 ± 1.6	>.5	844 ± 256 675 ± 260	>.3
Normal control in N_2 (PO_2 10 mmHg) vs. anaphylaxis in N_2	10	9.3 ± 1.1 4.4 ± 1.3	<.02	562 ± 80 428 ± 86	<.02
Normal control + SOD/CAT[a] vs. anaphylaxis + SOD/CAT	13	10.5 ± 0.5 9.9 ± 0.5	.5	1042 ± 43 1131 ± 42	>.2
Normal control vs. LTC_4 (2.3×10^{-8} M)	8	11.8 ± 2.1 18.9 ± 3.5	<.001	1012 ± 170 1244 ± 245	<.05
Normal control + SOD/CAT[b] vs. LTC_4 (2.3×10^{-8} M) + SOD/CAT	8	11.6 ± 1.4 12.8 ± 2.1	.7	1121 ± 199 1228 ± 189	.6

P = paired t test; N_2 = nitrogen; SEM = standard error of mean; SOD = superoxide dismutase; CAT = catalase.
[a] SOD/CAT = 500 U/ml each.
[b] SOD/CAT = 3000 U/ml each.
Source: Modified from E. B. Weiss, Toxic oxygen products alter calcium homeostasis in an asthma model. *J. Allergy Clin. Immunol.* In *press, 1984.*

cles exhibit a greater initial rate and absolute greater maximal relaxation when such muscles are exposed to a Ca^{++}-free buffer compared to normal (nonimmune activated) trachealis muscles (Table 65-4). A similar enhanced myorelaxation was observed following contractures induced in normal trachealis muscle by synthetic LTC_4. When the late plateau phase of anaphylaxis was inhibited by FPL 55712 (10^{-5} M), the augmented postanaphylactic myorelaxation phenomenon in a calcium-free medium was eliminated, indicating a causal role for the SRS-A products. In addition, when the ovalbumin-induced anaphylactic contraction was exposed either to hypoxia ($PO_2 = 10$ torr) or to pretreatment with superoxide dismutase/catalase, the postanaphylactic or LTC_4-enhanced myorelaxation in $0(Ca^{++})_E$ was also abolished. These observations support an entirely new hypothesis, namely that toxic oxygen products generated during anaphylaxis are due to or are associated with endogenous or exogenous leukotriene activity, and that these oxygen-toxic products induce an alteration in calcium homeostasis in airway smooth muscle in a model of allergic asthma. This approach provides a basis for clarifying some elements of the nonspecific airways reactivity to a variety of stimuli following a specific antigen-antibody immune-induced contraction. Figure 65-7 is suggested as a working model for this new hypothesis.

Other observations provide added evidence for this model. Volpi et al. [114], using rabbit peritoneal neutrophils, have reported that exogenous arachidonate metabolites cause a specific, rapid, and significant increase in the permeability of the plasma membrane to $^{45}Ca^{++}$. It has been proposed that acute pulmonary injuries as increased permeability pulmonary edema or pulmonary vascular endothelial cell damage might result from local toxic neutrophil products, including proteases, arachidonate products, and/or oxygen-derived toxic products [41]. A protective action by superoxide dismutase on free radical–mediated pulmonary vascular permeability has been described in dogs [84]. That a similar phenomenon involving airway smooth muscle might exist is prompted by our current observations and other studies. Holtzman et al. [61] have demonstrated that canine airways hyperreactivity induced by ozone inhalation correlated with airways inflammation, implying an association between increased numbers of inflammatory epithelial neutrophils and the ozone-induced hyperreactivity. Other information incriminates inflammatory mediators in airway smooth muscle reactivity: Lipoxygenase products augment the response to histamine in human bronchi [26]; methacholine responses in man can be increased by cyclooxygenase products [115].

It is interesting that some human survivors of the adult respiratory distress syndrome demonstrate increased pulmonary responsiveness to bronchial challenge [100]. Furthermore, granulocyte depletion in unanesthetized sheep exhibit a *reduced* pulmonary response to aerosol histamine, again suggesting some link between lung inflammation, hyperreactivity, and possibly oxygen-derived toxic products [59].

An added biochemical basis for an alteration in airways hyperreactivity through a membrane permeability to calcium has been suggested by Nath and colleagues [82]. Utilizing a nonimmune model of guinea pigs that were natively highly sensitive to histamine, they found that an increased tracheal lysophosphatidyl choline (LPC) content correlated with such airways sensitivity. Besides multiple other biochemical effects, increased membrane LPC could additionally alter membrane permeability to

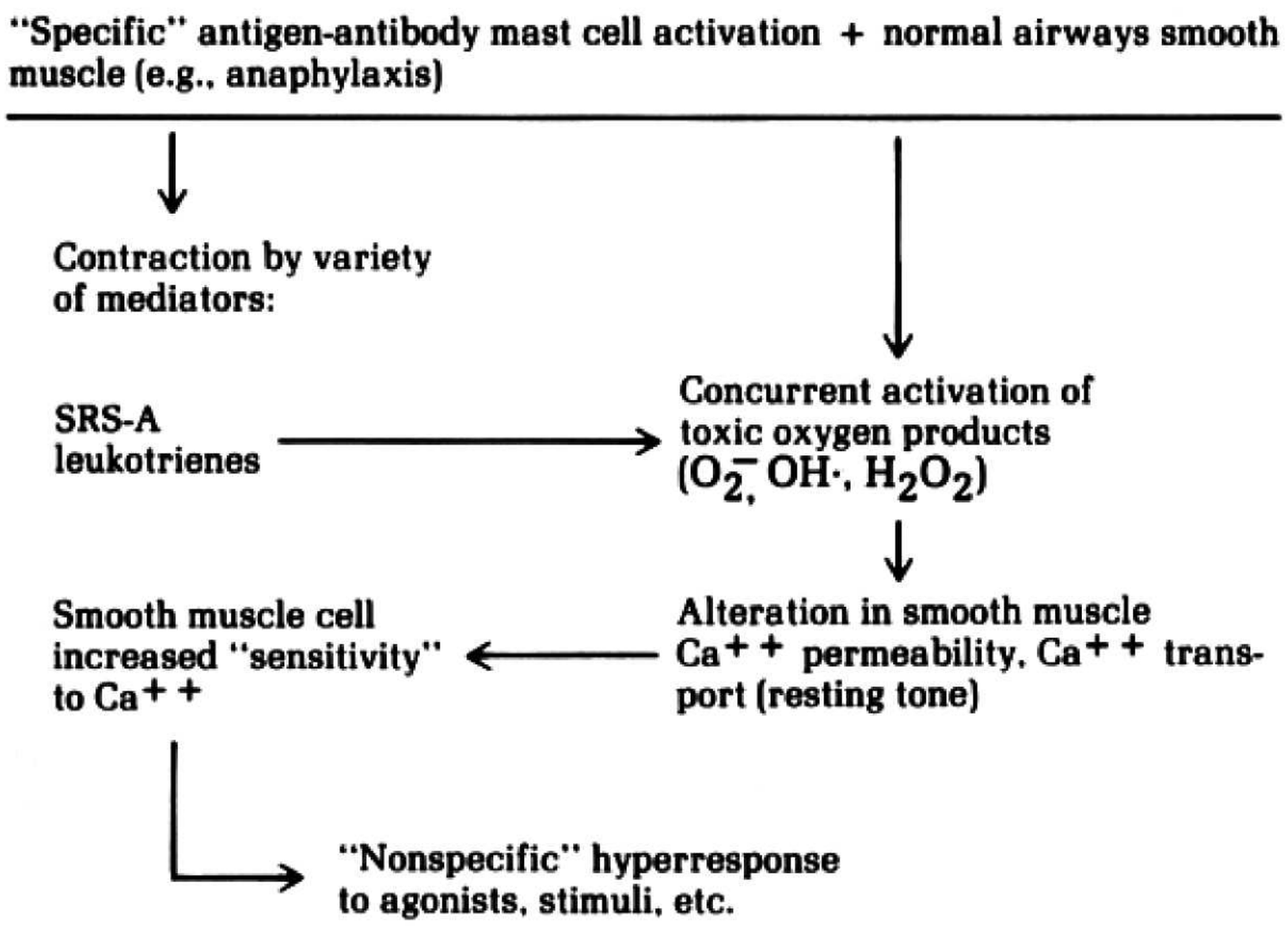


Figure 65-7. *A working model. Oxygen-toxic product interaction with calcium homeostasis in airways smooth muscle. [120].*

calcium, resulting in a higher concentration of intracellular calcium, and further contribute to airways hyperreactivity.

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