BRONCHIAL ASTHMA

Mechanisms and Therapeutics

Third Edition

Earle B. Weiss
Myron Stein

Bronchial Asthma

Mechanisms and Therapeutics

Third Edition

Edited by

Earle B. Weiss, M.D.
Visiting Professor of Anesthesia, Faculty of Medicine, Harvard Medical School; Senior Pulmonary Scientist, Department of Anesthesia Research, Brigham and Women's Hospital, Boston

Myron Stein, M.D.
Clinical Professor of Medicine, University of California, Los Angeles, UCLA School of Medicine, Los Angeles; Codirector, Department of Pulmonary Medicine, Brotman Medical Center, Culver City, California

Little, Brown and Company
Boston/Toronto/London

Andor Szentivanyi, M.D., D.Sc.
Distinguished Professor of Internal Medicine, University of South Florida College of Medicine, Tampa, Florida
14. Adrenergic Regulation, 33. Immune-Neuroendocrine Circuitry and Its Relation to Asthma

Donald P. Tashkin, M.D.
Professor of Medicine, University of California, Los Angeles, UCLA School of Medicine; Attending Physician, Division of Pulmonary/Critical Care Medicine, UCLA Medical Center, Los Angeles
55. Beta-Adrenergic Agonists

John H. Toogood, M.D., F.R.C.P.
Professor of Medicine, University of Western Ontario Faculty of Medicine; Director, Allergy Clinic, Victoria Hospital, London, Ontario, Canada
61. Aerosol Corticosteroids

Donald L. Uden, Pharm.D.
Associate Professor of Pharmacy Practice, University of Minnesota Medical School—Minneapolis
G. Guidelines for Intravenous Terbutaline Use in Status Asthmaticus

Martin D. Valentine, M.D.
Professor of Medicine, Johns Hopkins University School of Medicine; Attending Physician, Department of Medicine, Johns Hopkins Hospital, Baltimore
70. Immunotherapy

Paul P. VanArsdel, Jr., M.D.
Professor of Medicine, University of Washington School of Medicine; Head, Section of Allergy, University of Washington Medical Center, Seattle
34. Clinical Incitants

Adam Wanner, M.D.
Professor of Medicine, University of Miami School of Medicine, Miami; Chief, Pulmonary Division, Mount Sinai Medical Center, Miami Beach
30. Mucociliary Function

Andrew J. Wardlaw, Ph.D., M.R.C.P.
Senior Lecturer, Department of Medicine, Leicester University Medical School; Consultant Physician, Department of Respiratory Medicine, Glenfield Hospital, Leicester, England
20. Eosinophil

Miles M. Weinberger, M.D.
Professor of Pediatrics, University of Iowa College of Medicine; Director, Allergy and Pulmonary Division, Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, Iowa
58. Methylxanthines

Earle B. Weiss, M.D.
Visiting Professor of Anesthesia, Faculty of Medicine, Harvard Medical School; Senior Pulmonary Scientist, Department of Anesthesia Research, Brigham and Women's Hospital, Boston
71. Calcium, Calcium Antagonists, and Oxyradicals, 73. Status Asthmaticus

Scott T. Weiss, M.D., M.S.
Associate Professor Medicine, Harvard Medical School; Associate Physician, Pulmonary and Critical Care Division, Beth Israel Hospital and Brigham and Women's Hospital, Boston
2. Epidemiology and Natural History

John Wiggins, M.D.
Senior Medical Registrar, Royal Brompton and National Heart Hospital, London
60. Corticosteroids

Adrian J. Williams, M.B., F.R.C.P.
Professor of Clinical Medicine, University of California, Los Angeles, UCLA School of Medicine; Chief, Pulmonary Division, Veterans Affairs Medical Center, West Los Angeles
26. Physiology and Gas Exchange

M. Henry Williams, Jr., M.D.
Professor of Medicine, Albert Einstein College of Medicine of Yeshiva University; Director, Pulmonary Division, Bronx Municipal Hospital Center and Weiler Hospital, Bronx, New York
35. Clinical Evaluation

Archie F. Wilson, M.D., Ph.D.
Professor of Medicine, University of California, Irvine, College of Medicine; Chief, Pulmonary and Critical Care Medicine, University of California, Irvine, Medical Center, Orange, California
36. Methods of Assessing Bronchoreversibility: Site of Airway Obstruction and Bronchodilator Response, 56. Aerosol Delivery Systems

Richard Wood-Baker, M.B., B.S.
Clinical Research Fellow, University of Southampton, Southampton, England; Attending Physician, Department of Respiratory Medicine, Sir Charles Gardner Hospital, Nedlands, Perth, Western Australia
65. Histamine Antagonists

Robert S. Zeiger, M.D., Ph.D.
Clinical Professor of Pediatrics, University of California, San Diego, School of Medicine; Chief, Department of Allergy, Kaiser Permanente Medical Center, San Diego
67. Troleandomycin in Corticosteroid Dependency

Irwin Ziment, M.D., F.R.C.P.
Professor of Medicine, University of California, Los Angeles, UCLA School of Medicine; Chief, Department of Medicine, Olive View Medical Center, Sylmar, California
69. Hydration, Humidification, and Mucokinetic Therapy, 88. Inappropriate and Unusual Remedies

Calcium, Calcium Antagonists, and Oxyradicals

71

Wayne H. Anderson
Francis M. Cuss
Earle B. Weiss

CALCIUM AND THE CONTRACTILE PROCESS IN AIRWAY SMOOTH MUSCLE

Smooth muscle contraction, as in all muscle contraction, is initiated when the intracellular calcium $[Ca^{++}]_i$ concentration increases in response to a stimuli. Generally, depending on the smooth muscle type and method of measurement, the basal $[Ca^{++}]_i$ concentration is about 0.1 μM. In "skinned" tracheal smooth muscle, the threshold Ca^{++} concentration for increasing tension was 0.05 μM, with maximum tension occurring at 1.0 μM [292]. Estimates utilizing the fluorescent indicator fura-2 are in good agreement, demonstrating resting calcium levels of 0.165 μM, increasing to 0.5 μM on maximum response to carbachol [306]. Since the extracellular free calcium concentration is approximately 1 to 2 mM, this 10,000-fold gradient demonstrates the ability of smooth muscle to maintain a state of relative Ca^{++} impermeability. Perturbing the processes controlling the low $[Ca^{++}]_i$ concentration leads to contraction.

In striated muscle, Ca^{++} binds to the protein complex tropomyosin, which causes a conformational change allowing for the interaction of actin and myosin and the hydrolysis of adenosine triphosphate (ATP) [91, 92, 294]. The energy released during this hydrolysis is utilized for muscle contraction. In smooth muscle, in addition to a variety of structural differences including the organization of the actin and myosin, a tropomyosin complex has not been identified. Therefore, another mechanism for translating increases in $[Ca^{++}]_i$ to activation of muscle contraction is needed.

It is now established that phosphorylation of the light chain of smooth muscle myosin results in a rapid increase in ATP hydrolysis, a marker of myofibril activation [281], and that this process is reversible by dephosphorylation by phosphatases [228, 265] (Fig. 71-1). The enzyme primarily responsible for phosphorylation of myosin is myosin light-chain kinase, which is dependent on the activation by Ca^{++}-calmodulin [1]. Thus, an increase in $[Ca^{++}]_i$ activates calmodulin, which in turn can bind to and activate myosin light-chain kinase, resulting in the phosphorylation of myosin and energy utilization (ATP hydrolysis) for contraction. Ca^{++} and the phosphorylation of myosin light chains demonstrate a positive cooperativity, such that small increases in $[Ca^{++}]_i$ result in large increases in phosphorylation [306]. Myosin light chains can also be phosphorylated at the same site by a cyclic adenosine monophosphate (AMP)–dependent protein kinase, but the reaction is too slow to be considered physiologically significant [321] (Fig. 71-2).

In canine tracheal smooth muscle contracted with methacholine, a rapid increase in the phosphorylation of myosin can be observed; it precedes the generation of maximum tension and remains constant during maintained tension [76, 77]. The agonist dose-response curve and myosin phosphorylation determined at steady state are superimposable [77, 107]. In the absence of calcium, a transient increase in tension and myosin phosphorylation occurs. Readdition of calcium again results in parallel increases in tension and myosin phosphorylation, demonstrating the calcium dependence of the process [79]. The dependence on steady-state phosphorylation for tension maintenance is not universally accepted [148, 271] and has led to the hypothesis of latch bridges, which maintain force in the presence of declining or dephosphorylated myosin [83].

A second regulatory mechanism in smooth muscle involving Ca^{++}-calmodulin relates to the proposed actin-binding proteins caldesmon and calponin [320]. The inhibitory activity of caldesmon can be reversed by either binding Ca^{++}-calmodulin or by phosphorylation by a Ca^{++}-calmodulin–dependent protein kinase [202]. Both caldesmon and calponin inhibit actin-activated ATPase activity and bind actin and tropomyosin [320] (Fig. 71-3).

Not all phosphorylation of smooth muscle myosin, however, leads to contraction. Protein kinase C, which is activated by a lipid, diacylglycerol (DAG), also has been demonstrated to phosphorylate myosin at a site different from that of myosin light-chain kinase, and results in an inhibition of the ATPase activity [207].

$[Ca^{++}]_i$ can increase the following release from intracellular stores such as the endoplasmic reticulum or intracellular membrane–associated binding sites, or calcium can traverse the membrane down its electrochemical gradient through channels (Fig. 71-4). In airway smooth muscle, two types of channels are proposed to exist: voltage-operated channels (VOCs), which depend on a depolarizing stimuli, and receptor-operated channels (ROCs), which increase calcium flux following ligand-receptor activation.

Voltage-operated Channels

The resting membrane potential of airway smooth muscle is generally in the range of −45 to −60 mV [277]. A range of −20 to −45 mV has been reported for human airway smooth muscle [138, 336]. Some of the variability may depend on the source and viability of the tissue. Depolarizing stimuli to the cell results in an increase in calcium conduction through VOCs. Normally, in response to depolarizing stimuli, airway smooth muscle does not demonstrate action potentials, but depolarizes in a graded fashion, resulting in graded tension development [60, 61]. Two types of calcium channels have been identified in airway smooth muscle: transient (T) and long-lasting (L) [183]. Calcium entering the cell through the L-type channels is sensitive to the class of calcium antagonists exemplified by verapamil and dihydropyridine compounds such as nifedipine. L-channels in the human bronchus seem to be less sensitive to the inhibitory action of these drugs than are other types of smooth muscle [183]. Direct evidence for the existence of L-channels comes from binding studies demonstrating dihydropyridine-binding sites [49, 314]

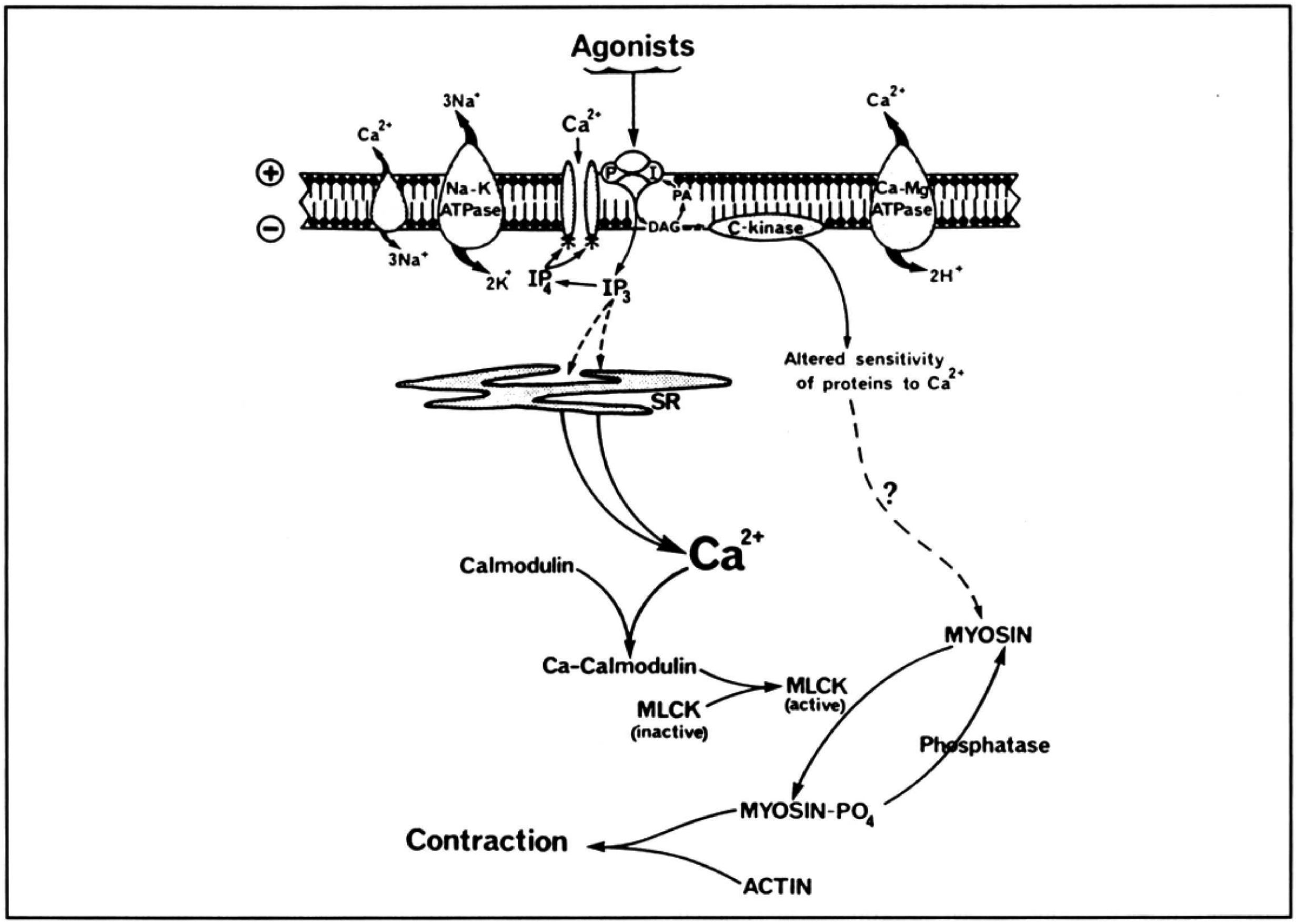


Fig. 71-1. *Summary of the events thought to be involved in excitation-contraction coupling in airway smooth muscle. The initial tension development (phasic response) is determined by the activity of the calmodulin/myosin light-chain kinase (MLCK) pathway, which is turned on as a consequence of the activator Ca^{++} released from the sarcoplasmic reticulum by inositol-1,4,5-trisphosphate (IP_3). Once generated, IP_3 is metabolized to inositol-1,3,4,5-tetraphosphate (IP_4) as a result of an IP_3 kinase. It has been suggested that IP_4 may be responsible for opening plasmalemmal ion channels, thus permitting a low level of influx of extracellular Ca^{++}. These Ca^{++} may be responsible for maintaining $[Ca^{++}]_i$ slightly above basal levels during maintained tension. The asterisks indicate the points on the inner surface of the membrane at which IP_4 might act. Protein C kinase may be responsible for enhancing the sensitivity of the contractile proteins to Ca^{++}. The C kinase pathway may, therefore, be involved in the maintenance of developed tension (tonic phase of contraction) at a time when $[Ca^{++}]_i$ is low. See text for a fuller description of these events. (Reprinted with permission from I. W. Rodger. Biochemistry of Airway Smooth Muscle Contraction. In P. J. Barnes, I. W. Rodger, and N. C. Thompson (eds.),* Asthma: Basic Mechanisms and Clinical Management. *New York: Academic Press, 1988. P. 269. Courtesy of Academic Press Inc.)*

and whole-cell patch clamping of human bronchial smooth muscle cells [183]. In guinea pig tracheal smooth muscle, which does exhibit some spontaneous tone, VOCs may be evident in the slow-wave electrical activity that is blocked by calcium antagonists [276]. Human airway smooth muscle appears to be electrically quiescent [227].

Most of the mediators associated with physiologically relevant stimuli (i.e., histamine, acetylcholine, 5-hydroxytryptamine, and leukotrienes) do not induce tension through electromechanical coupling [109], are relatively resistant to the removal of extracellular calcium, and do not generally cause increases in $^{45}Ca^{++}$ influx [251, 252]. While some membrane depolarization may be associated with these agents, generally it is poorly correlated with tension development, or only invokes small changes in membrane potential [109]. Since by definition, VOCs require depolarization as the opening stimulus, it would not be expected that the calcium antagonists have a major effect on contractions elicited by these agents. In canine tracheal smooth muscle, only responses to low concentrations of acetylcholine were sensitive to verapamil, implying that the strength of the stimulus may be related to different Ca^{++} pools [59]. In guinea pig airway tissue, calcium channel antagonists have been demonstrated to inhibit a portion of the responses to histamine, carbachol, and leukotrienes [50, 328]. While calcium antagonists demonstrated weak antagonist activity toward histamine and acetylcholine, they were more effective in reversing tissue precontracted with these agonists [4, 50]. These data suggest that extracellular calcium influx may be important during the sustained "latch bridge" phase of the response in airway smooth muscle.

Human airway tissue responses to these mediators demonstrate some sensitivity to calcium antagonists [89, 235], and voltage-dependent calcium currents have been demonstrated [183].

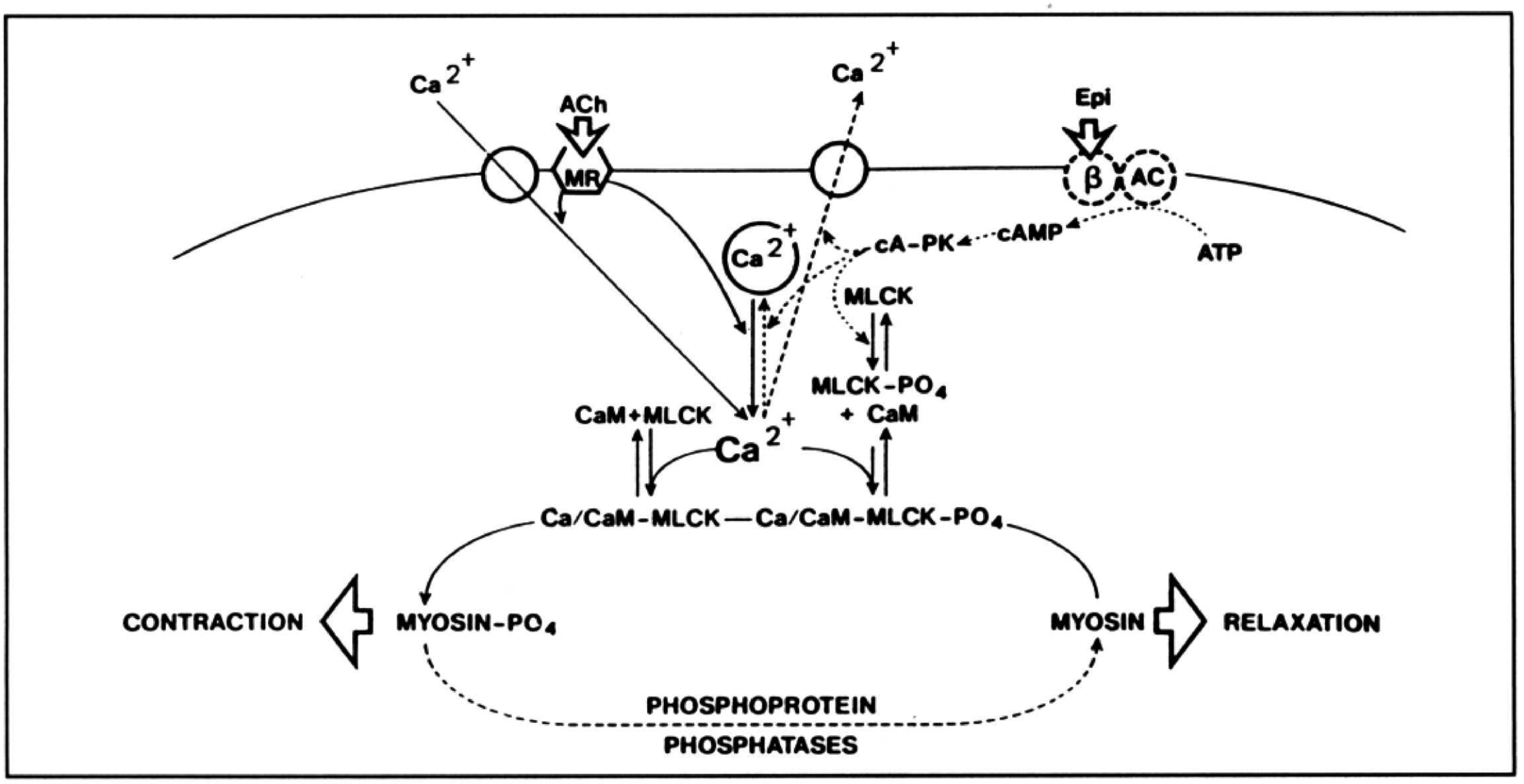


Fig. 71-2. *Cellular events following receptor activation of airway smooth muscle. The solid arrows depict the cellular events associated with contraction; the dotted arrows depict the events involved in relaxation, particularly those associated with an increase in cyclic AMP. The arrows pointing to mechanisms that lead to an increase or a decrease in intracellular calcium following stimulation of either receptor are not designed to specifically identify the underlying mechanisms. Note that the level of myosin phosphorylation is dependent on the balance between myosin light-chain kinase* (MLCK) *and myosin phosphoprotein phosphatase activity and that a shift in any of the equilibria shown (e.g., by phosphorylation of myosin light-chain kinase) can shift this balance and, hence, the force output of the muscle.* ACh = *acetylcholine;* MR = *muscarinic receptor;* Epi = *epinephrine;* β = *beta$_2$-adrenergic receptor;* AC = *adenylate cyclase;* cAMP = *cyclic AMP;* cA-PK = *cyclic AMP–dependent protein kinase;* MLCK-PO_4 = *phosphorylated MLCK;* CaM = *calmodulin;* myosin-PO_4 = *phosphorylated myosin. (Reprinted with permission from P. de Lanerolle. Regulation of Airway Smooth Muscle Responses. In D. Massaro (ed.),* Lung Cell Biology. *Vol. 41. New York: Marcel Dekker, 1989. P. 178. Courtesy of Marcel Dekker Inc.)*

The greatest inhibitory effects were against histamine, followed by methacholine and then leukotriene D_4, suggesting that human airway smooth muscle may utilize extracellular Ca^{++}, which enters the cells through L-type channels. The concentration of calcium antagonists utilized to affect responses in airway tissue is generally an order of magnitude greater than that required for similar inhibition in vascular tissue, despite the presence of high-affinity binding sites [314]. A comparison of the median inhibitory concentration (IC_{50}) of several calcium antagonists on vascular and tracheal smooth muscle using depolarizing potassium as the stimulus similarly demonstrated a 10- to 20-fold difference in potency in the two tissues [313]. One explanation could be the strong rectifying properties of airway smooth muscle due to potassium channel opening [109, 277]. The resistance to depolarization would maintain the receptor in a low-affinity state, reducing binding of the antagonist to the receptor [26, 260].

T-type voltage-dependent calcium channels have been described by whole-cell voltage clamp experiments in canine airway smooth muscle. T-type calcium currents are induced by depolarization and blocked by Mn^{++} and Cd^{++}, are not sensitive to the dihydropyridine calcium antagonist nifedipine, and demonstrated rapid inactivation [159].

Receptor-operated Channels

ROCs are postulated to open in response to agonist-receptor interactions and by definition are insensitive to the classic L-type calcium antagonists. There is, however, little direct evidence for ROCs in airway smooth muscle. Most agonists can cause contraction in the absence of extracellular Ca^{++} and, as measured by the uptake of $^{45}Ca^{++}$, do not induce calcium influx associated with the induction of tension [3, 237]. Schild plots for the antagonism of calcium in the presence of acetylcholine demonstrated a competitive antagonism for verapamil with a slope of 1 [15, 16]. This indicates the presence of a single affinity site. Since acetylcholine responses are postulated to utilize both VOCs and ROCs for Ca^{++} influx, the Schild plot data could be interpreted that the ROCs and the VOCs are the same site that can be modified by receptor-ligand interactions [252].

Intracellular Calcium

The lack of agonist-mediated calcium influx [3, 237], the relative resistance to the removal of extracellular calcium [60, 154, 235], and the increase in tension elicited by receptor activation in potassium-depolarized tissue [61, 155] collectively suggest that the primary source of receptor-mediated contractile calcium in airway smooth muscle is intracellular.

Many membrane receptor–mediated cellular events are coupled to an effector response by a guanosine triphosphate (GTP)–binding protein (G-protein). G-proteins consist of three subunits that in the unactivated state bind guanosine diphosphate (GDP). Receptor-ligand interactions facilitate the dissociation of GDP and the subsequent binding of GTP. Binding of GTP causes the dissociation of the alpha subunit from the remaining beta-gamma complex. The active alpha subunit interacts with a

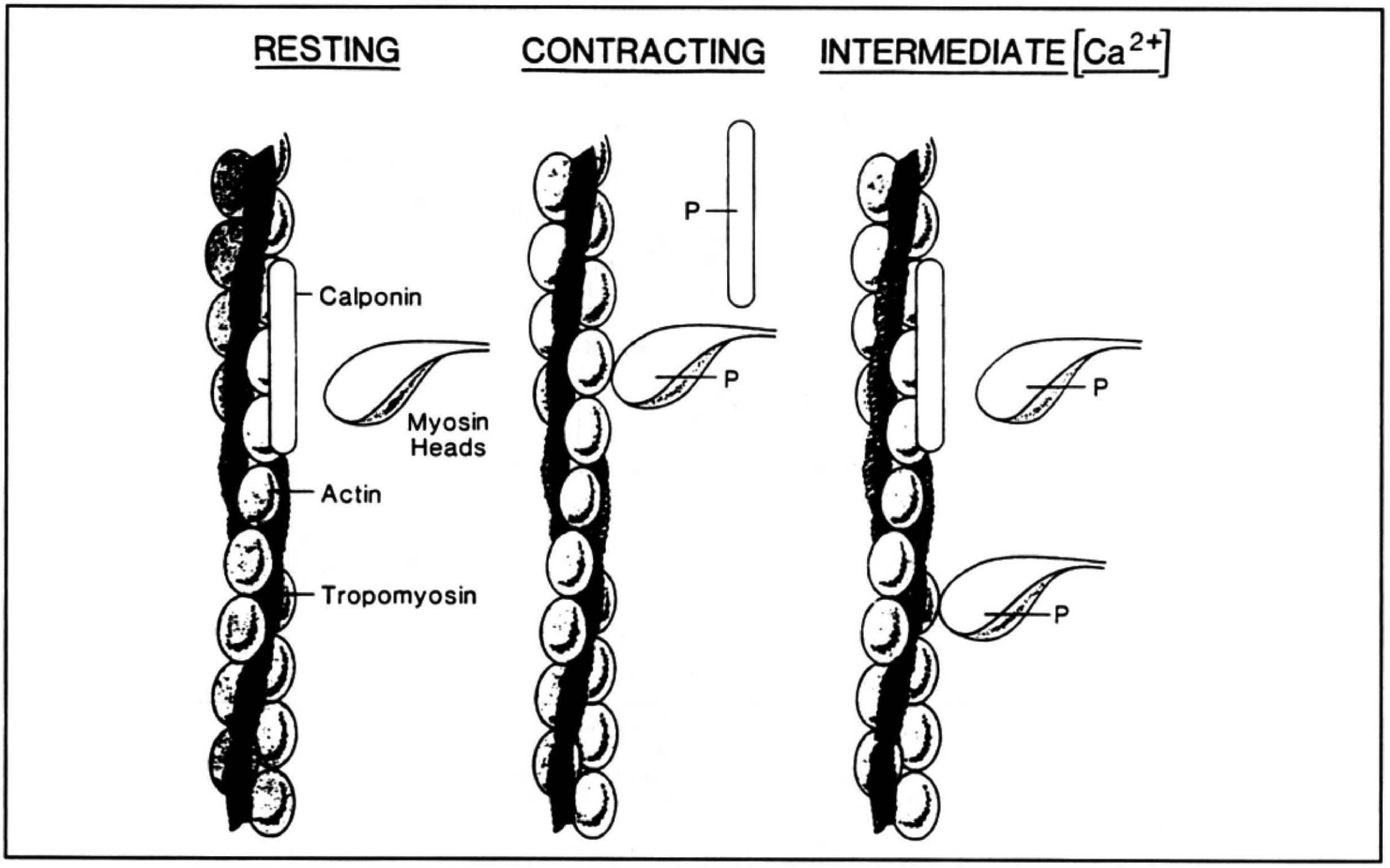


Fig. 71-3. *A model of the postulated physiologic role of calponin in the regulation of smooth muscle actin-myosin interaction. Calponin is shown spanning three actin monomers only because this is the maximum binding stoichiometry determined in vitro; the calponin content in situ is 1 mol per 7 actin monomers. Only the S-1 regions of myosin are included for simplicity.* P = *phosphorylation. (Reprinted with permission from M. P. Walsh. Calcium-dependent mechanisms of smooth muscle.* Biochem. Cell Biol. *69:791, 1991.)*

growing number of effector proteins. Hydrolysis of GTP by a GTPase present on the alpha subunit allows for reassembly of the trimeric inactive complex [111, 298].

Systems that have been identified to be associated with G-protein activation include activation and inhibition of adenylate cyclase [110], phosphodiesterase activation in rod outer segments [105], coupling of muscarinic receptors to potassium channels [37, 231], calcium channels in heart and skeletal muscle T-tubules [38], receptor-coupled phospholipase A_2 activation [13], and coupling of a large number of receptors to phospholipase C and the inositol phosphate second-messenger system [62, 63, 95, 172]. The activation of phospholipase C by receptor-activated G-proteins results in the formation of two intracellular messengers directly involved in $[Ca^{++}]_i$ regulation.

In canine tracheal smooth muscle, cholinergic receptor activation results in a graded fall in membrane phosphatidylinositol and a parallel increase in DAG and phosphatidic acid [24]. In bovine tracheal smooth muscle, a specific loss in phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate [51, 303], possibly indicates that the phosphorylation of phosphatidylinositol represents a rate-limiting step in the formation of inositol phosphate second messengers [55].

Phospholipase C hydrolysis of the phosphorylated membrane lipid phosphatidylinositol-4,5-bisphosphate results in the release of inositol-1,4,5-trisphosphate (IP_3) (Fig. 71-5). IP_3 can be either phosphorylated to inositol-1,3,4,5-tetraphosphate (IP_4) by a kinase or dephosphorylated by a 5-phosphatase to inositol-1,4-phosphate. Both IP_3 and IP_4 are sequentially dephosphorylated to inositol, which is recycled into the membrane. Inositol phosphate production is associated with a variety of agonist-induced contractions in airway tissue including cholinergic agonists [191], histamine [122], 5-hydroxytryptamine [167], bradykinin [52], tackykinins, substance P and neurokinins A and B [117], and leukotrienes C_4 and D_4 [115].

IP_3 causes Ca^{++} release from intracellular stores in a variety of smooth muscle, including tracheal smooth muscle [127, 284, 300]. IP_3 binds to a high-affinity stereospecific receptor, most likely on the endoplasmic reticulum. In bovine tracheal smooth muscle, IP_3 has a K_D of 3.8 nM and a B_{max} of 1,003 fmol/mg of protein [53]. Increasing calcium concentrations does not compete with IP_3 binding, as has been demonstrated in neuronal tissue by the calcium-binding protein calmedin [74].

In bovine tracheal smooth muscle, there is a dose-response relationship between receptor occupancy and inositol phosphate accumulation for both histamine and carbachol [114]. There is good agreement between histamine responses and inositol phosphate formation, but with carbachol, a maximum response could be obtained with as little as 20 percent of both inositol phosphate formation and receptor occupancy [114, 116]. It has also been established that the increases in inositol phosphate metabolism precedes contraction [90, 127, 196]. Other metabolites that have been proposed to play a role in calcium mobilization are 1,3,4,5-tetrakisphosphate, the cyclic inositol phosphates, and phosphatidic acid [78, 243].

The hydrolysis of phosphatidylinositol-4,5-phosphate also results in the formation of DAG. Contrary to the water-soluble inosi-

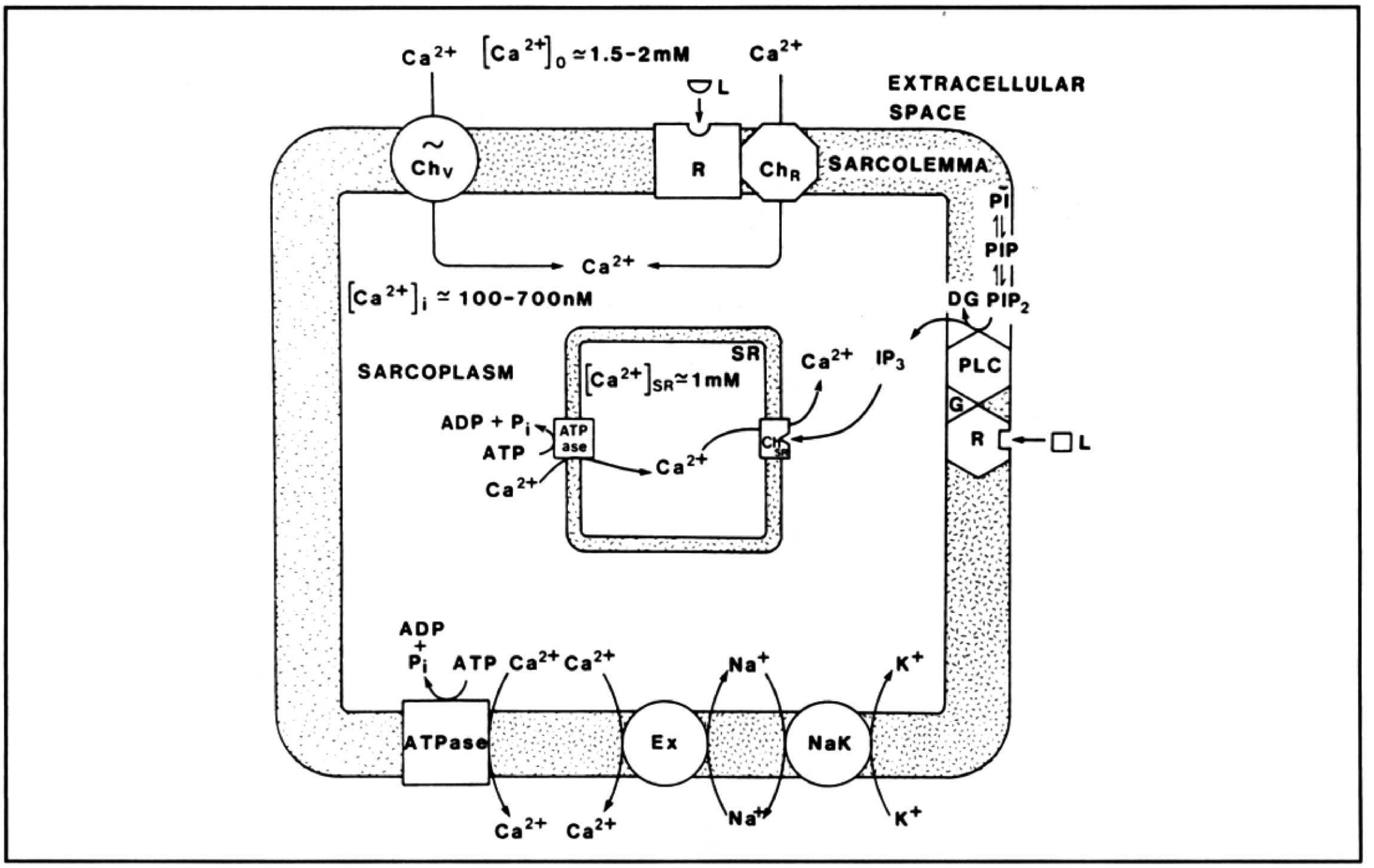


Fig. 71-4. *The principal mechanisms controlling Ca^{++} fluxes across the sarcolemmal and sarcoplasmic reticulum* (SR) *membranes in smooth muscle.* Ch_R = *receptor-operated* Ca^{++} *channel;* Ch_{SR} = *the SR* Ca^{++} *release channel–*IP_3 *receptor;* Ch_v = *voltage-dependent* Ca^{++} *channel;* DG = *1,2-diacylglycerol;* Ex = Na^+/Ca^{++} *exchanger;* G = *GTP-binding protein;* IP_3 = *inositol-1,4,5-trisphosphate;* L = *ligand; NaK* = Na^+-K^+-*transporting ATPase;* PI = *phosphatidylinositol;* PIP = *phosphatidylinositol-4-phosphate;* PIP_2 = *phosphatidylinositol-4,5-bisphosphate;* PLC = *phosphoinositide-specific phospholipase C;* R = *receptor. (Reprinted with permission from M. P. Walsh. Calcium-dependent mechanisms of smooth muscle.* Biochem. Cell Biol. *69:773, 1991.)*

tol phosphates that can diffuse through the cell, DAG is hydrophobic and remains associated with the membrane. DAG activates protein kinase C by markedly increasing its affinity for calcium [147, 208]. It has been proposed that one result of protein phosphorylation catalyzed by protein kinase C is the maintenance of smooth muscle tone following activation [102, 242]. Calcium entry induced by calcium ionophores is transient in airway smooth muscle, but when coupled with activators of protein kinase C a response similar to that induced with cholinergic agonists is obtained [213]. However, in cultured airway smooth muscle, increases in $[Ca^{++}]_i$ stimulated with histamine can be abolished by phorbol activators of protein kinase C [160], demonstrating that the role of protein kinase C in airway smooth muscle contraction is not fully understood.

Cyclic AMP and Relaxation

The process of maintaining low levels of $[Ca^{++}]_i$ is autoregulatory, since removing a contractile agonist results in a reversal of the response. There is good evidence in airway smooth muscle that the process of active relaxation in the presence of a contractile agonist can be induced, and that this process is mediated by cyclic AMP [309, 311] (Fig. 71-6).

Cyclic AMP activates a cyclic AMP–dependent protein kinase by binding to the regulatory subunits of the tetrameric protein (two regulatory subunits and two catalytic subunits), causing a conformational change and resulting dissociation of the regulatory subunits [163]. Cyclic AMP–dependent protein kinase exists as two isozymes that can vary in amounts and distribution in different airway smooth muscle [259, 310]. The physiologic significance of the difference of the two isozymes is not known. Two general mechanisms of cyclic AMP/protein kinase effects could result in a reduction in contraction, by mediating a reduction in $[Ca^{++}]_i$ or by altering the sensitivity of the effector contractile system to the Ca^{++} activation process.

Phosphorylation of myosin light-chain kinase reduces its affinity for calcium-calmodulin, thus resulting in a decrease in affinity for myosin light chains. Cyclic AMP–dependent protein kinase can phosphorylate myosin light-chain kinase, resulting in a decrease in phosphorylation of the myosin light chains and a decrease in muscle contraction [76, 78]. Similar results were not, however, observed in bovine tracheal smooth muscle, where no change in the sensitivity of myosin light-chain kinase to Ca^{++}-calmodulin was observed in the presence of the beta agonist isoproterenol [194].

Fura-2 studies on airway smooth muscle demonstrated a reduction in $[Ca^{++}]_i$ to both forskolin-activated adenylate cyclase and beta-receptor stimulation with isoproterenol [98], although a cyclic AMP–dependent, protein kinase–mediated increase in microsomal uptake of Ca^{++} was not observed, as seen in vascular smooth muscle [31, 258]. Alternative mechanisms of cyclic

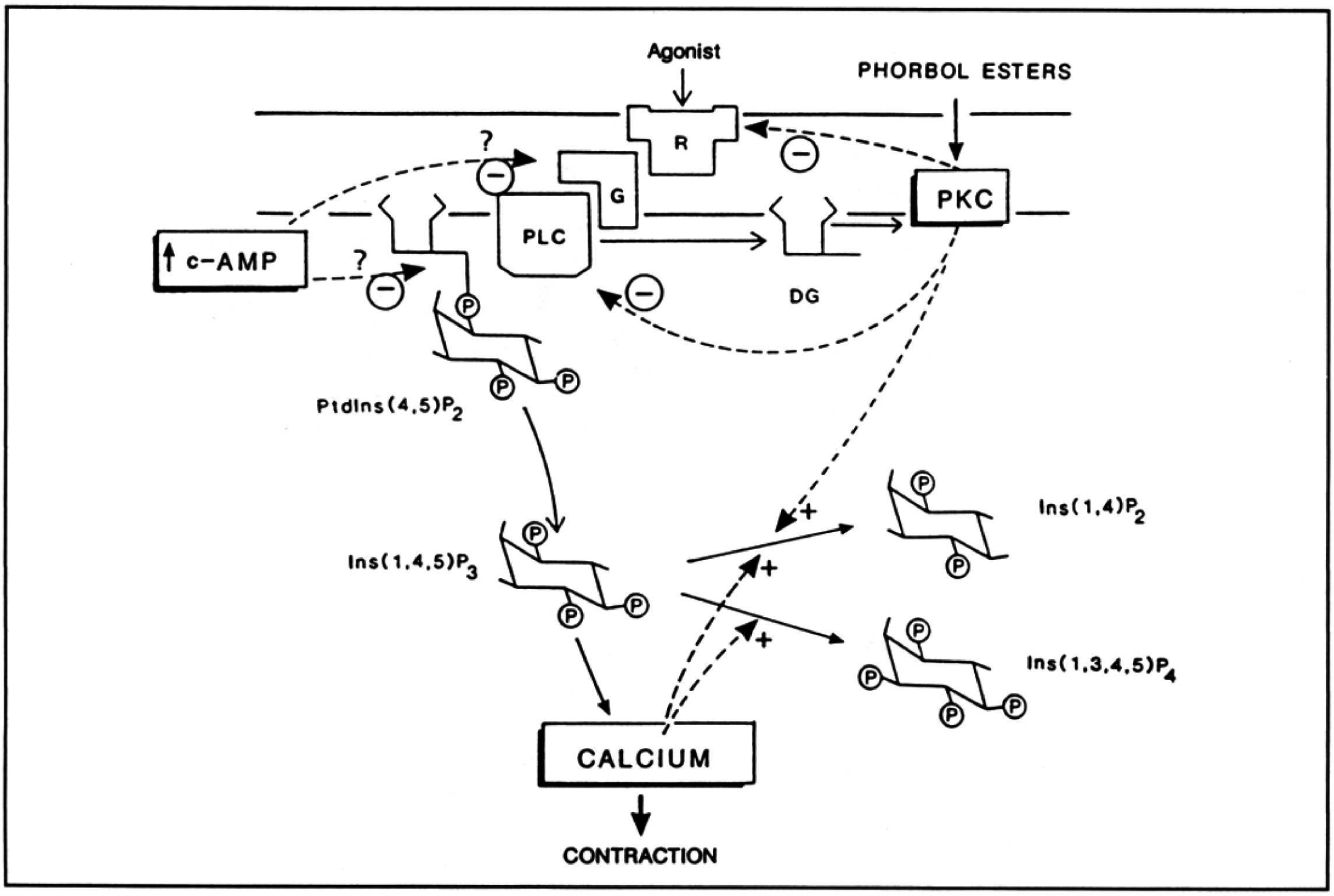


Fig. 71-5. *Proposed scheme for the regulation of inositol-1,4,5-trisphosphate formation and metabolism principally by $[Ca^{++}]_i$, protein kinase C* (PKC), *and cyclic AMP* (cAMP). PLC = *phosphoinositide-specific phospholipase C;* G = *GTP-binding protein;* R = *receptor;* DG = *1,2-diacylglycerol. (Reprinted with permission from E. R. Chilvers and S. R. Nahorski. Phosphoinositide metabolism in airway smooth muscle.* Am. Rev. Respir. Dis. *141:S139, 1990.)*

AMP–mediated decreases in $[Ca^{++}]_i$ include activation of a membrane Na-K-ATPase resulting in hyperpolarization [121], inhibition of Ca^{++} influx via VOCs [189], and a reduction in inositol phosphate [122].

For additional information the reader is referred to two recent symposia examining in detail newer basic views on calcium fluxes in cells and recent advances in calmodulin research [44a, 331a].

CALCIUM CHANNEL BLOCKERS IN THE TREATMENT OF ASTHMA

It has been proposed that an anomaly in calcium homeostasis might be an underlying cause of asthma and that any drug that reduced $[Ca^{++}]_i$ concentrations might have therapeutic benefits [193, 329]. Early in their development, it was realized that calcium channel blockers lacked the adverse respiratory effects associated with other antihypertensive drugs [10, 142, 245]. Since they were known to cause vasodilatation by preventing the entry of calcium into vascular smooth muscle, it was logical to assess their effects on bronchial smooth muscle. An increase in $[Ca^{++}]_i$ concentration is the final common pathway of many of the pathologic features of asthma, so it was postulated that in addition to bronchodilatation, calcium channel blockers might have other beneficial effects. Initially studies on models of induced bronchospasm showed calcium channel blockers to be very effective [22, 45] and this encouraged further exploration of their effects on other aspects of the asthmatic process. Subsequent studies, however, particularly in clinical asthma, did not confirm the early promise. As new compounds have become available, further studies have been performed in the hope of showing significant therapeutic effects and a more selective action on pulmonary tissue. There have been more than 100 published studies on the effects of calcium channel blockers, but neither formal development programs nor large-scale clinical studies have been performed with this class of drugs in the treatment of asthma.

RATIONALE FOR USE

Calcium ions have a central role in excitation-contraction coupling, stimulus-secretion coupling, and nerve impulse conduction; all are important mechanisms in the pathogenesis of asthma. Thus, it was proposed that there may be an abnormality of calcium homeostasis in asthma that might be amenable to treatment with the broad class of calcium antagonists [193]. The most effective and best known of these are a specific class of heterogeneous compounds called *calcium channel blockers*, which cause vasorelaxation by reducing the entry of calcium ions into the cell through the voltage-sensitive channel in the cell membrane. In vitro studies in smooth muscle showed that calcium channel

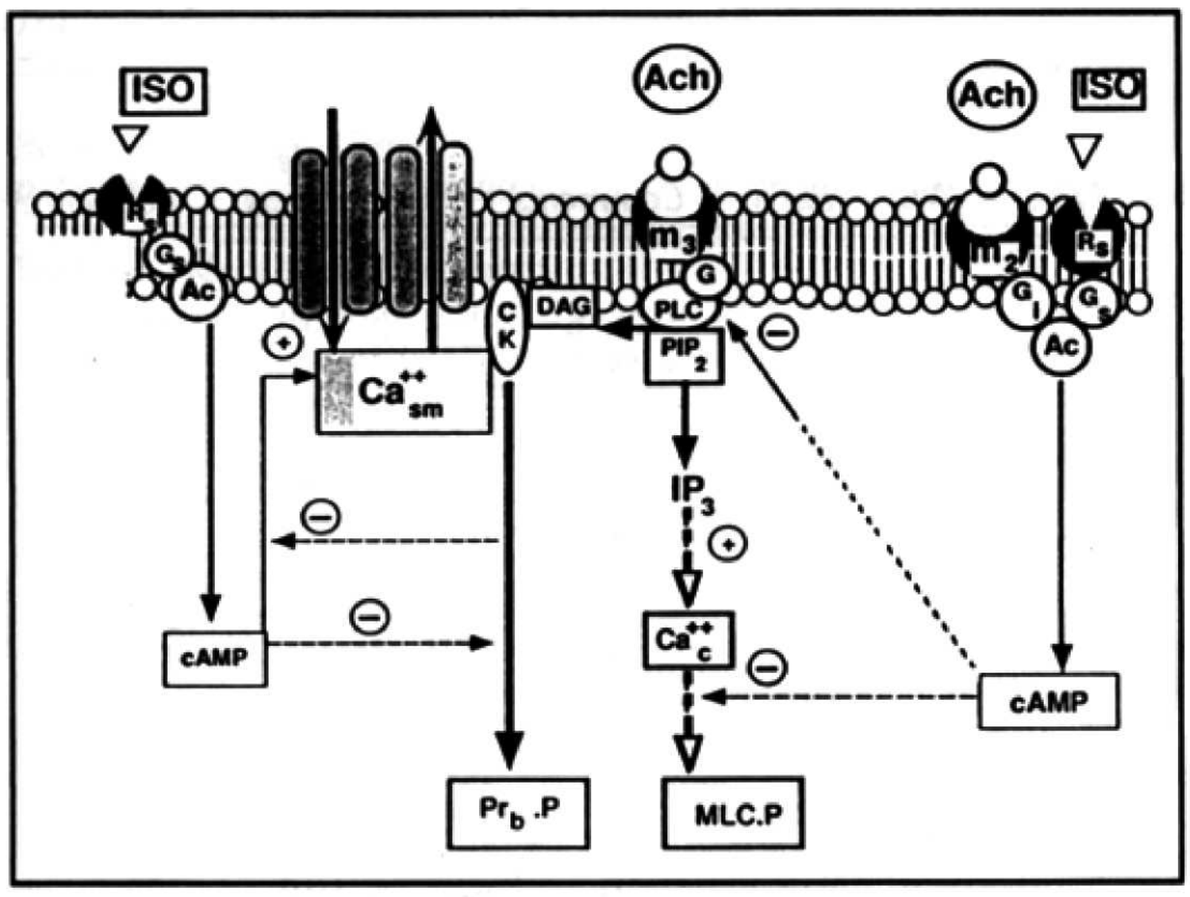


Fig. 71-6. *A schematic model depicting interrelationships between Ca^{++} and cyclic AMP* (cAMP) *messenger systems in control of airway smooth muscle tone. The plasma membrane (at top) possesses beta-adrenergic receptor* (R_s) *and two types of muscarinic* (M) *cholinergic receptors: M_2 linked via an inhibitory G-protein* (G_i) *to adenylate cyclase* (AC), *and M_3 linked via a G-protein to a phosphoinositol-specific phospholipase C* (PLC). *When the M_3 receptor is activated by acetylcholine* (ACh), *there is the production of inositol-1,4,5-trisphosphate* (IP_3) *and diacylglycerol* (DAG). *The IP_3 stimulates a release of Ca^{++} from an intracellular Ca^{++} pool, resulting in a transient rise in cytosolic free Ca^{++} concentration* (Ca^{++})$_c$. *This rise in $[Ca^{++}]_c$ activates myosin light-chain kinase, thereby bringing about a transient increase in the phosphorylation of myosin light chain* (MLC.P). *This phosphorylation is the key event in initiating muscle contraction. A rise in $[Ca^{++}]_c$ along with an increase in DAG content of the plasma membrane leads to a translocation of protein kinase C* (CK) *to the plasma membrane where it exists in a Ca^{++}-sensitive form. Activation of M_3 receptor also causes an increase in Ca^{++} influx and hence Ca^{++} cycling, across the plasma membrane, leading to an increase in Ca^{++} concentration in a submembrane domain* ($Ca^{++}{}_{sm}$). $[Ca^{++}]_{sm}$ *regulates CK activity. An increase in CK activity leads directly or indirectly to phosphorylation of a number of proteins* (Pr_b.P), *which are involved in sustaining the response. When M_2 receptor is activated, adenylate cyclase activity decreases and cyclic AMP content falls. When a beta-adrenergic agonist such as isoproterenol* (ISO) *acts, it stimulates adenylate cyclase and causes an increase in cyclic AMP concentration. A rise in cyclic AMP acts at several sites to inhibit events in both branches of the Ca^{++} messenger system: It inhibits ACh-induced hydrolysis of phosphatidylinositol-4,5,-bisphosphate* (PIP_2). *It acts to inhibit the extent of phosphorylation of both MLC.P and some but not all of the late-phase phosphoproteins* (Pr_b.P). *This involves either an inhibition of several kinases and/or an activation of phosphoprotein phosphatase activity. A rise in cyclic AMP also inhibits the ACh-induced increase in Ca^{++} influx by an unknown mechanism. Also, cyclic AMP per se increases Ca^{++} influx, but this effect is markedly inhibited when protein kinase is activated. Hence, a rise in cyclic AMP content in a contracted muscle leads to a decrease in $[Ca^{++}]_{sm}$ in DAG content of membrane and a decrease in state of phosphorylation of both MLC.P and Pr_b.P. All of these effects appear to participate in relaxing the muscle. (Reprinted with permission from H. Rasmussen, G. Kelley, and J. S. Douglas. Interactions between Ca^{2+} and cAMP messenger system in regulation of airway smooth muscle contraction.* Am. J. Physiol. (Lung Cell. Mol. Physiol.) *258:L285, 1990.)*

blockers are most effective at inhibiting K^+-induced contractions whereas leukotriene-, histamine-, and methacholine-induced contractions are increasingly resistant to the effects of these compounds, presumably because they initiate the release of calcium from alternative sites in the cell [312]. The most obvious action of these drugs would be the attenuation of smooth muscle contraction but there is evidence for modulation of mast cell degranulation [166], sputum clearance [18], and neurotransmission [73].

ASSESSMENT OF CLINICAL UTILITY

The assessment of the therapeutic value of calcium channel blockers is based on their pharmacology, their pharmacokinetics, and their safety and efficacy. The published studies were performed with different compounds, with different trial designs, under different conditions, and with different populations of asthmatic patients, and therefore comparisons have to be made with caution. However, even from such a heterogeneous data base, patterns can be discerned because a large number of studies are available for study.

Clinical Pharmacology

The aim of clinical pharmacology studies is to determine whether a compound shows activity in humans and whether these effects are comparable to those seen in animals. Bronchodilator studies and more complicated inhalational challenge tests have been performed with calcium channel blockers, and are discussed in detail below.

Approximately 100 studies in which pulmonary function was measured after the administration of calcium channel blockers have been performed, but in only a small proportion has statistically significant bronchodilatation been seen either acutely (Table 71-1) or after chronic treatment (Table 71-2). Indeed in three studies, verapamil [100] and diltiazem [131] aerosols and verapamil powder [238] caused significant bronchoconstriction in some patients (see Table 71-1). Bronchodilatation seen after acute administration even in studies showing "positive" responses was clinically insignificant. Overall, none of the calcium channel blockers tested have consistently shown significant bronchodilatation, even when the plasma levels achieved are sufficient to induce vasoactive effects including a fall in blood pressure. While it is a quick and relatively easy test of activity to see if a calcium channel blocker causes bronchodilatation, interpretation of the results can be difficult. For instance, in normal subjects, resting tone is the result of vagal activity, as evidenced by bronchodilatation after the use of anticholinergic drugs [20]. In asthmatic patients, the increased tone may be caused by a release of histamine or leukotrienes, as demonstrated by improvements seen after the administration of specific inhibitors [139, 239]. Although calcium channel blockers have been relatively ineffective in reducing resting tone, nifedipine did attenuate the bronchoconstriction seen after deep inspiration [254]. It is important to note that a lack of acute bronchodilatation does not rule out clinical efficacy; for example, cromolyn has no acute effect on lung function but is efficacious in asthma after longer-term use.

If bronchoconstriction with nonspecific inhalational agents such as histamine and methacholine, which act directly on bronchial smooth muscle, is attenuated, this demonstrates "functional" antagonism of muscle contraction. Protection against exercise-induced bronchospasm may indicate a useful therapeutic effect in those patients with exercise-induced asthma. Bronchial responses that follow the administration of antigen are more complex. The early response is a result of mast cell degranulation, whereas the fall in lung function that occurs in some patients a few hours after antigen administration is associated with an increase in both cellular inflammation and bronchial responsiveness. It has been suggested that therapeutic modification of the late antigen response may be more predictive of an antiinflammatory action and beneficial effects in clinical asthma than other models of induced bronchospasm. Similarly, if a reduction in bronchial responsiveness is observed after prolonged treatment of clinical asthma, it is postulated that this could be a result of a concomitant reduction in bronchial inflammation [257]. This may indicate an important antiasthmatic property of the drug.

Table 71-1. Acute administration

No. of patients[a]	Drug/ formulation		Dose/ frequency	Time before measurement (hr)	Design	Control	FEV_1	FVC	PEFR	Comments	Side effects	Reference
Controlled studies												
10	NIF	o	20 mg	2	DX	Alb/PL	[b]	—	—	3/10 FEV_1 >10%	2/10	263
11	NIF	o	10 mg	½	DX	PL	NS	NS	NS	SGaw[bd]	—	217
18	NIF	o	20 mg	1½	SX	PL	—	—	[d]	—	—	142
15	NIF	o	10 mg	1½	SX	None	NS	NS	—	From baseline	7/15	198
15	NIF	o	20 mg	1½	SX	None	[b]	[b]	—	From baseline	7/15	198
15	Ver	Neb	20 mg	Immed	SX	PL	NS	[b]	[b]	From baseline	8/15	268
8n	Ver	Neb	2 mg	½–1½	SX	PL	—	—	—	SGaw[d]	—	233
10	Ver	Neb	1 mg	½–1½	SX	PL	—	—	—	SGaw[d]	—	233
10	Ver	Neb	2 mg	½–1½	SX	PL	—	—	—	SGaw[d]	—	233
12	PY	o	150 mg/ 3 days	1¼	DX	PL	[b]	[b]	—	FEF_{25-75} [b]	5/12	28
Open studies												
5n	NIF	o	20 mg	2	Open	None	NS	NS	—	—	—	140
25	NIF	o	20 mg	2	Open	None	[b]	[c]	—	Decrease PaO_2	1/10	140
15n	NIF	sl	20 mg	⅔	Open	None	NS	NS	—	FEF_{25-75} NS	26/60	203
15a	NIF	sl	20 mg	⅔	Open	None	[b]	[b]	—	FEF_{25-75} NS	26/60	203
15c	NIF	sl	20 mg	⅔	Open	None	[b]	[b]	—	FEF_{25-75} [b]	26/60	203
15	NIF	sl	20 mg	⅔	Open	None	[b]	[b]	—	FEF_{25-75} [b]	26/60	203
15	NIF	sl	20 mg	¾	Open	Amino	—	—	—	Equiv. with amino	8/15	47
Bronchoconstriction												
10	DIL	Neb	5–60 mg	⅙	SX	PL	NS	Bronchoconstriction at 60 mg			—	131
9	Ver	Neb	5–12.5 mg	⅔	DX	PL	NS	Bronchoconstriction at 12.5 mg			0/9	100
24	Ver	Powder	5 mg	½	DX	PL	NS	Bronchoconstriction in 10/24			—	238

NIF = nifedipine; Ver = verapamil; DIL = diltiazem; PY = PY 108-068; Alb = albuterol; Amino = aminophylline; PL = placebo; o = oral; sl = sublingual; Neb = nebulized; D = double-blind; S = single-blind; X = crossover; Immed = immediate; — = not done; NS = not significant; FEV_1 = 1-second forced expiratory volume; FVC = forced vital capacity; PEFR = peak expiratory flow rate; SGaw = specific airway conductance; FEF = forced expiratory flow.

[a] All patients are asthmatic unless indicated otherwise. c = chronic obstructive pulmonary disease; a = angina; n = normal.

[b] $p < .05$.

[c] $p < .02$.

[d] $p < .01$.

Table 71-2. Chronic administration[a]

No. of patients	Drug/ formulation		Dose/ frequency	Duration	Design	Control	PFT	PEFR	Symptoms	Concomitant medication	Side effects	Reference
Controlled studies												
11	NIF	o	10 mg tid	4 days	DX	PL	—	[b]	NS	—	2/11	217
9	NIF	o	10 mg qid	2 wk	DX	PL	—	NS	NS	NS	—	106
17	NIF	o	20 mg bid	2 wk	SX	PL	[b]	—	—	—	6/7	142
10	NIF	sr	20 mg tid	2 wk	DX	PL	—	NS	NS	—	2/10	279
11	NIF	o	10 mg qid	2 wk	DX	PL	—	NS	[b]	NS	0/11	171
11	NIF	o	20 mg tid	3 wk	DX	PL	NS	NS	[b]	[b]	5/11	212
14	NIF	o	10 mg tid	4 wk	DX	PL	—	NS	NS	NS	7/14	216
21	NIF	o	40–120 mg/day	4 wk	DX	—	NS	NS	NS	NS	—	57
12	NIF	o	20 mg bid	30 days	DX	PL	NS	—	NS	[b]	—	293
15	NIF	o	10 mg qid	8 wk	DX	PL	—	NS	NS	[c] (steroids)	4/15	8
12	Ver	o	160–240 mg/day	4 wk	DX	Capt	NS	NS	—	—	11/12	247
17	Ver	o	160–240 mg/day	4–6 wk	DX	Capt	NS	NS	NS	NS	15/17	246
21	DIL	o	240–480 mg/day	4 wk	DX	—	NS	NS	NS	NS	—	57
Open studies												
15c	NIF	o	20 mg tid	2 wk	Open	N	N	NS	NS	—	3/15	203
10	NIC	o	20 mg tid	3 mo	Open	N	NS	—	—	—	—	108
47	NIF	sr	20 mg bid	3–18 mo	Open	N	—	—	—	—	14/47	41
Pediatric study												
22	NIF	o	10 mg tid	4 wk	DX	PL	—	—	NS	NS	0/22	275

NIC = nicardipine; sr = slow-release; Capt = captopril; N = none.

[a] See Table 71-1 footnotes for abbreviations not explained here.

[b] $p < .05$.

[c] $p < .02$.

Table 71-3. Exercise-induced and cold air–induced bronchospasm[a]

No. of patients	*Drug/ formulation*	*Dose*	*Time before challenge (hours)*	*Design*	*Significance*	*Reference*
Exercise-induced bronchospasm						
10	NIF o	20 mg	¾	S	[b]	45
15	NIF o	20 mg	½	D	[b]	218
12	NIF o	30 mg	2	D	[b]	210
11	NIF o	10 mg	½	D	NS	240
11	NIF o	20 mg	½	D	[b]	240
11	NIF o	30 mg	½	D	[b]	240
8	NIF sl	20 mg	½	D	[b]	22
8	NIF sl	20 mg	½	D	[b]	68
8	NIF sl	20 mg	½	D	[b]	224
19	NIF sl	20 mg	½	D	[b]	269
4	NIF sl	20 mg	1	D	NS	113
8	Ver Neb	3 mg	½	S	[b]	221
10	Ver Neb	3 mg	½	S	[b]	221
10	Ver Neb	3 mg	½	D	[b]	226
10	GAL Neb	3 mg	½	D	[b]	226
10	GAL Neb	1 mg	½	D	NS	184
10	GAL Neb	10 mg	½	D	[b]	184
15	GAL Neb	10 mg	½	D	[b]	185
10	DIL o	60 mg	4	D	NS	175
10	DIL o	120–180 mg	1⅔	D	[b]	125
15	DIL o	120 mg	1½	D	NS	184
10	DIL Neb	20–45 mg	¼	D	[c]	131
10	DIL Neb	10 mg	¼	D	NS	131
9	FEL o	10 mg	10	D	[b]	225
12	PY o	75 mg	2	D	NS	210
12	PY o	150 mg	2	D	[c]	210
12	PY o	150 mg	1¼	D	[c]	28
4	FLO o	25 mg	1	D	NS	113
4	FLO o	50 mg	1	D	NS	113
Pediatric studies						
15	Ver Neb	5 mg/2 ml	½	D	NS	34
9	Ver Neb	10 mg/4 ml	½	D	NS	34
7	DIL Neb	5 mg	⅓	D	NS	103
7	DIL Neb	10 mg	⅓	D	NS	103
13	Ver Neb	5 mg/2 ml	½	D	[d]	35
12	Ver Neb	10 mg/2 ml	½	D	NS	35
Cold air–induced bronchospasm						
8	NIF o	20 mg	¾	D	[c]	283
8	NIF sl	20 mg	½	D	[b]	133
24	Ver Neb	5 mg	½	D	NS	238
8	Ver IV	Infusion	½	D	NS	283
10	DIL o	60 mg	4	D	NS	175
Normal subjects						
8	NIF sl	20 mg	½	D	NS	133

GAL = gallopamil; FEL = felodipine; FLO = floridipine; IV = intravenous.
[a] See Table 71-1 footnotes for abbreviations not explained here.
[b] $p < .01$.
[c] $p < .05$.
[d] $p < .02$.

The first study to test calcium channel blockers in exercise-induced bronchospasm showed that oral nifedipine almost totally blocked the bronchoconstrictive response [45]. Subsequent studies (Table 71-3) using calcium channel blockers by both the oral and inhaled routes showed that while the degree of bronchoconstriction after exercise, compared to that with a placebo, was in general significantly reduced, the proportion of patients with total protection was less than in the earlier studies (Fig. 71-7). When nifedipine was administered after bronchospasm was induced by exercise, it had no effect [181]. Overall pretreatment with verapamil and nifedipine was as effective as pretreatment with cromolyn [68, 225] but less than with beta agonists (see Fig. 71-7). The effects of nifedipine were dose related but with a plateau beyond which larger doses had no greater effect [240]; diltiazem was less effective and showed no dose effect [125, 131, 185]. The protection afforded by diltiazem disappeared relatively quickly and this may explain the negative observations made in some studies [175]. Nifedipine prevents the increase in histamine seen after exercise, suggesting that mediator release inhibition may be as important as smooth muscle effects [22]. In children [34, 35, 103], the effects of neither diltiazem nor verapamil were statistically significantly different from those of placebo, while other comparative drugs were effective in the same studies. Cold air–induced bronchospasm, which is considered to cause bronchoconstriction by a similar mechanism as exercise, has also been studied (see Table 71-3). Nifedipine attenuated the effect of cold air inhalation [133, 283] although verapamil and diltiazem did not [175, 238, 283]. Nifedipine provided no protection in normal subjects.

The effects of calcium channel blockers on bronchoconstric-

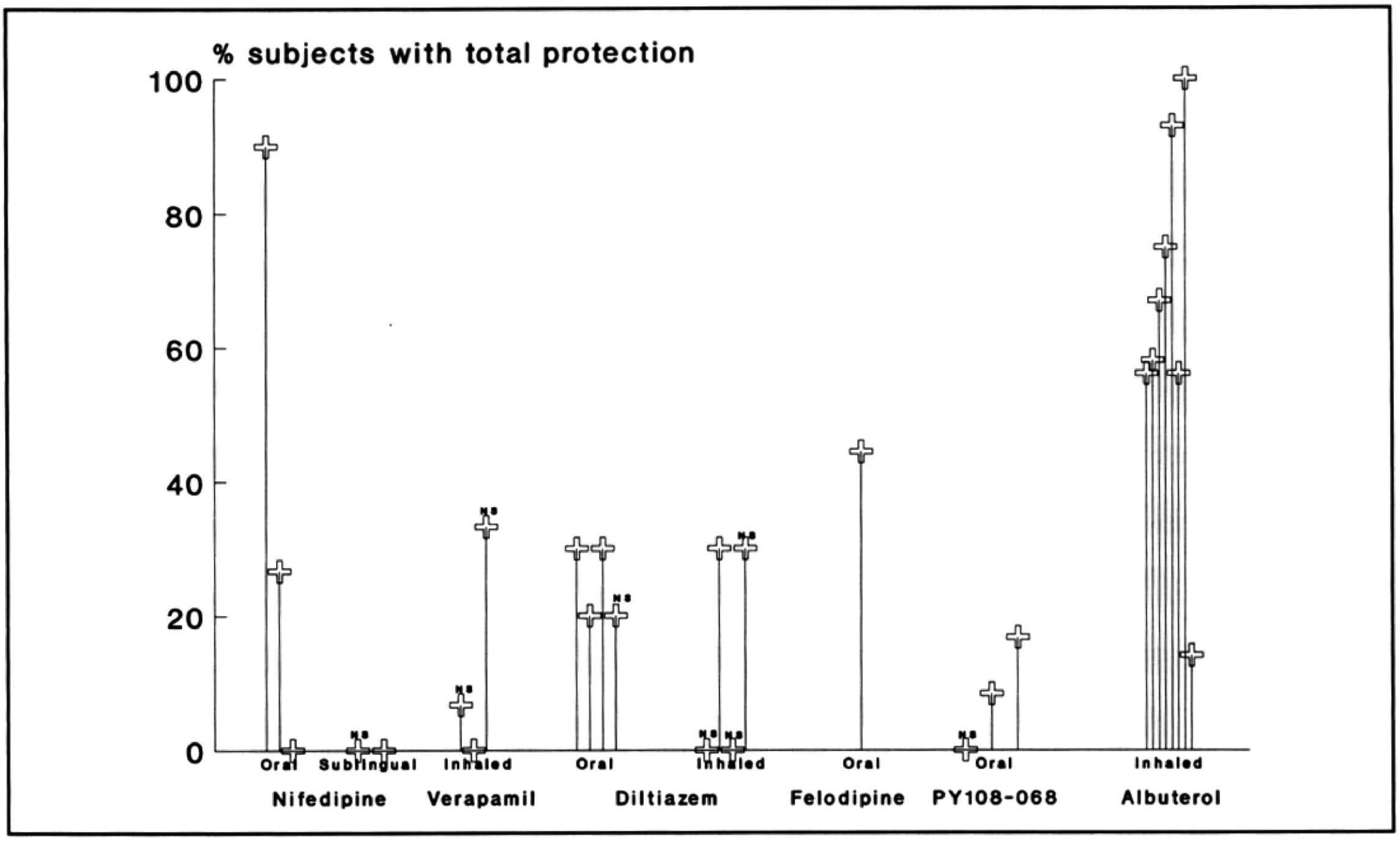


Fig. 71-7. *The effect of calcium channel blockers on exercise-induced bronchospasm. Each cross represents the results of a single published study. The proportion of subjects showing total protection is shown, that is, those subjects whose forced expiratory volume in 1 second (FEV_1) after treatment does not drop below 10 percent of the total fall in FEV_1 after placebo. The group mean is significantly different from the mean with a placebo unless indicated by "NS." The results from published studies conducted with albuterol are included for comparison.*

tion by nonspecific agents are variable but generally positive (Tables 71-4, 71-5, and 71-6). Oral nifedipine appears to offer the greatest degree of protection, although this effect is modest compared to albuterol administered under similar conditions (Figs. 71-8 and 71-9). The effects in normal subjects were also variable; nebulized verapamil was better in normal subjects [6, 233] while oral diltiazem was better in asthmatic subjects [132]. Nebulized verapamil did not significantly shift the provocative dose (PD_{20}) of histamine required to cause a 20 percent drop in forced expiratory volume (FEV_1) in 1 second but did increase the threshold dose needed to cause bronchoconstriction [322], and the administration of nifedipine by aerosol, which would be expected to deliver a greater concentration of the drug to the airways, gave no greater protection than other routes of administration [71]. When the tone was artificially increased with inhaled histamine, nifedipine by aerosol returned pulmonary function to baseline quicker than the control [193]. There was not a dose-related inhibition of methacholine-induced bronchospasm with either oral diltiazem [125] or inhaled gallopamil [185]. In only one [238] out of five studies [126, 132, 220, 248] was any protection noted in normal subjects. In pediatric studies from the same group, nebulized verapamil gave significant protection on one occasion [36] but not on the other [47]. If the degree of effectiveness of these compounds against different bronchoconstricting agents is compared, histamine-induced bronchospasm seems marginally more susceptible to attenuation. However, in one direct comparison [100], nebulized verapamil attenuated methacholine- but not histamine-induced bronchoconstriction. Nebulized verapamil attenuates leukotriene D_4–induced bronchoconstriction in normal [248] but not asthmatic subjects [249]. These data suggest that leukotriene D_4 has a different action in asthmatic subjects, but since verapamil is only able to block bronchoconstriction in normal subjects, this effect is unlikely to be of much value in asthma. Nifedipine has no effect on adenosine-induced bronchoconstriction [69] but is more effective at blocking bronchoconstriction induced by hypotonic saline solution [152] than is nebulized verapamil [153].

Inhalational challenges with various antigens have been performed, although only one study on the effects of calcium channel blockers on the late response has been performed [138a]. Four [5, 67, 134, 256] out of eight controlled studies showed a significant shift in the early response to antigen (Table 71-7), again with nifedipine and gallopamil being more effective than verapamil. The degree of protection was generally modest (Fig. 71-10), although the effects of inhaled gallopamil in one study were considerably superior to those of cromolyn [5]. Overall, these effects presumably reflect blockade both of mediator release and of bronchial smooth muscle contraction. Gallopamil by aerosol had no effect on the late antigen response [138a], and verapamil had no effect on the late response induced by toluene diisocyanate [180].

In summary, therefore, the protective effects of calcium channel blockers against induced bronchoconstriction are modest. In general, the relative potency of individual drugs and their effects in different studies are predictable from in vitro studies. Nifedipine and gallopamil are more effective in these models than the other drugs tested. Therapeutic effects do not appear to be limited by dose since a "plateau" of effect appears to be reached

Table 71-4. Histamine-induced bronchospasm[a]

No. of patients	*Drug/ formulation*	*Dose*	*Time before challenge (hours)*	*Design*	*Significance*	*Reference*
Asthmatic subjects						
8	NIF o	10 mg	⅔	S	NS	308
8	NIF sl	20 mg	1½	D	[b]	22
15	NIF sl	20 mg	1	D	[b]	332
10	NIF sl	20 mg	½	D	[c]	176
10	NIF sl	20 mg	½	D	NS	222
8	NIF sl	20 mg	½	D	[c]	65
11	NIF sl	20 mg	½	D	[c]	132
6	NIF Neb	10 mg	¼	D	[b]	71
10	Ver Neb	3 mg	⅓	D	NS	224
10	Ver Neb	6 mg	⅓	D	NS	224
8	Ver Neb	5 mg	½	D	[b]	188
10	Ver Neb	3 mg	⅓	D	NS	219
8	Ver Neb	1 mg	1	S	NS	233
8	Ver Neb	2 mg	1	S	NS	233
8	Ver Neb	4 mg	¼	D	[c]	322
9	Ver Neb	5 mg	⅔	D	NS	100
9	Ver Neb	12.5 mg	⅔	D	NS	100
7	DIL o	60 mg	¾	D	[b]	126
Normal subjects						
8	NIF sl	20 mg	½	D	[c]	132
8	Ver Neb	3 mg	⅓	D	NS	219
7	Ver Neb	1 mg	1	S	NS	233
7	Ver Neb	2 mg	1	S	[b]	233
8	DIL o	60 mg	¾	D	NS	126

[a] See Table 71-1 footnotes for explanation of abbreviations.
[b] $p < .05$.
[c] $p < .01$.

before side effects become intolerable. Importantly, gallopamil, a potent drug, administered topically in maximal doses, had no effect in the model most predictive of clinical efficacy, the late antigen response.

Pharmacokinetics and Drug Interactions

Since modest and variable effects are the hallmark of studies with calcium channel blockers, it is important to determine their pharmacokinetics to ensure that a sufficient amount of drug is reaching the site of action. Plasma concentrations were not monitored in the early trials, but more recent studies have shown that there is a threshold below which protective effects are lost as a function of either dose used or time of testing [184, 185]. There is also a plateau beyond which larger doses cause no increase in effect [185, 240]. There is, however, considerable intersubject variability. These studies strongly suggest that higher oral doses are unlikely to offer any further benefits, with the additional risk that vasoactive side effects will become increasingly evident. Theoretically, administration of the compound by the inhaled route may reduce the systemic side effects; however, studies using this method have not shown greater efficacy [71, 185].

Interactions with other antiasthmatic medications have also been studied. Nifedipine was reported to reduce the concentrations of theophylline in one study [279] but not in two others [56, 69]. Diltiazem has no obvious interaction with theophylline [56]. In vitro studies suggested that calcium channel blockers may enhance the effects of beta agonists, and a small additive effect has been shown with nifedipine [70, 168, 301] but not with verapamil [118, 250] in studies in humans. This improvement was minimal and a similar clinical effect would be achieved by increasing the dose of the beta agonist by a small amount.

Clinical Studies

A significant effect on "asthma in the wild" rather than on models of asthma is perhaps the acid test of the efficacy of calcium channel blockers. Thirteen controlled and three open studies have been conducted for periods ranging from 4 days to 3 months in adults (see Table 71-2) and 4 weeks in children [275]. The number of patients in these studies have been very small, ranging from 9 to 21 in controlled studies. Measurements of lung function, patient symptoms, and concomitant medication have been used to assess clinical efficacy, and adverse effects have also been noted. One of the earliest studies showed a significant reduction in the diurnal peak expiratory flow rate after 4 days of nifedipine treatment [217]; however, subsequently the same group found no significant clinical effects after treating patients with nifedipine for 4 weeks [216]. In children, nifedipine had no effect on the diurnal variation in pulmonary function [275]. A large dose of nifedipine given for 3 weeks showed reductions in symptoms and concomitant medication but no changes in lung function [212], while a similar dose given for 2 weeks produced a reduction in symptoms only [171]. Neither verapamil [246, 247], nifedipine [106, 279], nor diltiazem [39] had any significant effect on any clinical parameters in the remaining studies. When nifedipine was given in addition to theophylline, there was a significant reduction in concomitant beta-agonist usage [293] or a reduction in theophylline levels [283] without a change in efficacy. Nifedipine, 10 mg administered 4 times a day for 16 weeks to oral corticosteroid–dependent patients, allowed 12 of the 15 treated patients to significantly reduce their dose of corticosteroids [8], and in a further study the condition of two patients deteriorated after stopping nifedipine treatment [93]. However, these results are not representative of the other clinical studies and should be interpreted with caution. Reductions in blood pressure were noted in most of the studies, especially in those patients with preexisting hypertension. In addition, a variable proportion of patients, ranging from 0 to 95 percent, noted vascular side effects such as flushing, headaches, and peripheral edema.

Overall, the results from the clinical studies showed an inconsistent and small effect in clinical asthma, with a significant num-

Table 71-5. Methacholine-induced bronchospasm[a]

No. of patients	Drug/ formulation	Dose	Time before challenge (hours)	Design	Significance	Reference
13	NIF o	20 mg tid/3 days	½–¾	D	[b]	19
8	NIF o	10 mg	⅔	S	[c]	308
12	NIF o	10 mg tid	5 days	D	[b]	99
8	NIF o	20 mg	1	S	NS	186
8	NIF sl	20 mg	⅙	D	NS	39
11	NIF sl	20 mg	½	D	[c]	132
8	NIF sl	10 mg	?	?	[b]	157
7	Ver Neb	3 mg	⅙	S	NS	249
8	Ver Neb	2 mg	1	S	NS	233
8	Ver Neb	1 mg	1	S	NS	233
5	Ver Neb	3 mg	⅓	D	NS	220
9	Ver Neb	5 mg	⅔	D	NS	100
9	Ver Neb	12.5 mg	⅔	D	[c]	100
19	DIL o	120 mg tid	7 days	D	NS	170
8	DIL o	60 mg	¾	D	[c]	126
12	DIL o	60 mg tid	5 days	D	NS	99
9	DIL o	30–180 mg	1⅔	S	NS	125
12	DIL Neb	5–60 mg	⅙	S	NS	131
11	GAL Neb	1–20 mg	½	S	[b]	185
10	NIC sl	20 mg	Immed	D	NS	25
12	NIS o	10 mg	3	D	[b]	317
Normal subjects						
8	NIF sl	20 mg	½	D	NS	132
7	Ver Neb	1 mg	1	S	NS	233
6	Ver Neb	3 mg	¼	S	NS	248
7	Ver Neb	2 mg	1	S	[c]	233
5	Ver Neb	3 mg	⅓	D	NS	220
5	DIL o	60 mg	¾	D	NS	126
Pediatric studies						
15	Ver Neb	5 mg	½	D	[b]	36
13	Ver Neb	5 mg/2 ml	½	D	NS	35
12	Ver Neb	10 mg/2 ml	½	D	NS	35
Open studies						
9	NIF o	10 mg tid	3 days	Open	[b]	112
14	NIF sl	20 mg	⅓	Open	[b]	230
9	NIF sl	20 mg	¾	Open	[b]	199

GAL = gallopamil; NIC = nicardipine; NIS = nisoldipine; ? = not recorded.
[a] See Table 71-1 footnotes for abbreviations not explained here.
[b] $p < .01$.
[c] $p < .05$.

Table 71-6. Bronchospasm induced by other inhalational agents[a]

No. of patients	Drug/ formulation	Dose	Time before challenge (hours)	Type of challenge	Design	Significance	Reference
6	Ver Neb	3 mg	¼	LTD_4	S	NS	249
6n	Ver Neb	3 mg	¼	LTD_4	S	[b]	248
10	NIF o	20 mg	½	HS	D	[c]	152
10	Ver Neb	20 mg	½	HS	D	NS	153
7	NIF sl	20 mg	?	Ad	D	NS	69
5	NIF sl	20 mg	¾	TDI	D	[c] (early response)	200
6	Ver sr	120 mg bid	7 days	TDI	D	NS (late response)	180

LTD_4 = leukotriene D_4; HS = hypertonic saline; Ad = adenosine; TDI = toluene diisocyanate.
[a] See Table 71-1 footnotes for abbreviations not explained here.
[b] $p < .01$.
[c] $p < .05$.

ber of side effects in many of the studies. The small numbers of patients participating in these studies mean that it is possible that a statistically significant effect could have been missed; however, it remains unlikely that such a result would be clinically significant.

In conclusion, published studies, when reviewed as a group, indicate that presently available calcium channel blockers produce only marginal therapeutic effects in asthma, at the price of systemic vascular effects in some patients. While this precludes a recommendation for their use in asthma, calcium channel

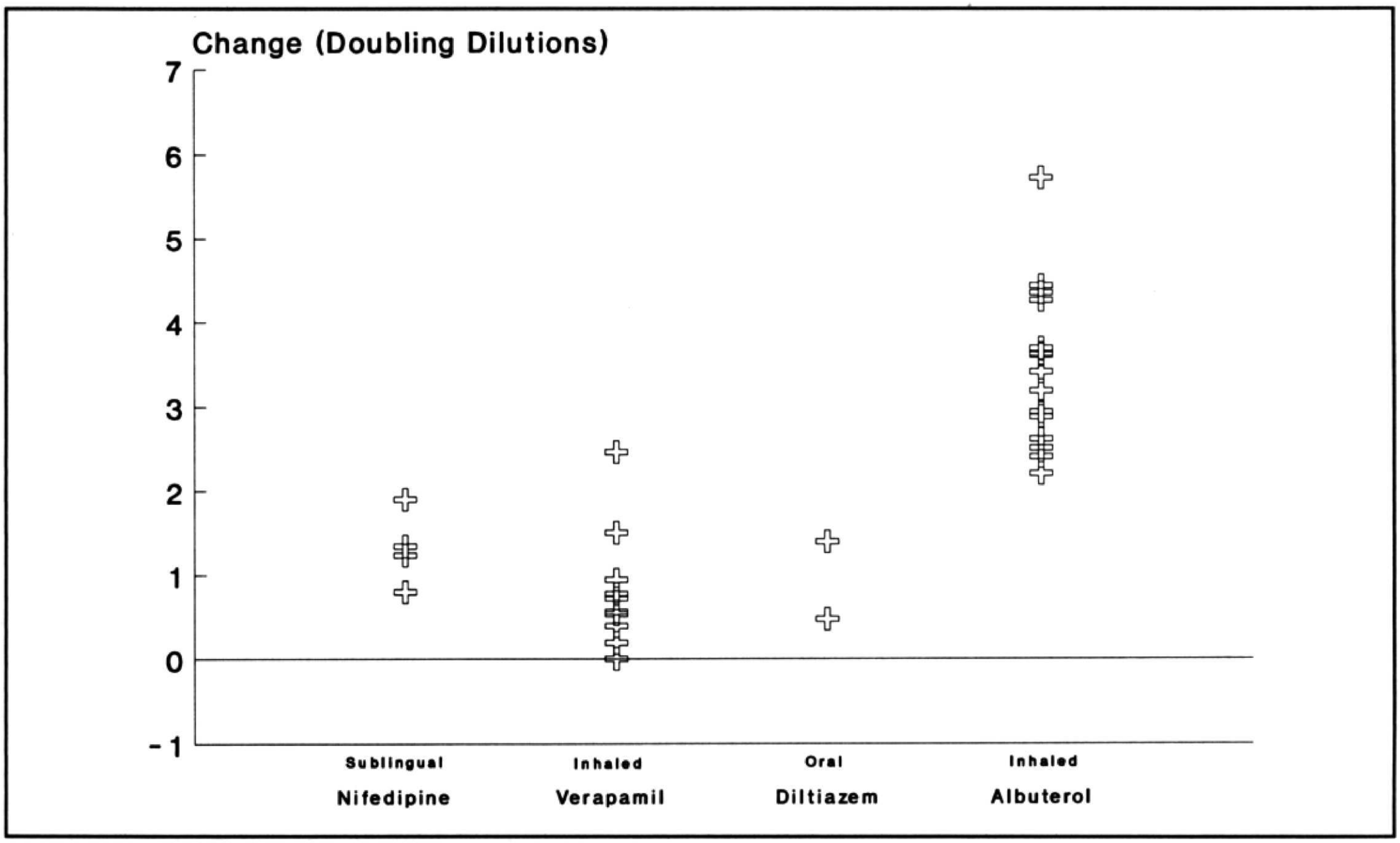


Fig. 71-8. *The effect of calcium channel blockers on histamine-induced bronchospasm. Each cross represents the results of a single published study. The geometric mean change in the dose-effect curve (expressed as the number of doubling dilutions of histamine) is shown following each treatment compared to placebo. The results from published studies conducted with albuterol are included for comparison.*

blockers may offer advantages over other antihypertensive drugs in patients with both hypertension and asthma. Presently available calcium channel blockers have failed to show pulmonary selectivity in clinical trials but this might have been predicted from simple in vitro studies with smooth muscle (Fig. 71-11) [313]. At this stage of our knowledge, it would seem prudent to obtain convincing data showing pulmonary selectivity in vitro before embarking on further studies with calcium channel blockers in humans.

THE ROLE OF CALCIUM IN AIRWAY REACTIVITY

The important action of $[Ca^{++}]_i$ in the muscle excitation-contraction process places it in a potentially critical role for altered airway smooth muscle responses in asthma. The contractile mechanism, indeed even cellular viability, of airway smooth muscle cells is dependent on a critical regulation of free $[Ca^{++}]_i$ concentration. Normally, at rest, airway smooth muscle cell $[Ca^{++}]_i$ concentrations are in the order of 10^{-7} M (about 1/10,000 the concentration [10^{-3} M] of the extracellular fluid); maximal contraction occurs at $[Ca^{++}]_i$ levels of about 10^{-5} M. This calcium concentration differential is achieved by the plasma membrane and by a series of energy-dependent extrusion processes involving sodium-calcium ionic exchange, calcium efflux, and intracellular organelle sequestration. Although contraction in both airway smooth muscle and skeletal muscle is activated by calcium, the mechanisms by which this occurs in smooth muscle are still not definitively established. In airway smooth muscle, an influx of calcium from the extracellular fluid and a release of calcium from internal pools in stored or bound intracellular sites are likely sources of internal free activator calcium—this in contrast to the reservoir of calcium sequestered in the sarcoplasmic reticulum of skeletal muscle. Calcium fluxing from the extracellular fluid or intracellular sites then diffuses throughout the smooth muscle cells where the smooth muscle fibers are relatively small, conditions sufficient to activate the contractile process. In smooth muscles with a more extensive sarcoplasmic reticulum, a greater rate of contraction results since transmembrane entry is a slower event than is the mobilization of calcium from the sarcoplasmic reticulum. Caveolae, minute cell membrane invaginations in the smooth muscle cell, probably enhance the release of calcium from sarcoplasma tubules following the appropriate stimulus. Additional entry of calcium utilizable for contraction may be via receptor-activated calcium channels in response to hormones or drugs. Overall, an impairment or alteration in any of the critical regulatory processes of calcium balance could result in an increased level of free $[Ca^{++}]_i$ and hence a variety of abnormal responses including those affecting or involving the myocontractile apparatus; abnormal regulation might also include impairment of the myorelaxation process [255].

As discussed, an increase in available or free myoplasmic calcium could contribute to the heightened reactivity of airway smooth muscle to a variety of stimuli, specific or nonspecific, a feature characteristic of the asthmatic airway. A possible abnormality of calcium homeostasis in the pathogenesis of airway smooth muscle reactivity was first reported in 1979 by Weiss and Viswanath [329]. These authors observed an increased sensitivity

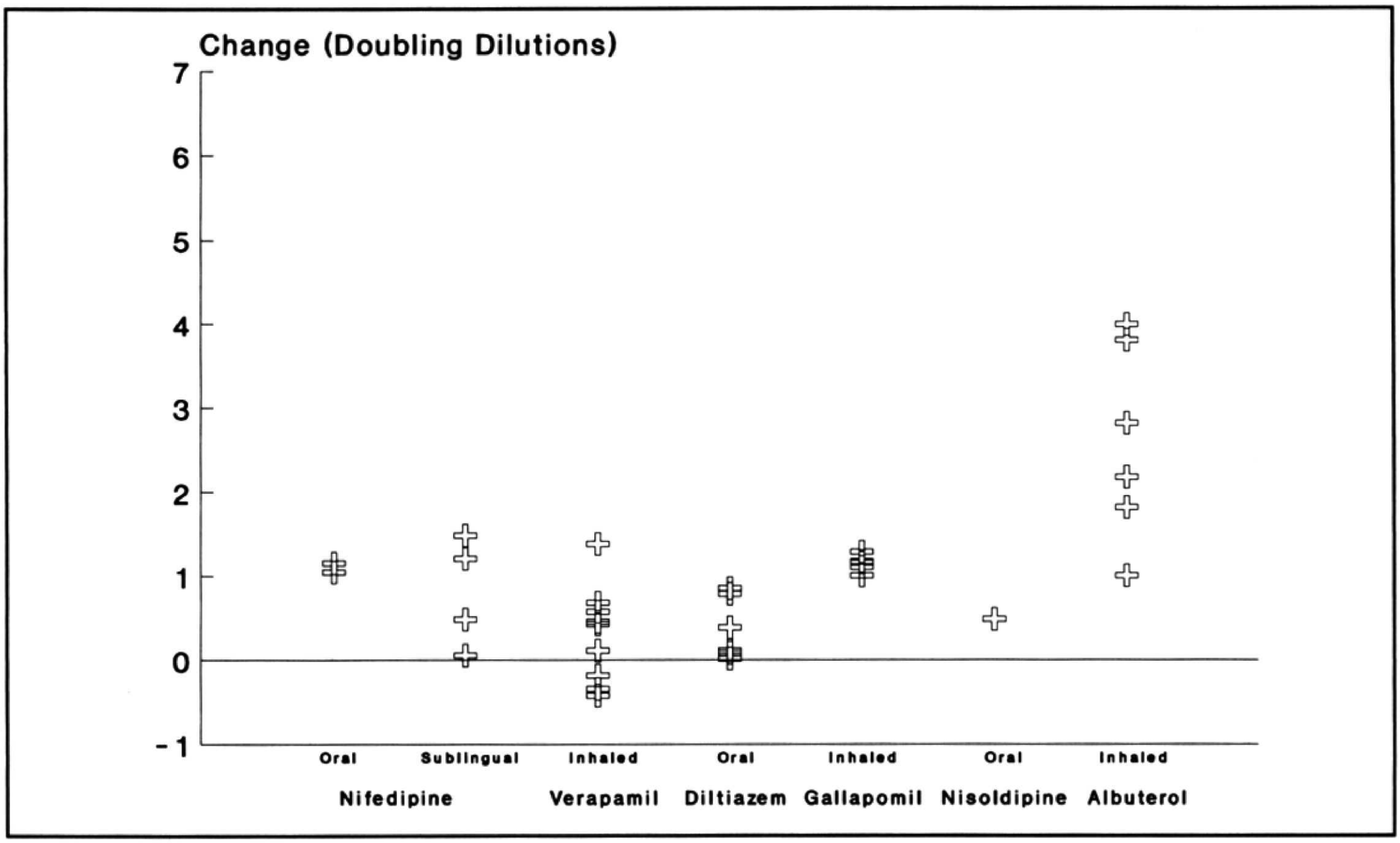


Fig. 71-9. *The effect of calcium channel blockers on methacholine-induced bronchospasm. Each cross represents the results of a single published study. The geometric mean change in the dose-effect curve (expressed as the number of doubling dilutions of methacholine) is shown following each treatment compared to placebo. The results from published studies conducted with albuterol are included for comparison.*

Table 71-7. Antigen-induced bronchospasm[a]

No. of patients	*Drug/ formulation*	*Dose*	*Time before challenge (hours)*	*Significance*	*Reference*
Controlled studies					
Early response					
12	NIF o	20 mg	¾	[b]	256
8	NIF sl	20 mg	½	[c]	134
7	NIF sl	20 mg	½	NS	215
9	NIF sl	20 mg	½	[c]	67
9	Ver Neb	5 mg	⅔	NS	100
9	Ver Neb	12.5 mg	⅔	NS	100
12	Ver o	160 mg	¾	NS	256
9	GAL Neb	1.05 mg	Immed	[b]	5
Late response					
6	GAL Neb	20 mg	½	NS	138a
Open studies					
23	NIF o	20 mg	½	[b]	158
8	NIF sl	20 mg	½	NS	280
8	Ver Neb	2 mg	½	NS	280
8	Ver Neb	3 mg	Immed	NS	223
4	Ver Neb	6 mg	Immed	NS	223
10	Ver Neb	20 mg	Immed	[d]	6
10	Ver o	160 mg	¾	NS	6
8	Ver o	80 mg tid	½	[c]	192

GAL = gallopamil.
[a] See Table 71-1 footnotes for abbreviations not explained here.
[b] $p < .05$.
[c] $p < .01$.
[d] $p < .02$.

to extracellular calcium following in vitro anaphylaxis in the guinea pig trachealis. The data suggested the following: Normal airway smooth muscle → airway smooth muscle "injury" (e.g., anaphylactic reaction) → acquired calcium homeostatic defect (? increased membrane permeability, altered intracellular binding, etc.) → increased free $[Ca^{++}]_i$ → increased basal muscle tone → nonspecific airway smooth muscle hyperreactivity.

In this scheme the airway smooth muscle becomes altered by some pathophysiologic event(s); its reactivity is not per se the sole consequence of extramyocytic events. The contribution of resting basal muscle tonus to airway reactivity was proposed by Benson [29]. Alterations in excitation-contractile coupling in airway smooth muscle were suggested in a theoretic discussion by Andersson as a basis for bronchial hyperreactivity [11].

While it is emphasized that this concept is currently speculative, several observations have suggested a calcium defect in reactive airway pathogenesis. Dhillon and Rodger [82] observed changes attributable to the utilization or binding of calcium following histamine interaction in calcium-free buffer in the airways of ovalbumin-sensitized guinea pigs. Hedman and Andersson, assaying lungs from sensitized guinea pigs, reported a small but statistically significant difference in ^{45}Ca microsomal binding compared to that in control animals [129]. However, Creese and Bach [66] viewed 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 revealing leukotriene-induced hyperresponsiveness to other bronchoconstrictor agents required low (0.1 mM) extracellular calcium conditions for analysis.

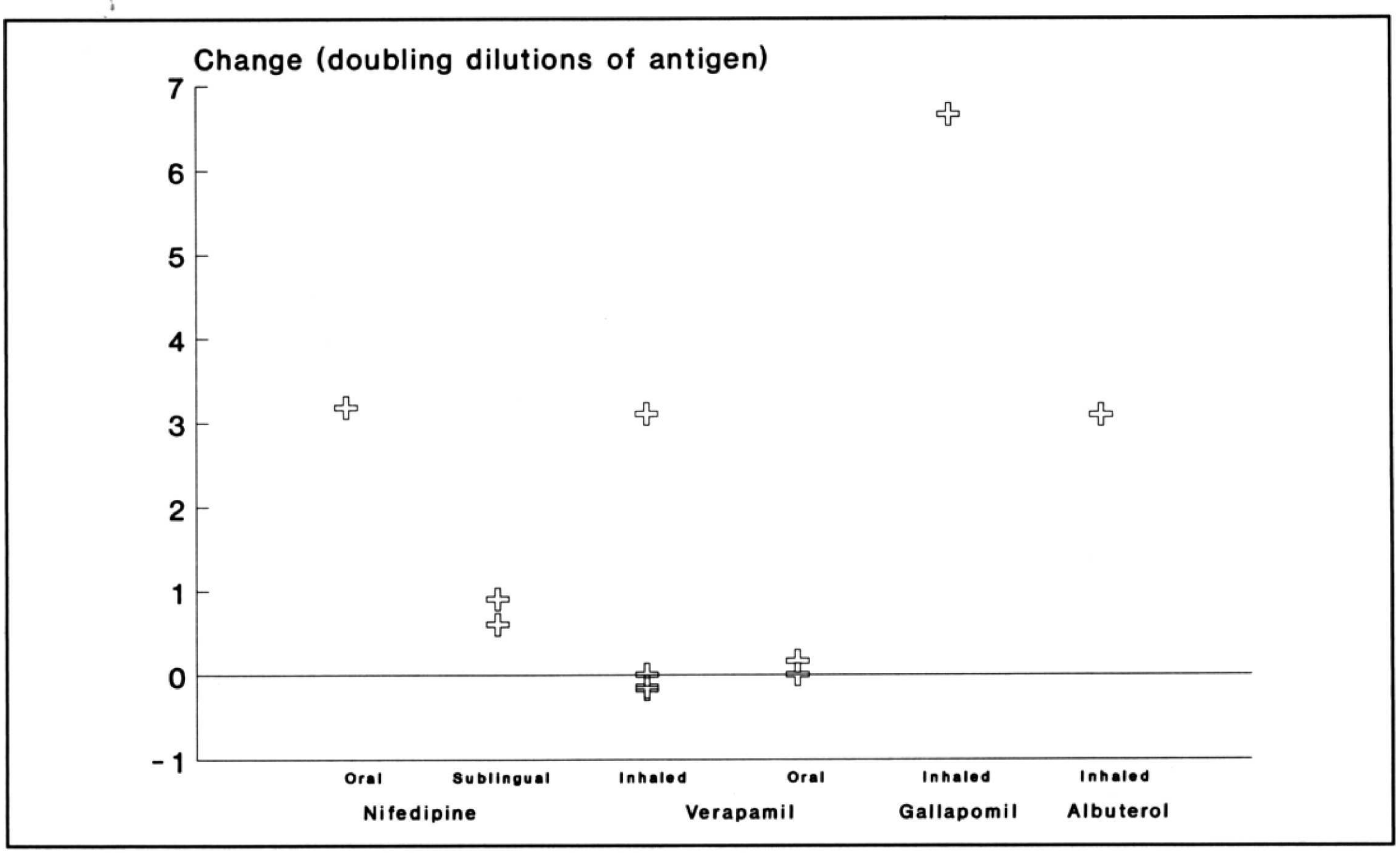
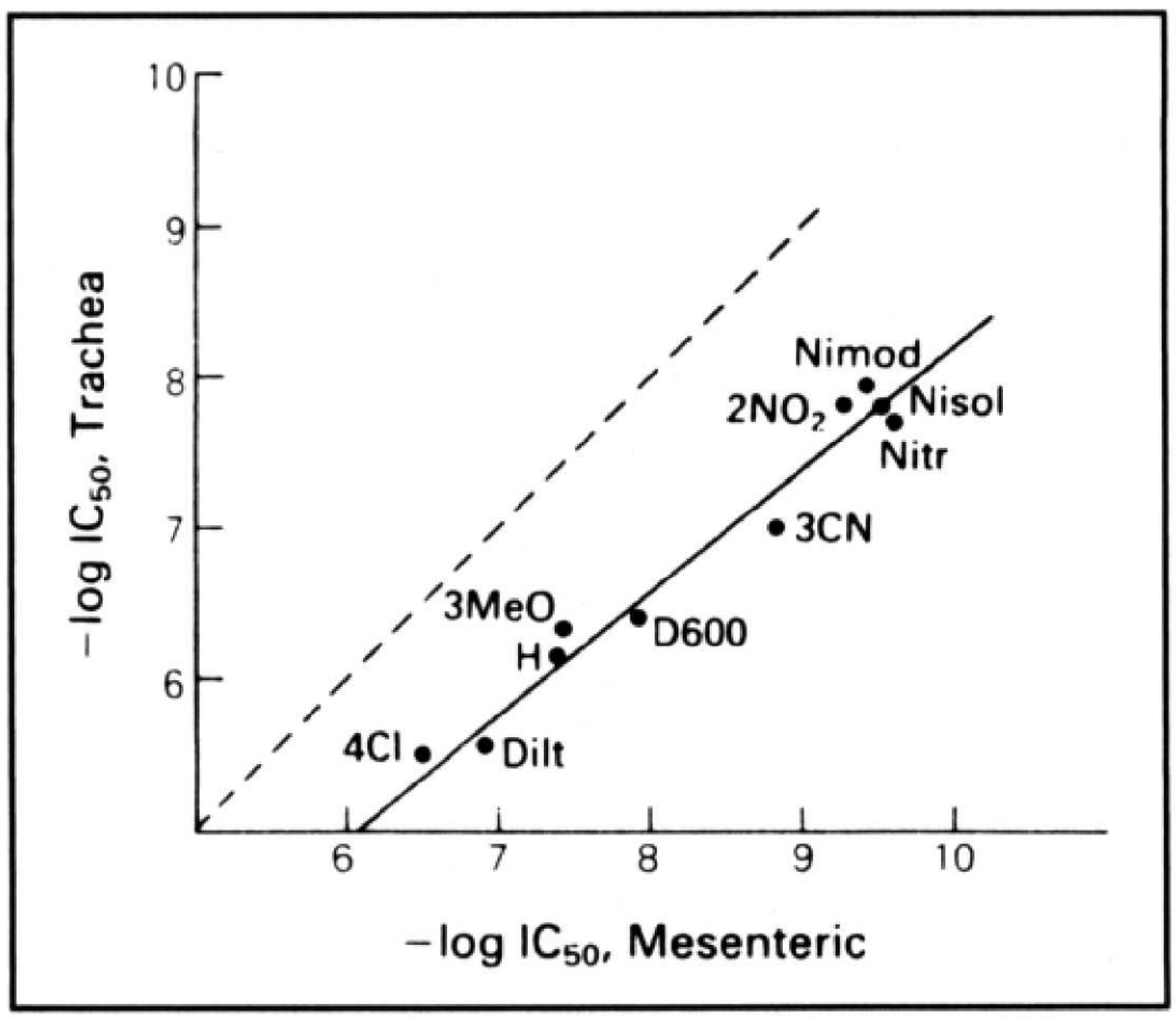


Fig. 71-10. *The effect of calcium channel blockers on antigen-induced bronchospasm. Each cross represents the results of a single published study. The geometric mean change in the dose-effect curve (expressed as the number of doubling dilutions of antigen) is shown following each treatment compared to placebo. The results from published studies conducted with albuterol are included for comparison.*

Fig. 71-11. *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 analogs (*$2NO_2$ = *nifedipine). (Reprinted with permission from D. S. Triggle. Calcium ions and respiratory smooth muscle function.* Br. J. Clin. Pharmacol. *20:2185, 1985.)*



Souhrada and Souhrada [288, 290] reported that sensitization alone alters the electrophysiologic properties of animal airways. Utilizing guinea pig trachealis cells, it was observed that ovalbumin sensitization induced membrane hyperpolarization, an effect demonstrable in vivo and in vitro [285, 289]. While it is possible that agents present in the serum of sensitized animals might produce such changes in membrane potential, these ovalbumin-sensitized trachealis muscles were shown to be hyperresponsive to histamine [286, 287]. It is proposed that an increase in membrane sodium permeability to causative antibodies results in an increase in intracellular sodium; a secondary rise of $[Ca^{++}]_i$ could then explain the above-cited airway smooth muscle response to agonists. In a similar observation, Black and colleagues [33] found that passive sensitization alone with serum derived from *Dermatophagoides farinae* allergen in human bronchial tissue in vitro altered the contractile responses to histamine and concurrently increased the involvement of the calcium voltage-dependent channel (VDC) when exposed to potassium chloride, suggesting an altered calcium mobilization in airway smooth muscle. BAY K8644, a voltage-dependent channel entry agonist, was employed by Raeburn and associates [236] to similarly show an alteration in calcium homeostasis in human bronchial muscle following passive sensitization with antibodies of the house dust mite, *Dermatophagoides pteronyssimus*. A functional difference in the sensitivity to responses mediated by an influx of calcium through VDCs of normal and sensitized bronchial smooth muscles was proposed, with sensitized airways more dependent on calcium entry. The existence of voltage-dependent calcium channels in human bronchial smooth muscles, as determined by the whole-cell patch clamp technique, has been verified by Marthan

and colleagues [182]. Small and Foster [278] reviewed the electrophysiologic features of normal and sensitized airway smooth muscle. Stephens and coworkers [295] focused on the role of the mechanical properties of airway smooth muscle in relation to hyperreactivity. In a recent report, an increased shortening capacity of allergic canine tracheal smooth muscle was described. The mechanism for this augmented shortening was speculated to possibly reside in a cytoskeletal protein [295]. The interested reader is directed to Stephens's chapter (Chap. 25) in this textbook for additional information.

Perpina and coworkers [229] investigated the effect of verapamil on contractions of guinea pig lung parenchymal strips to a variety of agonists in unsensitized and sensitized animals. The inhibition of $CaCl_2$-, potassium chloride-, acetylcholine-, and histamine-induced contractions by verapamil in sensitized tissues was considered consistent with an increased calcium influx related to airway hyperreactivity [229]. However, other studies have failed to reveal hyperresponsiveness or only minimal changes employing in vitro models [46, 179, 318, 331].

Animal data in vivo or studies in asthmatic patients analyzing alterations in calcium handling in relation to airway hyperreactivity are limited. Gugger and associates [120] examined ionized plasma calcium concentrations in 12 patients with exercise-induced asthma (EIA) and 20 other asthmatic patients without EIA and compared these with 42 healthy subjects: Plasma ionized calcium concentrations in 12 EIA patients averaged 1.16 ± 0.01 (standard error) mmol/L ($p < .001$) and in 20 asthmatics without EIA, 1.16 ± 0.01 mmol/L ($p < .001$), compared with 1.24 ± 0.01 mmol/L in 42 normal nonexercise controls and 1.20 ± 0.02 mmol/L in 7 normal exercise controls. As shown, there was a small but statistically significant decrease in mean plasma ionized calcium in the asthma subjects, this taking into account changes in inorganic magnesium and phosphorus concentrations, pH, and plasma volumes. However, while a causal relationship between ionized calcium concentrations and bronchial reactivity is possible from these data, as the authors stressed, the relationship is not a simple one and other factors might be involved or operative [120]. Another study utilizing basophils of asthmatic patients revealed the inhibition of calcium ionophore–induced histamine release by nifedipine to be less than observed with normal controls, suggestive of a defective cellular regulation of calcium in asthma [27]. Finally, Downes and Hirshman demonstrated that aerosols of calcium-chelating agents in Basenji dogs increase airway responsiveness to methacholine [88].

At present, the hypothesis of an abnormality in calcium homeostasis contributing to airway smooth muscle hyperresponsiveness in asthma is unresolved. However, there is preliminary information to suggest an increased responsiveness of airway smooth muscle to some defect in calcium control processes following a disturbance in environmental conditions such as in vitro anaphylaxis, chelation, plasma ionized calcium concentrations, or passive sensitization of airway smooth muscle. For a variety of reasons, the limited clinical efficacy of calcium channel inhibitory drugs may have no necessary direct relationship to a fundamental role of calcium disturbance in airway reactivity. Additional studies of this subject are clearly indicated.

OXYRADICALS AND ASTHMA

While oxygen is essential for aerobic life, its activated intermediates are cytotoxic and may be involved in the pathogenesis of a variety of diseases. Oxygen toxic intermediates are free radicals defined as any atom or group of atoms containing one or more unpaired electrons; the term *free* is considered by some as unnecessary. The sequential univalent reduction of molecular oxygen (O_2) yields superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2; by definition not a free radical), and the hydroxyl radical ($OH\cdot$):

$$O_2 \xrightarrow{e^-} O_2^{\cdot-} \xrightarrow{e^- + 2H^+} H_2O_2 \xrightarrow{e^- + H^+} OH\cdot \xrightarrow{e^- + H^+} H_2O$$

$$H_2O_2 \rightarrow H_2O$$

Overall,

$$O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O$$

Superoxide anion radical is very unstable, spontaneously dismutating to yield O_2 and H_2O_2 by the reaction: $2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$. While superoxide is cytotoxic, its low reactivity suggests that additional biologic effects reside with more reactive, derived metabolites. Superoxide and hydrogen peroxide can yield the more potent and toxic hydroxyl free radical ($OH\cdot$) in the presence of certain hemoproteins (hemoglobin, transferrin), metal chelates, or transition metals in the iron catalyzed Harber-Weiss or $O_2^{\cdot-}$-driven Fenton reaction:

$$O_2^{\cdot-} + Fe^{+++} \rightarrow O_2 + Fe^{++}$$

$$H_2O_2 + Fe^{++} \rightarrow HO\cdot + OH^- + Fe^{+++}$$

$$\text{Net:} \quad O_2^{\cdot-} + H_2O_2 \rightarrow HO\cdot + OH^- + O_2$$

Other reactive oxygen species include singlet oxygen and lipid peroxides. Oxyradicals may also be associated with the metabolism of the arachidonic prostaglandin-thromboxane and lipoxygenase pathways, and superoxide production via cytochrome P_{450} oxygenase metabolism of arachidonate [165, 334]. Chemically or metabolically generated, such radicals exert oxidative stress or biochemical effects in a wide variety of tissues and biologic molecules. For example, free radical effects on proteins result in oxidation of sulfhydryl bound enzymes; on lipids, with peroxidation of membrane fatty acids altering membrane fluidity and permeability; on nuclear DNA, leading to DNA strand modification and even result in cell death (Fig. 71-12).

Biologically active oxyradicals are characterized by a rather brief existence (microseconds), a small radius of toxic activity (30 Å intracellularly), and a low concentration (100 μM–1 nM); hence difficulties may exist in their detection and in clarifying their relationship to disease processes. All mammalian cells possess defense mechanisms to protect against the potentially damaging action of these reactive oxygen species, and a homeostatic balance exists between generation and inactivation processes. The latter antioxidant mechanisms limit or prevent oxidative injury from oxygen reactive products generated during normal metabolic or abnormal pathologic activities. Oxidative damage may ensue where there is either a relative oxidant excess (e.g., hyperoxia, radiation) and/or an insufficient antioxidant capacity. Some of the major protective mechanisms of oxidative stress, whether enzymatic or chemically reactive, include a variety of intracellular and extracellular oxyradical scavengers. Intracellular enzymatic defenses, for example, include catalase ($2\,H_2O_2 \rightarrow 2H_2O + O_2$), superoxide dismutase (SOD) $2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$), and glutathione peroxidases ($H_2O_2 + 2GSH \rightarrow 2H_2O +$ GSSG, where GSH is reduced glutathione and GSSG is oxidized glutathione). Nonenzymatic antioxidants include vitamin E (cell membrane and extracellular fluids), ascorbic acid (extracellular and intracellular), ceruloplasmin, beta-carotene (membranes of certain tissues), uric acid, and glutathione (-SH compounds). The reader is referred to the text by Halliwell and Gutteridge for a recent detailed review of this subject [123]. The text of Greenwald provides an excellent resource for methodology in oxygen radical research [119]. Doelman and Bast reviewed the subject of oxyradicals in lung diseases [84]; Barnes summarized current information concerning airway inflammation and reactive oxygen species [21].

Sufficient data exist to incriminate reactive oxygen metabolites in some human diseases. In asthma, a significant source of oxy-

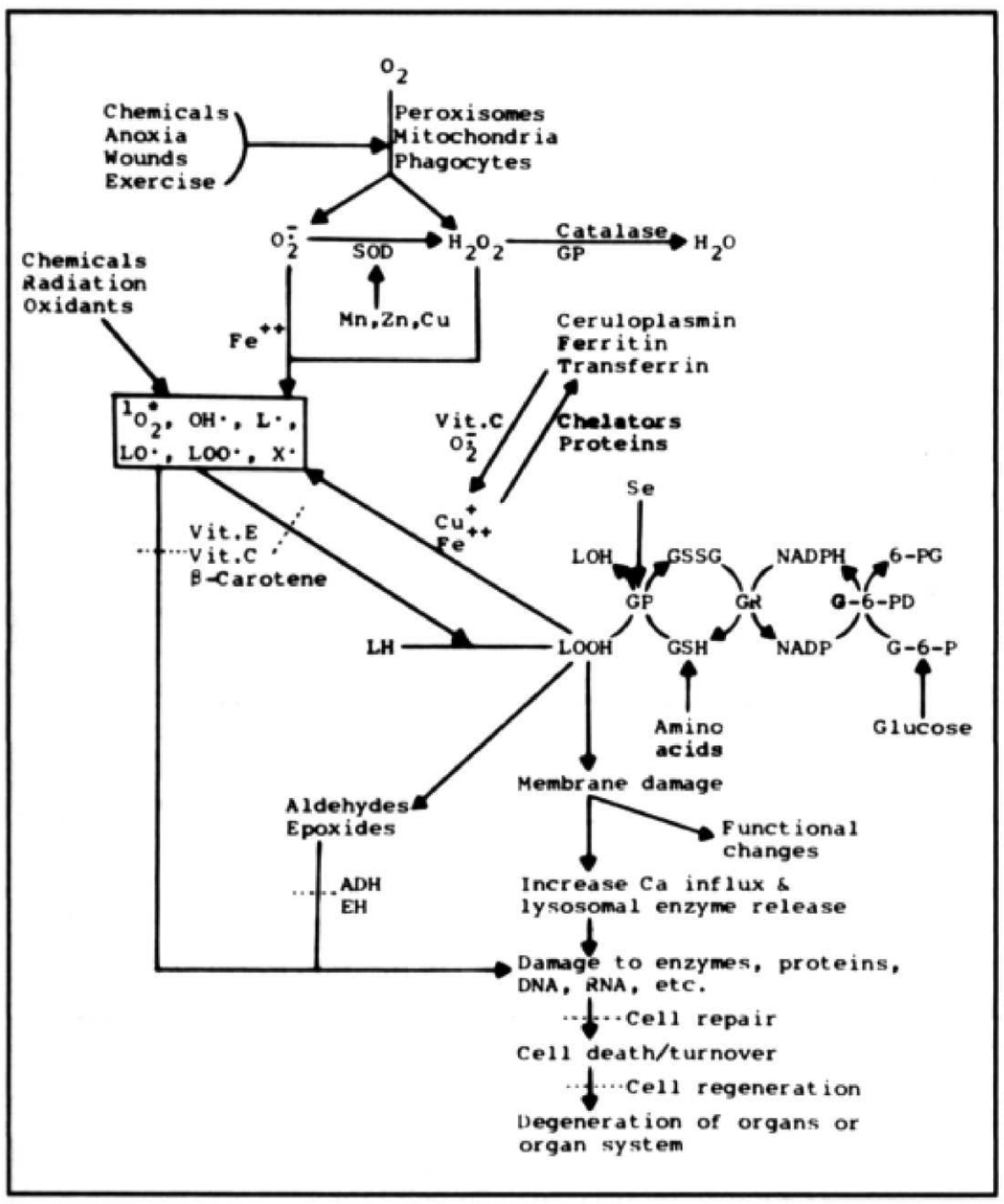


Fig. 71-12. *Possible scheme of free radical–induced lipid peroxidation tissue damage and antioxidant defense.* LH = *membrane or polyunsaturated lipids;* LOOH = *lipid hydroperoxides;* LOH = *hydroxy acid;* LOO· = *peroxyl radical;* L· = *alkyl radical;* LO· = *alkoxy radical;* OH· = *hydroxyl radical;* O_2^- = *superoxide radical;* X· = *other free radicals;* $^1O_2$ = *singlet oxygen;* H_2O_2 = *hydrogen peroxide;* SOD = *superoxide dismutase;* GSH = *reduced glutathione;* GSSG = *oxidized glutathione;* GP = *GSH peroxidase or phospholipid hydroperoxide GSH peroxidase;* GR = *GSSG reductase;* G-6-PD = *glucose 6-phosphate dehydrogenase;* 6-PG = *6-phosphogluconate;* ADH = *aldehyde dehydrogenase/oxidase;* EH = *epoxide hydrolase;* vit. = *vitamin;* NADPH *or* NADP = *reduced or oxidized nicotinamide adenine dinucleotide phosphate. The dotted lines denote interruption of process or event. (Reprinted with permission from C. K. Chow. Vitamin E and oxidative stress.* Free Radic. Biol. Med. *11:215, 1991.)*

radicals are the resident and migratory cells associated with the inflammatory processes of the airways, including neutrophils, eosinophils, monocytes, mast cells, and alveolar macrophages. The increased activity of the 5-lipoxygenase pathway and the increased complement receptor expression suggest that neutrophils from asthmatics are activated [12, 197]. Not only do activated neutrophils and eosinophils release superoxide anion but also its production and release may be greater in the atopic state [299]. Release of O_2^- by activated peritoneal mast cells has also been described [164]. When confronted with an invasive microorganism, polymorphonuclear cells and macrophages become activated and utilize large amounts of oxygen (the "respiratory burst") to generate cytotoxic superoxide anion, which then is converted to other species as H_2O_2, OH·, and singlet oxygen. This conversion is catalyzed at the external cell membrane and in phagocytic vacuoles via bound NADPH-reduced nicotinamide-adenine dinucleotide phosphate. Potent bactericidal hypochlorous acid (HOCl) is subsequently generated from H_2O_2 and various halides by cellular myeloperoxidase. Cellular activation of cytotoxic oxyradicals resulting from viruses or opsonized bacteria as well as immune complexes, immunoglobulins, and chemotactic peptides has been reported to directly damage lung structures, stimulate histamine release [178], activate the arachidonic acid cascade or platelet-activating factor (PAF) biosynthesis [169], induce smooth muscle contraction [23], and increase vascular permeability or produce secondary bronchoconstricting mediators following lipid peroxidation [80, 81].

Studies utilizing peripheral blood or bronchoalveolar lavage (BAL) cells from asthmatic patients have revealed oxyradical formation and additionally some correlation to airway hyperreactivity. In a 1978 study [135], immune and nonimmune stimulation of human lung mast cells resulted in a parallel release of histamine and superoxide anion. More recently, alveolar macrophages recovered by BAL and assayed by luminol-enhanced chemiluminescence were found to be activated, with the amount of oxyradical release correlating with asthma severity [58, 151]. Calhoun and colleagues reported a greater alveolar macrophage release of superoxide anion from patients with symptomatic asthma contrasted to normal volunteers [43]. Comparing peripheral blood neutrophil O_2^- production by *N*-formyl-methionyl-leucyl-phenylanine (FMLP) with asthma severity, as indexed by methacholine PD_{20}, a significant inverse association was observed [190]. A correlation between the extent of airway hyperresponsiveness and stimulated neutrophil production of H_2O_2 was observed in asthmatic children [75]. In asthmatic adults a similar correlation was observed between neutrophilic superoxide anion release and reduction in expiratory airflow rates; moreover, patients with either an asthma exacerbation or a greater duration of disease exhibited greater changes in FEV_1 reduction and O_2^- generation [149] (Fig. 71-13). In another study blood leukocyte O_2^- generation was augmented in asthmatic children [205], and was associated with a significant parallel histamine release to the calcium ionophore A23187 and to inhaled histamine bronchoprovocation. Hypodense eosinophils, found in increased numbers in asthma, exhibit augmented inflammatory potential and correlate with airway obstructive severity. Such cells, isolated from the peripheral blood of seven asthmatics, exhibited a small release of O_2^- when activated by FMLP or opsonized zymosan; however, eosinophilic heterogeneity was observed dependent on a variety of factors such as cell source and stimulus [264]. Eosinophils from asthmatic subjects appear to be more responsive to PAF release of superoxide anion than are neutrophils, a difference that might contribute to a relationship between PAF and asthma [335]. Furthermore, this eosinophilic release of O_2^- is dependent on both transmembrane influx as well as intracellular mobilization of calcium. In patients with chronic obstructive pulmonary disease (COPD), a significant correlation exists between nonspecific airway hyperresponses to aerosol histamine and polymorphonuclear leukocyte O_2^- production [234]. In contrast to the above-cited observations, Chilvers and coworkers were unable to demonstrate circulating products of oxygen-derived free radicals in the peripheral blood of patients with acute severe asthma [54]. It should be emphasized that the observations of airway inflammation, enhanced cellular release of oxyradicals, and associated airway obstruction or airway responses to bronchoactive agents in asthmatic patients do not currently provide direct evidence of a causal relationship or explain the mechanism(s) of oxyradical-associated bronchial hyperresponsiveness.

A number of reports on whole-animal studies or in vitro conditions revealed activation and association of oxyradicals with airway responses. For example, H_2O_2 contracts bovine trachealis or canine parenchymal lung strips [296], while in guinea pig tracheal strips, similar contractile responses to H_2O_2 are augmented by removal of the contiguous epithelium [23, 244] (Fig. 71-14); the existence of an epithelial relaxant factor or the removal of antioxidants affecting the H_2O_2 contraction is suggested by the latter finding. In rat lung parenchymal strip models, H_2O_2 causes an initial contractile response followed by a gradual return to resting isometric tension baseline [161]. Concurrently, in rat tracheal

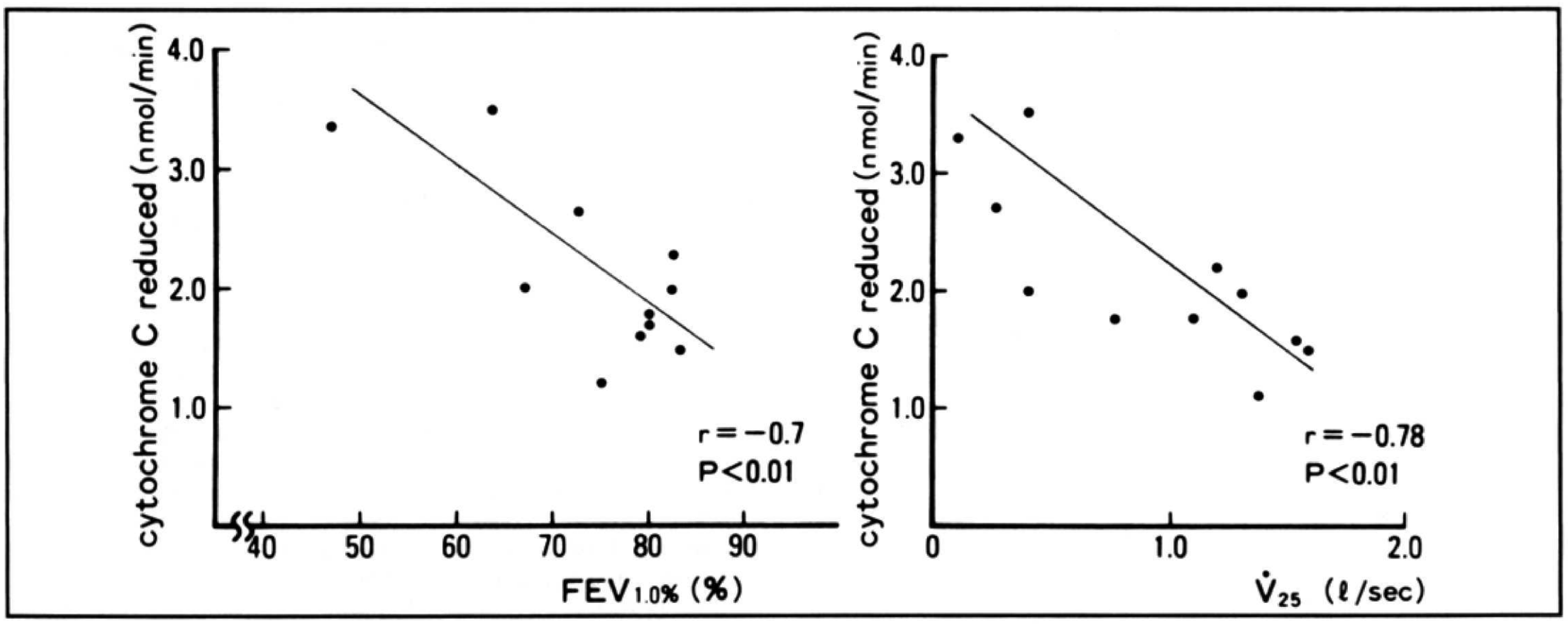


Fig. 71-13. *Correlation between pulmonary function test results and superoxide anion production (ordinate) after stimulation with phorbol myristate acetate. (Reprinted with permission from H. Kanazawa, et al. Role of free radicals in asthmatic patients.* Chest *100:1319, 1991.)*

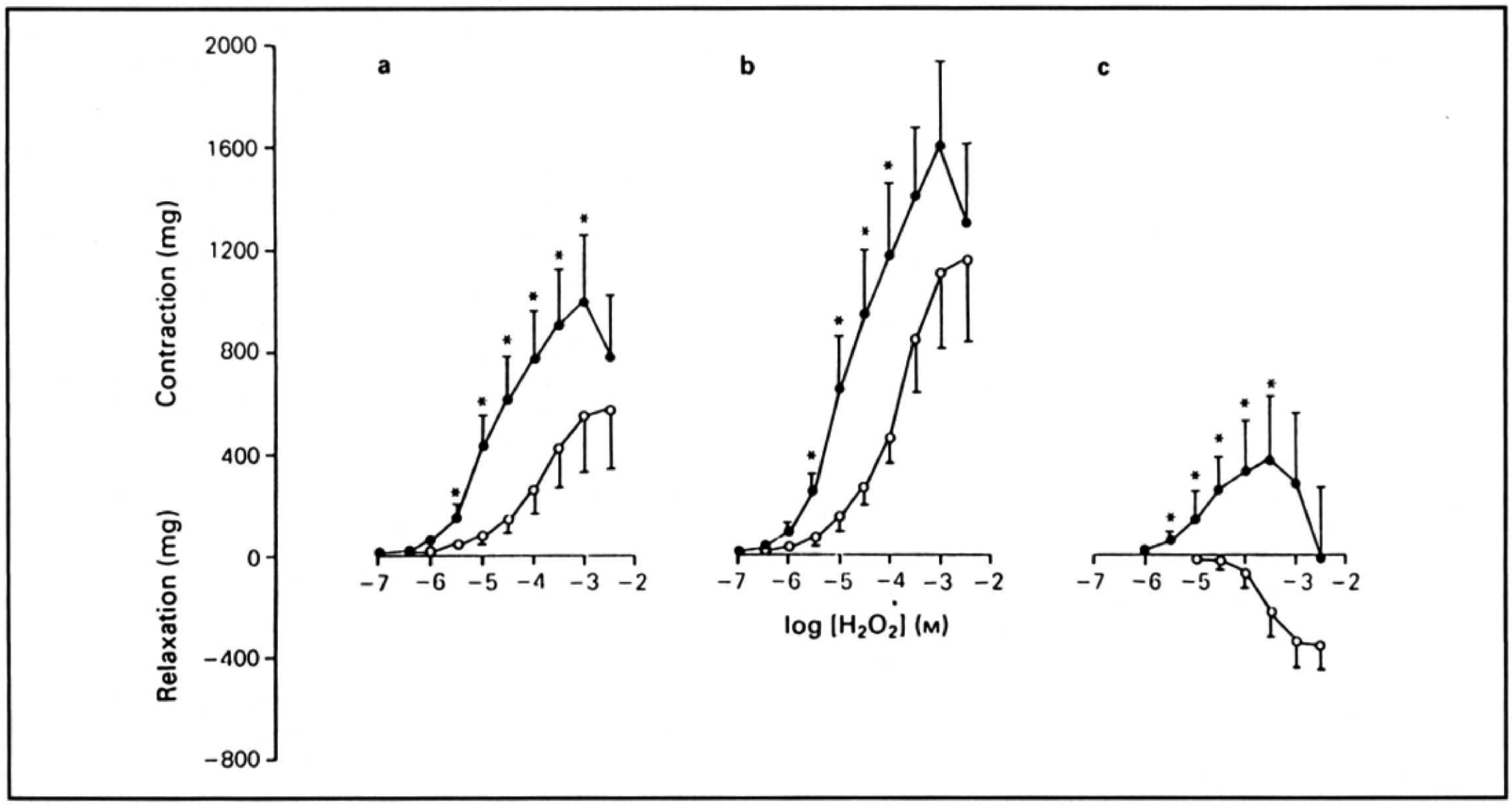


Fig. 71-14. *Effect of hydrogen peroxide (H_2O_2) on guinea pig trachea in the presence (○) and absence (●) of epithelium. a. Mean values of 17 preparations are shown. b. The 10 of 17 preparations, where contraction was observed. c. The 5 of 17 preparations where a relaxation response was observed in the intact preparation. (Reprinted with permission from K. J. Rhoden and P. J. Barnes. Effect of hydrogen peroxide on guinea pig tracheal smooth muscle.* Br. J. Pharmacol. *98:325, 1990.)*

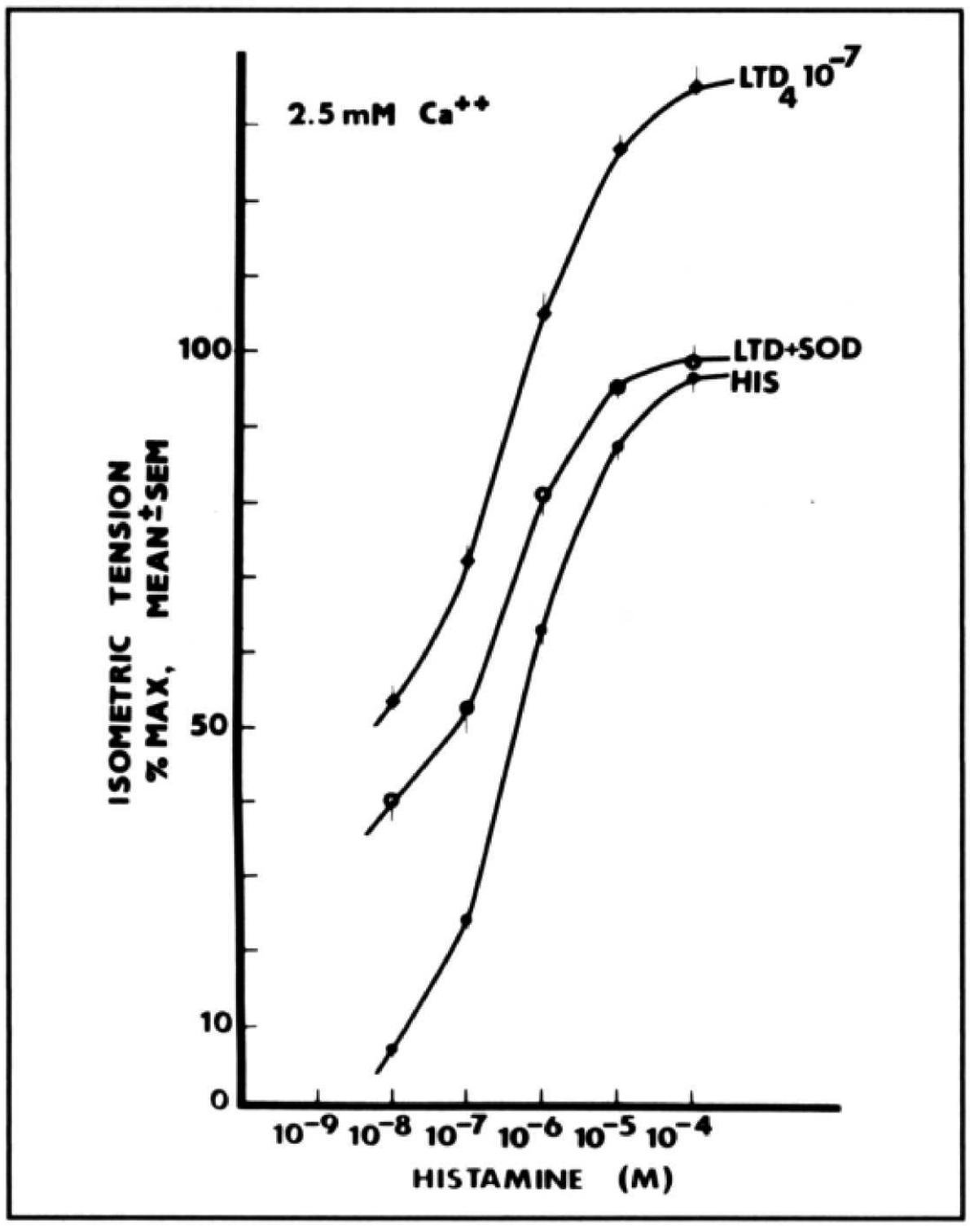
strips, 1 mM H_2O_2 depressed methacholine tension responses by 39 percent (maximal effect) without shifting the PD_2, indicative of an oxidant effect on muscarinic receptors [84]. In other reports cyclooxygenase inhibition by indomethacin of contractile responses induced by low-concentration H_2O_2 in guinea pig trachealis implied activation of bronchoconstricting cyclooxygenase products such as prostaglandin $F_{2\alpha}$ or thromboxane; deepithelialized preparations exposed to H_2O_2 exhibited myorelaxation ascribed to direct or indirect factors [21, 296]. Since direct in vivo measurement of superoxide anion in tissue can be complex (e.g., electron spin resonance [ESR] spin-trapping), specific enzymes as SOD are often employed to evaluate the presence and effect of $O_2^{\cdot-}$. For example, a direct bronchoconstrictor action of the xanthine/xanthine oxidase superoxide generation system was shown as an increase in pulmonary resistance in a feline model; this effect was significantly inhibited by pretreat-

ment with SOD or catalase [150]. Finally, while a direct biphasic contraction induced by superoxide radical in guinea pig trachea has been described [206], others have not observed such O_2^- contractile responses [21].

Actual concentrations of oxidants at tissue sites that may affect smooth muscle are not precisely known. In the catalytic reduction of xanthine (1 mM) to uric acid by xanthine oxidase (0.01 U/ml), superoxide anion is generated at a rate of ~5 μM/L/min. Human blood neutrophils (10^6 cells) are reported to release $\sim 10^{-5}$ M H_2O_2 over 2 hours and 14.8 nM O_2^- over 5 minutes [190, 307]. In guinea pig trachealis muscle, superoxide anion formation was found to reach a maximum of 15.1 nM over 60 minutes following 10^{-6} M leukotriene D_4 and ~4.85 nM over 30 minutes during in vitro anaphylaxis [324, 326].

Several studies have examined the effect of oxyradicals during immunogenic activation and their relationship to airway reactivity. A direct role for superoxide anion was shown by Weiss where a histamine- and leukotriene D_4–associated trachealis muscle hyperreactivity was induced by superoxide anion; in this model a leftward shift of the median effective concentration (EC_{50}) and an absolute increase in maximal isometric tension were reversed by the O_2^- scavenger SOD (Fig. 71-15). The observation proposes a defect in antioxidant protective mechanisms and/or a direct role for O_2^- in causing airway hyperreactivity [324]. Generation of O_2^- during experimental in vitro immunogenic anaphylaxis has been demonstrated, providing a basis for leukotriene D_4 and O_2^- generation, release, and interaction in airway smooth muscle

Fig. 71-15. *Interaction of histamine with leukotriene D_4* (LTD$_4$) *(10^{-7} M) in 2.5 mM $(Ca^{++})_E$ and the effect of pretreatment with superoxide dismutase* (SOD): *SOD (300 U/ml) inhibited the leukotriene D_4–induced histamine hyperresponse. Both maximal isometric tension and EC_{50} returned to control levels following SOD. (Reprinted with permission from E. B. Weiss. Leukotriene associated toxic oxygen metabolites induce airway hyperreactivity.* Chest *89:709, 1986.)*



under these conditions [326]. In addition, lipoxygenase product augmentation of histamine responses in human bronchi has been reported [64]. As discussed previously, Katsumata and associates demonstrated direct bronchoconstriction in feline airways in vivo to aerosol xanthine/xanthine oxidase [150]. In this study, concurrent hyperresponsiveness to aerosolized acetylcholine was observed, and this effect was inhibited by vagotomy; under conditions of airway aerosol deposition of a superoxide anion–generating system, these observed bronchoconstrictive and bronchohyperresponsive effects may largely reflect vagally mediated, and not direct myogenic, responses. Airway hyperresponses may be additionally augmented by oxyradical activation of the arachidonic acid cascade, PAF release, and endogenous release of histamine [169, 178]. Other putative mediators or inflammatory cells may be associated with a free radical–asthma process, for example, eosinophil-generated oxyradical epithelial damage by PAF in asthmatics [48], damage to respiratory epithelium from the H_2O_2–halide ion–eosinophil peroxidase system [14], stimulation of PAF biosynthesis by H_2O_2 as demonstrated in bovine endothelial cells [169], H_2O_2 epithelial injury in rat trachea [86], augmented mucus secretion in cultured epithelial cells in the form of high-molecular-weight glycoconjugates in response to superoxide [2], augmented mucus secretion in sheep trachea following ozone exposure [232], and microvascular endothelial disruption injury and increased permeability [81, 144, 304]. In view of the current interest of the potential role of the respiratory epithelium to asthmatic reactivity, some of the above-cited observations appear to implicate interactive effects of the airway structures, beyond the airway smooth muscle itself. Finally, a recent study in ragweed-sensitive patients with allergic rhinitis but without a clinical history of asthma employed BAL and ragweed segmental bronchoprovocation to evaluate superoxide anion production and cellular characteristics following antigen challenge [43a]. The data were consistent with a relatively acellular initial response with activation of alveolar macrophages immediately following ragweed challenge and a delayed (48 hr) appearance of high density alveolar macrophages, which have potentiated superoxide anion release in the late-phase airway response.

Oxygen reactive molecules may react with and may alter cell membrane constituents including beta-adrenoceptor sulfhydryl/disulfide bonds. For example, following exposure to oxyradical-generating systems, beta-adrenergic receptor density in rat lung is decreased, both in vivo and in vitro [161]. In another model the amount of O_2^- formed by alveolar macrophages exhibited a high positive correlation with the extent of deterioration of beta-adrenoceptor function in guinea pig trachea [173]. In addition, exposure of rat trachea to H_2O_2 results in a loss of beta-receptor sensitivity to isoproterenol, an effect enhanced by the dietary depletion of antioxidant vitamin E or selenium (reduction of glutathione peroxidase) [84, 85]. In another study, combining isolated tracheal muscle strips and isolated alveolar macrophages, Engels and associates were able to demonstrate hydroxyl radical generation that presumably attenuated tracheal beta-adrenoreceptor function; both catalase and thiouran (hydroxyl scavenger) inhibited this effect [94]. However, Rhodan and coworkers were unable to establish an effect on muscarinic receptors to lipid peroxidation with 4-hydroxy-2,3-transnonenal at concentrations (100 μM) that did reduce beta-adrenoreceptor activity [244]. Doelman and Bast also described a differential sensitivity to oxidative stress preferentially affecting the beta-adrenergic receptor as compared to the muscarinic receptor response [84]. Hence, within the environment of the asthmatic airway, phagocytic/inflammatory cells or free polyunsaturated fatty acids may, via generation of reactive oxygen products, contribute to beta-adrenergic dysfunction presumably by lipid peroxidation or receptor sulfhydryl interaction [162].

Beyond the above-cited factors dealing with the possible inter-

relationship of oxidative injury in asthma, a small number of observations have involved the role of antioxidants in asthma or asthma models. Two studies reported a reduced selenium concentration in the whole blood of asthmatics [101, 297], trace element selenium being a cofactor for the antioxidant enzyme glutathione peroxidase. The activity of the enzyme glutathione peroxidase, which is cytoprotective of oxyradical injury and involved in arachidonic acid metabolic regulation, is reported to be reduced in whole blood and platelets of asthmatic patients, including those with food and aspirin intolerance [128, 177]. In contrast, Jadot and colleagues observed elevated erythrocytic plasma concentrations of copper-containing and manganese SODs and glutathione peroxidase in all of 29 patients experiencing an asthma exacerbation [141]. Not directly related to asthma, the mucolytic *N*-acetylcysteine (a glutathione precursor) exhibits some antioxidant properties by its reaction with hypochlorous acid and hydroxyl radicals in dogs exposed to hyperoxic pulmonary stress [319]. A protective action by SOD on free radical–mediated pulmonary vascular permeability is described in a dog model [214]. In the absence of an efficient O_2^- scavenger, the ascorbate system may, as a component of its action, act as a sink for superoxide, and some human and animal studies of experimental asthma cited a beneficial role for ascorbic acid [209, 262]. In addition, infection-associated or infection-induced airway hyperreactivity may also be ameliorated by ascorbate [130]. Alveolar macrophages that release oxidants are also rich in antioxidants [137]; effective scavenging can limit H_2O_2-mediated lung injury [187]. The relationship of a low selenium soil content to a dietary deficiency culpable in an increased relative risk of asthma, as noted in New Zealand, must be currently considered conjectural [101]. While one report observed a decreased alpha-tocopherol activity in asthmatic patients, the effects of vitamin E, which inhibits lipid peroxidation, and beta-carotene, a scavenger, have not been sufficiently evaluated in asthma [9]. Of clinical interest, unrelated to a scavenger role, is the inhibition of superoxide radical generation from guinea pig, rabbit, and human neutrophils by azelastine [42].

Studies examining the effect of oxyradicals on calcium homeostasis in asthma are limited. As discussed, the cytosolic concentration of calcium must be maintained at a low level and within tolerable physiologic limits; concurrently oxyradicals can injure cell membranes and other structures via lipid peroxidation, and overwhelm or injure tightly controlled intracellular regulatory processes [96, 104]. In one paradigm employing a paired experimental sequence, an enhanced (initial rate and maximal reduction) relaxation of resting isometric tension in guinea pig trachealis was observed in muscles previously immunogenically activated and then exposed to ~0 mM extracellular calcium [325]. Inhibition of the late phase of ovalbumin-induced anaphylaxis by FPL 55712 (10 μM) eliminated this enhanced postanaphylactic relaxation following low calcium exposure. Other tracheal muscles, exposed to synthetic leukotriene C_4, exhibited the same enhanced relaxation in a low extracellular calcium environment. Pretreatment with SOD or isocapnic hypoxia (P_{O_2} 10 ± 4 torr) abolished the postanaphylactic and leukotriene C_4–augmented relaxation. Although exposure to low extracellular calcium concentrations may transiently increase cytosolic $[Ca^{++}]$, sequential immersion of control muscles (nonimmune activated) in ~0 mM extracellular calcium did not exhibit any altered effect [302]. It was proposed that an alteration in calcium homeostasis developed following anaphylaxis (or LTD_4 exposure) which affected resting airway isometric muscle tone [325]. Lansing and coworkers [165a] have subsequently demonstrated oxygen-radical induction (employing aerosolized xanthine/xanthine oxidase) of reversible airflow obstruction and hyperresponsiveness to carbachol in a sheep model. An interrelationship between oxyradicals and secondary generation of lipid mediators was proposed to contribute to the free oxygen radical–induced bronchoconstriction and airway hyperreactivity. Hence, while it is reported that oxidants may induce cellular influx and oxidized fatty acids may exhibit calcium ionophore effects, further studies are needed to delineate the relationships between oxidative stress and calcium in cellular injury or in calcium homeostasis in asthma [169, 266].

OTHER CONSIDERATIONS

Sodium and Na^+-K^+-ATPase

A few studies have related the dietary intake of sodium to alterations in bronchial responses to histamine [40, 143]. Other reports indicated a reduced activity of the sodium-potassium–stimulated ATPase in platelet membranes of allergic patients including those with asthma [273]. A circulating Na^+-K^+-ATPase inhibitor responsible for a transport enzyme defect in platelets of allergic patients could affect intracellular cation concentrations [274].

Magnesium

Intravenous magnesium sulfate has been proposed for treatment in patients with severe, intractable asthma (see Chap. 73) [272]. In asthmatic patients, protection against methacholine- or histamine-induced bronchoprovocation by magnesium has been observed [253]. Hypomagnesemia in acute asthma is uncommon and may result from limited gastrointestinal intake or losses (nasogastric suction, diarrhea), use of corticosteroids or diuretics, or other factors. Magnesium, a major intracellular cation, is involved in a variety of processes including neuromuscular activity, phosphorylation reactions, membrane stability, and modulation of ionic calcium transients. While its action may be similar to that of calcium channel antagonists, the observation of a decrease in nerve terminal acetylcholine release suggests that its spasmolytic mechanism may be complex. Extracellular concentrations above 1.2 mM/L inhibit smooth muscle contraction. There is no evidence that magnesium activates the arachidonic acid cascade, but it may enhance prostacyclin release [323].

Potassium

The activity and mechanism of airway smooth muscle potassium channels, which are associated with hyperpolarization and relaxant processes, are presented in detail in Chapter 16 and recently reviewed in [337]. Blockade of K^+ channels could be a contributory mechanism in airway smooth muscle hyperresponsiveness and drugs that open K^+ channels represent a new class of myorelaxants with potential clinical application [7, 124]. Drugs that open potassium channels may cause relaxation by antagonism of intracellular ATP, which functions normally to keep these channels closed [241]. In a recent study, BRL 38227 (lemakalim), the active L-enantiomer of cromakalim, was shown to be an effective relaxant in human bronchi to agonist histamine, neurokinin A, carbachol, resting isometric tone, and contraction provoked by electrical field stimulation [32]. BRL 38227 also protected against morning dipping in asthmatic patients and inhibited histamine-induced bronchoconstriction in healthy volunteers [17, 211]. Interestingly, brain microsomal K^+ channels may be implicated in the regulation of $[Ca^{++}]_i$ and some properties of lemakalim may affect $[Ca^{++}]_i$ [267]. In guinea pig and bovine trachealis muscle, the action of cromakalim in opening K^+ channels was recently reported not to involve the intracellular accumulation of cyclic nucleotides [30]. From studies with human airways Miura and coworkers [197a] recently suggested that charbdotoxin (an inhibitor of smooth muscle large conductance calcium-activated potassium channels)-sensitive potassium channels participate in beta-adrenergic-agonist- and theophylline-induced

bronchodilation. Additional studies, both basic and clinical, are warranted to further define the mechanisms and role of K^+ channels and to determine what effect their antagonism has in asthma.

Calmodulin Antagonism

Trifluoperazine, a calmodulin antagonist, exhibits an inhibitory action to a variety of agonists (5-hydroxytryptamine, acetylcholine, histamine, potassium chloride, $CaCl_2$) in sensitized and nonsensitized guinea pig lung strips [261]. Inhibition of calmodulin-calcium binding with subsequent activation of myosin light-chain kinase may afford therapeutic potential.

General Anesthetics

Volatile anesthetics, notably halothane, isoflurane, and enflurane, affect airways by a variety of complex and possibly interrelated mechanisms, including direct smooth muscle relaxation, inhibition of bronchoreactive mediators, and central neural or airway neural reflex blockade (see Chap. 82). The subject was recently reviewed by Hirshman and Bergman [136]. Whatever the fundamental mechanism(s), the clinical use of inhalational anesthetics is generally limited to acute severe asthma when other conventional methods have failed (see Chap. 73). General anesthetics may inhibit oxidative metabolism and calcium mobilization. For example, halothane, enflurane, and isoflurane were shown to inhibit superoxide production by decreased mobilization of $[Ca^{++}]_i$ in human neutrophils stimulated by FMLP [204]. Two recent preliminary reports suggested halothane-mediated relaxant effects in airway smooth muscle to be partially due to a decreased mobilization of $[Ca^{++}]_i$ following cholinergic stimulation; a parallel rise in cytosolic AMP may influence this action [145, 333].

Local Anesthetics

Local anesthetic agents have been administered to asthmatic patients and examined in a variety of experimental conditions. Their action may be multifactorial involving a direct myogenic role, interruption of neural reflexes, and even inhibition of mast cell histamine release, albeit at rather high concentrations [327]. An asthmalytic effect has been observed in some patients but not in others, and aerosol lidocaine is often complicated by bronchospasm in asthmatics [87, 195]. It is suggested that a bronchodilator such as metaproterenol be added to topical lidocaine anesthetic solutions for use in patients with hyperreactive airways [156]. In neural structures, local anesthetics inhibit excitation by decreasing cell membrane permeability to sodium ions, thereby inhibiting cell membrane depolarization. However, in smooth muscle, the spasmolytic action of local anesthetics is not clarified, although some effect on cellular calcium flux or binding has been proposed [44, 97, 338].

REFERENCES

1. Adelstein, R. S., and Klee, C. B. Purification and characterization of smooth muscle myosin light chain kinase. *J. Biol. Chem.* 256:7501, 1981.
2. Adler, K. B., Holden-Stauffer, W. J., and Repine, J. E. Oxygen metabolites stimulate release of high-molecular-weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via an arachidonic acid-dependent mechanism. *J. Clin. Invest.* 85:75, 1990.
3. Ahmed, F., et al. Some features of the spasmogenic actions of acetylcholine and histamine in guinea-pig isolated trachea. *Br. J. Pharmacol.* 83: 227, 1984.
4. Ahmed, F., Foster, R. W., and Small, R. C. Some effects of nifedipine in guinea pig isolated trachealis. *Br. J. Pharmacol.* 84:861. 1985.
5. Ahmed, T., et al. Inhibition of antigen-induced bronchoconstriction by a new calcium antagonist, gallopamil: Comparison with cromolyn sodium. *J. Allergy Clin. Immunol.* 81:852, 1988.
6. Ahmed, T., et al. Comparative effects of oral and inhaled verapamil on antigen-induced bronchoconstriction. *Chest* 88:176, 1985.
7. Akasaka, K., et al. Electromyographic studies of bronchial smooth muscle in bronchial asthma. *Tohoku J. Exp. Med.* 117:55, 1975.
8. al-Waili, N. S. Nifedipine in corticosteroid-dependent asthma: Preliminary study. *Clin. Exp. Pharmacol. Physiol.* 16:715, 1989.
9. Amatuni, V. G., and Safarian, M. D. Metabolite aspects of the differential diagnosis of asthmatic bronchitis and bronchial asthma. *Ter. Arkh.* 58: 12, 1986.
10. Anavekar, A. N., et al. A double-blind comparison of verapamil and labetalol in hypertensive patients with co-existing chronic obstructive airways disease. *J. Cardiovasc. Pharmacol.* 3:S374, 1982.
11. Andersson, K. E. Airway hyperreactivity smooth muscle and calcium. *Eur. J. Respir. Dis.* 131(Suppl. 1):49, 1983.
12. Arm, J. P., Walport, J. J., and Loe, T. H. Expression of complement receptors type I (CR1) and type 3 (CR3) on circulating granulocytes in experimentally provoked asthma. *J. Allergy Clin. Immunol.* 83:649, 1989.
13. Axelrod, J., Burch, R. M., and Jelsema, C. L. Receptor mediated activation of phospholipase A_2 via GTP-binding proteins: Arachidonic acid and its metabolites as second messengers. *Trends Neurosci.* 11:117, 1988.
14. Ayars, G. H., et al. Injurious effect of the eosinophil peroxidase-halide system and major basic protein in human nasal epithelium in vitro. *Am. Rev. Respir. Dis.* 140:125, 1989.
15. Baba, K., et al. Effects of verapamil on the contractions of guinea-pig tracheal muscle induced by Ca, Sr and Ba. *Br. J. Pharmacol.* 84:203, 1985.
16. Baba, K., et al. Effects of verapamil on the response of the guinea-pig tracheal muscle to carbachol. *Br. J. Pharmacol.* 88:441, 1986.
17. Baird, A., et al. Cromakalim, a potassium channel activator inhibits histamine induced bronchoconstriction in healthy volunteers. *Br. J. Clin. Pharmacol.* 25:114, 1988.
18. Balfre, K. The effects of calcium and calcium ionophore A23187 on mucin secretion and potential difference in the isolated chicken trachea. *J. Physiol.* 275:80P, 1978.
19. Ballester, E., et al. Effect of nifedipine on arterial hypoxaemia occurring after methacholine challenge in asthma. *Thorax* 41:468, 1986.
20. Barnes, P. J. Neural control of human airways in health and disease. *Am. Rev. Respir. Dis.* 134:1289, 1986.
21. Barnes, P. J. Reactive oxygen species and airway inflammation. *Free Radic. Biol. Med.* 9:235, 1990.
22. Barnes, P. J., et al. A calcium antagonist, nifedipine, modifies exercise-induced asthma. *Thorax* 36:726, 1981.
23. Barnes, P. J., et al. The effect of oxygen derived free radicals on airway smooth muscle responses. *Br. J. Pharmacol.* 90(Suppl.):142, 1987.
24. Baron, C. B., et al. Pharmacomechanical coupling in smooth muscle may involve phosphatidylinositol metabolism. *Proc. Natl. Acad. Sci. USA* 81:6899, 1984.
25. Baronti, A., et al. Effects of acute nicardipine (YC93) on carbachol-induced bronchoconstriction. *Curr. Ther. Res.* 34:142, 1983.
26. Bean, B. P. Nitrendipine block of cardiac calcium channels: High affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. USA* 81:6388, 1984.
27. Bedard, R. M., and Busse, W. W. Inhibition of basophil histamine release by the calcium channel antagonist nifedipine in asthma. *Am. Rev. Respir. Dis.* 129:A9, 1984.
28. Ben-Dov, I., et al. Bronchodilatation and attenuation of exercise-induced bronchospasm by PY 108-068, a new calcium antagonist. *Am. Rev. Respir. Dis.* 133:116, 1986.
29. Benson, M. K. Bronchial hyperreactivity. *Br. J. Dis. Chest* 69:227, 1975.
30. Berry, J. L., et al. Mechanical, biochemical and electrophysiological studies of RP49356 and cromakalim in guinea pig and bovine trachealis muscle. *Pulmonol. Pharmacol.* 4:91, 1991.
31. Bhalla, R. C., et al. Role of cyclic AMP in rat aortic microsomal phosphorylation and calcium uptake. *Am. J. Physiol.* 234:H508, 1978.
32. Black, J. L., et al. The action of a potassium channel activator, BRL 38227 (lemakalim) on human airway smooth muscle. *Am. Rev. Respir. Dis.* 142:1384, 1990.
33. Black, J. L., et al. Sensitization alters contractile responses and calcium influx in human airway smooth muscle. *J. Allergy Clin. Immunol.* 84:440, 1989.
34. Boner, A. L., et al. Comparison of the effects of inhaled calcium antagonist verapamil, sodium cromoglycate and ipratropium bromide on exer-

cise-induced bronchoconstriction in children with asthma. *Eur. J. Pediatr.* 146:408, 1987.
35. Boner, A. L., et al. Effects of different dosages of the calcium antagonist verapamil in exercise- and methacholine-induced bronchospasm in children with chronic asthma. *J. Asthma* 24:81, 1987.
36. Boner, A. L., et al. Nebulised sodium cromoglycate and verapamil in methacholine induced asthma. *Arch. Dis. Child* 62:264, 1987.
37. Breitwieser, G. A., and Szabo, G. Uncoupling of cardiac muscarinic and β-adrenergic receptors from ion channels by a guanine nucleotide analog. *Nature* 317:538, 1985.
38. Brown, A. M., and Birnbaumer, L. Direct G protein gating of ion channels. *Am. J. Physiol. (Heart Circ. Physiol.)* 254:H401, 1988.
39. Burghuber, O. C. et al. Inhibition of acetylcholine-induced bronchoconstriction in asthmatics by nifedipine. *Respiration* 50:265, 1986.
40. Burney, P. G. J., et al. Response to inhaled histamine and 24 hour sodium excretion. *Br. Med. J.* 292:1483, 1986.
41. Bursztyn, M., et al. Long-acting nifedipine in moderate and severe hypertensive patients with serious concomitant diseases. *Am. Heart J.* 110: 96, 1985.
42. Busse, W., et al. The effect of azelastine on neutrophil and eosinophil generation of superoxide. *J. Allergy Clin. Immunol.* 81:212, 1988.
43. Calhoun, W. J., et al. Increased superoxide release from alveolar macrophages in symptomatic asthma (abstract). *Am. Rev. Respir. Dis.* 135: A224, 1987.
43a. Calhoun, W. J., et al. Enhanced superoxide production by alveolar macrophages and air-space cells, airway inflammation, and alveolar macrophage density changes after segmental antigen bronchoprovocation in allergic subjects. *Am. Rev. Respir. Dis.* 145:317, 1992.
44. Campbell, A. K. Intracellular Calcium, Its Universal Role as Regulation. Wiley, 1983, P. 32.
44a. Carafoli, E., and Klee, C. New developments in the calmodulin field. *Cell Calcium* 13:353, 1992.
45. Cerrina, J., et al. Inhibition of exercise-induced asthma by a calcium antagonist, nifedipine. *Am. Rev. Respir. Dis.* 123:156, 1981.
46. Cerrina, J., et al. Comparison of human bronchial muscle response to histamine in vivo with histamine and isoproterenol agonists in vitro. *Am. Rev. Respir. Dis.* 134:57, 1986.
47. Chandra, R., et al. Comparative effect of sub-lingual nifedipine and intravenous aminophylline in allergic bronchial asthma. *Indian J. Chest Dis. Allied Sci.* 29:233, 1987.
48. Chanez, P., et al. Increased eosinophil responsiveness to platelet-activating factor in asthma. *Clin. Sci.* 74:5, 1988.
49. Cheng, J. B., Bewtra, A., and Townley, R. G. Identification of calcium antagonist receptor binding sites using (3H)-nitrendipine in bovine tracheal smooth muscle membranes. *Experientia* 40:267, 1984.
50. Cheng, J. B., and Townley, R. G. Pharmacological characterization of effects of nifedipine on isolated guinea pig and rat tracheal smooth muscle. *Arch. Int. Pharmacodyn. Ther.* 263:228, 1983.
51. Chilvers, E. R., Barnes, P. J., and Nahorski, S. R. Muscarinic receptor stimulated turnover of polyphosphoinositides and inositol phosphates in bovine tracheal smooth muscle. *Br. J. Pharmacol.* 95:778P, 1988.
52. Chilvers, E. R., Barnes, P. J., and Nahorski, S. R. Characterization of agonist-stimulated incorporation of [^{3}H]myo-inositol into phospholipids and [^{3}H]inositol phosphate formation in tracheal smooth muscle. *Biochem. J.* 262:739, 1989.
53. Chilvers, E. R., et al. Characterization of stereospecific binding sites for inositol 1,4,5-triphosphate in airway smooth muscle. *Br. J. Pharmacol.* 99:297, 1990.
54. Chilvers, E. R., et al. Absence of circulating product of oxygen-derived free radicals in acute severe asthma. *Eur. Respir. J.* 2:950, 1989.
55. Chilvers, E. R., and Nahorski, S. R. Phosphoinositide metabolism in airway smooth muscle. *Am. Rev. Respir. Dis.* 141:S137, 1990.
56. Christopher, M. A., et al. Clinical relevance of the interaction of theophylline with diltiazem or nifedipine. *Chest* 95:309, 1989.
57. Christopher, M. A., et al. Efficacy of maintenance therapy with calcium channel blockers for chronic asthma (abstract). *Am. Rev. Respir. Dis.* 137:37, 1988.
58. Cluzel, M., et al. Luminol-dependent chemiluminescence in asthma. *J. Allergy Clin. Immunol.* 80:195, 1987.
59. Coburn, R. F. The airway smooth muscle cell. *Fed. Proc.* 36:2692, 1977.
60. Coburn, R. F. Electromechanical coupling in canine trachealis muscle: Acetylcholine contractions. *Am. J. Physiol.* 236:C177, 1979.
61. Coburn, R. F., and Yamaguchi, T. Membrane potential-dependent and -independent tension in the canine tracheal muscle. *J. Pharmacol. Exp. Ther.* 201:276, 1977.
62. Cockcroft, S. Polyphosphoinositide phosphodisterase: Regulation by a novel guanine nucleotide binding protein, G_p. *Trends Biochem. Sci.* 12: 75, 1987.
63. Cockcroft, S., and Gomperts, B. D. Role of guanine nucleotide binding protein in the activation of phosphoinositide phosphodiesterase. *Nature* 314:534, 1985.
64. Copas, J. L., Borgeat, P., and Gardiner, P. J. The actions of 5-, 12-, and 15-HETE on tracheobronchial smooth muscle. *Prostaglandins Leukotr. Med.* 8:105, 1982.
65. Corris, P. A., et al. Nifedipine in the prevention of asthma induced by exercise and histamine. *Am. Rev. Respir. Dis.* 128:991, 1983.
66. Creese, B. R., and Bach, M. K. Hyperreactivity of airways smooth muscle produced in vitro by leukotrienes. *Prostaglandins Leukotr. Med.* 11:161, 1983.
67. Crimi, N., et al. Effect of nifedipine on allergen-induced bronchoconstriction. *Allergol. Immunopathol. (Madr.)* 14:263, 1986.
68. Crimi, N., et al. Effect of a calcium antagonist, nifedipine, in exercise-induced asthma. *Respiration* 45:262, 1984.
69. Crimi, N., et al. Effect of sodium cromoglycate and nifedipine on adenosine-induced bronchoconstriction. *Respiration* 53:74, 1988.
70. Cuss, F. M., and Barnes, P. J. Nifedipine prolongs isoprenaline-induced bronchodilatation in normal subjects (abstract). *Clin. Sci.* 67(Suppl. 9): 62P, 1984.
71. Cuss, F. M., and Barnes, P. J. The effect of inhaled nifedipine on bronchial reactivity to histamine in man. *J. Allergy Clin. Immunol.* 76:718, 1985.
72. Cuss, F. M., and Barnes, P. J. Inhaled nifedipine and histamine-induced bronchoconstriction (abstract). *Am. Rev. Respir. Dis.* 131(Suppl.):A53, 1985.
73. Cuss, F. M., et al. Effect of nifedipine on autonomic control of airway smooth muscle in vitro. *Am. Rev. Respir. Dis.* 131(Suppl.):A283, 1985.
74. Danoff, S. K., Supattapone, S., and Snyder, S. H. Characterization of a membrane protein from brain mediating the inhibition of inositol(1,4,5) triphosphate receptor binding by calcium. *Biochem. J.* 254:701, 1988.
75. Degenhart, H., et al. Oxygen radicals and their production by leukocytes from children with bronchial hyperresponsiveness. *Clin. Respir. Physiol.* 22:100, 1986.
76. de Lanerolle, P. cAMP, myosin dephosphorylation, and isometric relaxation of airway smooth muscle, *J. Appl. Physiol.* 64:705, 1988.
77. de Lanerolle, P., et al. Myosin phosphorylation, agonist concentration and contraction of tracheal smooth muscle. *Nature* 298:871, 1982.
78. de Lanerolle, P., et al. Increased phosphorylation of myosin light chain kinase after an increase in cyclic AMP in intact smooth muscle. *Science* 223:1415, 1983.
79. de Lanerolle, P., and Stull, J. T. Myosin phosphorylation during contraction and relaxation of tracheal smooth muscle. *J. Biol. Chem.*. 255:9993, 1980.
80. Del Maestro, R. F., et al. Oxygen Derived Free Radicals: Their Role in Inflammation. In P. Verge (ed.), *The Inflammatory Process*. Stockholm: A. Lindquist and Wiskell International, 1989. Pp. 113–143.
81. Del Maestro, R. F., Bjork, J., and Arfors, K. E. Increase in microvascular permeability induced by enzymatically generated free radicals. I. In vivo study. *Microvasc. Res.* 22:239, 1981.
82. Dhillon, D. S., and Rodger, I. W. Hyperreactivity of guinea pig isolated airway smooth muscle (abstract). *Br. J. Pharmacol.* 74:180, 1981.
83. Dillon, P. F., et al. Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle. *Science* 211:495, 1981.
84. Doelman, C. J. A., and Bast, A. Oxygen radicals in lung pathology. *Free Radic. Biol. Med.* 9:381, 1990.
85. Doelman, C. J. A., et al. Vitamin E and selenium regulate the balance between β-adrenergic and muscarinic responses in rat lungs. *FEBS Lett.* 233:427, 1988.
86. Doelman, C. J. A., et al. Mineral dust exposure and free radical-mediated lung damage. *Exp. Lung Res.* 16:41, 1990.
87. Downes, H., and Hirshman, C. A. Lidocaine aerosols do not prevent allergic bronchoconstriction. *Anesth. Analg.* 60:28, 1981.
88. Downes, H., and Hirshman, C. A. Calcium chelators increase airway responsiveness. *J. Appl. Physiol.* 59:92, 1985.
89. Drazen, J. M., Fanta, C. H., and Lacouture, P. G. Effect of nifedipine on constriction of human tracheal strips in vitro. *Br. J. Pharmacol.* 78:687, 1983.
90. Duncan, R. A., et al. Polyphosphoinositide metabolism in canine tracheal smooth muscle (CTSM) in response to a cholinergic stimulus. *Biochem. Pharmacol.* 36:307, 1987.

91. Ebashi, S. Regulation of muscle contraction. *Proc. R. Soc. Lond.* [*Biol.*] 207:259, 1980.
92. Eisenberg, E., and Hill, T. L. Muscle contraction and free energy transduction in biological systems. *Science* 227:999, 1985.
93. Eliraz, A., et al. Exacerbation of asthmatic symptoms after cessation of nifedipine therapy. *Ann. Allergy* 52:125, 1984.
94. Engels, F., Oosting, R. S., and Nijkamp, F. P. Dual effects of *Haemophilus influenzae* on guinea pig tracheal β-adrenergic receptor: Involvement of oxygen-centered radicals from pulmonary macrophages. *J. Pharmacol. Exp. Ther.* 241:994, 1987.
95. Fain, J. N., Wallace, M. A., and Wojcikiewicz, J. H. Evidence for involvement of guanine nucleotide-binding regulatory proteins in the activation of phospholipases by hormones. *FASEB J.* 2:2569, 1988.
96. Farber, J. L. The role of calcium in cell death. *Life Sci.* 29:1289, 1981.
97. Feinstein, M. B. Inhibition of contraction and calcium excitability in rat uterus by local anesthetics. *J. Pharmacol. Exp. Ther.* 152:516, 1966.
98. Felbel, J., et al. Regulation of cytosolic calcium by cAMP and cGMP in freshly isolated smooth muscle cells from bovine trachea. *J. Biol. Chem.* 263:16764, 1988.
99. Ferrari, M., et al. Differential effects of nifedipine and diltiazem on methacholine-induced bronchospasm in allergic asthma. *Ann. Allergy* 63:196, 1989.
100. Fish, J. E., and Norman, P. S. Effects of the calcium channel blocker, verapamil, on asthmatic airway responses to muscarinic, histaminergic, and allergenic stimuli. *Am. Rev. Respir. Dis.* 133:730, 1986.
101. Flatt, A., et al. Reduced selenium in asthmatic subjects in New Zealand. *Thorax* 45:95, 1990.
102. Forder, J., Scriabine, A., and Rasmussen, H. Plasma membrane calcium flux, protein kinase C activation and smooth muscle contraction. *J. Pharmacol. Exp. Ther.* 232:267, 1985.
103. Foresi, A., et al. Effect of two doses of inhaled diltiazem on exercise-induced asthma. *Respiration* 51:241, 1987.
104. Fuller, B. J., Gower, J. D., and Green, C. J. Free radical damage and organ preservation: Fact or fiction. *Cryobiology* 25:377, 1988.
105. Fung, B. K.-K., Hurley, J. B., and Stryer, L. Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. USA* 78:152, 1981.
106. Garty, M., et al. Effect of nifedipine and theophylline in asthma. *Clin. Pharmacol. Ther.* 40:195, 1986.
107. Gerthoffer, W. T. Calcium dependence of myosin phosphorylation and airway smooth muscle contraction and relaxation. *Am. J. Physiol.* 250: C597, 1986.
108. Gianotti, A., et al. Some effects of prolonged treatment with nicardipine in chronic bronchial asthma. *Curr. Med. Res. Opin.* 10:422, 1987.
109. Giembycz, M. A., and Rodger, I. W. Electrophysiological and other aspects of excitation-contraction coupling in mammalian airway smooth muscle. *Life Sci.* 41:111, 1987.
110. Gilman, A. G. G proteins and dual control of adenylate cyclase. *Cell* 36: 577, 1984.
111. Gilman, A. G. G proteins: Transduces of receptor-generated signals. *Annu. Rev. Biochem.* 56:615, 1987.
112. Gonzalez, J. M., et al. Inhibition of airway reactivity by nifedipine in patients with coronary artery disease. *Am. Rev. Respir. Dis.* 127:155, 1983.
113. Gordon, E. H., et al. Comparison of nifedipine with a new calcium channel blocker, flordipine, in exercise-induced asthma. *J. Asthma* 24:261, 1987.
114. Grandordy, B. M., and Barnes, P. J. Phosphoinositide turnover. *Am. Rev. Respir. Dis.* 136(Suppl.):S17, 1987.
115. Grandordy, B. M., et al. Leukotriene C_4 and D_4 induce contraction and formation of inositol phosphates in airways and lung parenchyma. *Am. Rev. Respir. Dis.* 133:A239, 1986.
116. Grandordy, B. M., et al. Phosphatidylinositol response to muscarinic agonist in airway smooth muscle: Relationship to contraction and receptor occupancy. *J. Pharmacol. Exp. Ther.* 238:273, 1986.
117. Grandordy, B. M., et al. Tachykinin-induced phosphoinositide breakdown in airway smooth muscle and epithelium: Relationship to contraction. *Mol. Pharmacol.* 33:515, 1988.
118. Greenspon, L. W., and Levy, S. F. The effect of aerosolized verapamil on the response to isoproterenol in asthmatics. *Am. Rev. Respir. Dis.* 137: 722, 1988.
119. Greenwald, R. A. *Handbook of Methods for Oxygen Radical Research.* Boca Raton, Fl.: CRC Press, 1985.
120. Gugger, M., et al. Low plasma concentrations of ionized calcium in patients with asthma. *J. Appl. Physiol.* 64:1354, 1988.
121. Gunst, S. J., and Stropp, J. Q. Effect of Na-K adenosine triphosphatase activity on relaxation of canine tracheal smooth muscle. *J. Appl. Physiol.* 64:635, 1988.
122. Hall, I. P., and Hill, S. J. B_2-adrenoceptor stimulation inhibits histamine-stimulated inositol phospholipid hydrolysis in bovine tracheal smooth muscle. *Br. J. Pharmacol.* 95:1204, 1988.
123. Halliwell, B., and Gutteridge, J. M. C. *Free Radicals in Biology and Medicine.* Oxford: Clarendon, 1989.
124. Hamilton, T. C., and Weston, A. H. Cromakalim, nicorandil and pinacidil: Novel drugs which open potassium channels in smooth muscle. *Gen. Pharmacol.* 20:1, 1989.
125. Harman, E., et al. The effect of oral diltiazem on airway reactivity to methacholine and exercise in subjects with mild intermittent asthma. *Am. Rev. Respir. Dis.* 136:1179, 1987.
126. Hartmann, V., and Magnussen, H. Effect of diltiazem on histamine- and carbachol-induced bronchospasm in normal and asthmatic subjects. *Chest* 87:174, 1985.
127. Hashimoto, T., Hirata, M., and Ito, Y. A role for inositol 1,4,5-trisphosphate in the initiation of agonist induced contractions of dog tracheal smooth muscle. *Br. J. Pharmacol.* 86:191, 1985.
128. Hasselmark, L., et al. Lowered platelet gluathione peroxidase activity in patients with intrinsic asthma. *Allergy* 45:523, 1990.
129. Hedman, S. E., and Andersson, R. G. G. Studies on the contracting mechanism induced by slow reacting substance of anaphylaxis (SRS-A) interaction with calcium. *Acta Pharmacol. Toxicol.* (*Copenh.*) 50:379, 1980.
130. Heffner, J. E., and Repine, J. E. Pulmonary strategies of antioxidant defense. State of the art. *Am. Rev. Respir. Dis.* 140:531, 1989.
131. Hendeles, L., et al. Dose-response of inhaled diltiazem on airway reactivity to methacholine and exercise in subjects with mild asthma. *Clin. Pharmacol. Ther.* 43:387, 1988.
132. Henderson, A. F., and Costello, J. F. The effect of nifedipine on bronchial reactivity to inhaled histamine and methacholine: A comparative study in normal and asthmatic subjects. *Br. J. Dis. Chest* 82:374, 1988.
133. Henderson, A. F., et al. Effect of nifedipine on bronchoconstriction induced by inhalation of cold air. *Thorax* 38:512, 1983.
134. Henderson, A. F., et al. Effects of nifedipine on antigen-induced bronchoconstriction. *Am. Rev. Respir. Dis.* 127:549, 1983.
135. Henderson, W. R., and Kaliner, M. Immunologic and nonimmunologic generation of superoxide from mast cells and basophils. *J. Clin. Invest.* 61:187, 1978.
136. Hirshman, C. A., and Bergman, W. A. Factors influencing intrapulmonary airway calibre during anesthesia. *Br. J. Anaesth.* 65:30, 1990.
137. Hoidal, J. R., et al. Altered oxidative metabolic responses in vitro of alveolar macrophages from asymptomatic cigarette smoke. *Am. Rev. Respir. Dis.* 123:85, 1981.
138. Honda, K., and Tomita, T. Electrical activity in isolated human tracheal muscle. *Jpn. J. Physiol.* 37:333, 1987.
138a. Hoppe, M., et al. The effect of inhaled gallopamil, a potent calcium channel blocker, on the late-phase response in subjects with allergic asthma. *J. Allergy Clin. Immunol.* 89:688, 1992.
139. Hui, K. P., and Barnes, N. C. Lung function improvement in asthma with cysteinyl-leukotriene receptor antagonist. *Lancet* 337:1062, 1991.
140. Ikeda, H., et al. Acute effects of nifedipine on patients with chronic obstructive lung disease. *Arzneimittelforschung* 35:518, 1985.
141. Jadot, G., et al. Antioxidants in asthma. *Bull. Acad. Natl. Med.* 172:693, 1988.
142. Jaiprakash, S. S., et al. Efficacy of nifedipine in the treatment of angina pectoris and chronic airways obstruction. *Postgrad. Med. J.* 56:624, 1980.
143. Javaid, A., Cushley, M. J., and Bone, M. F. Effect of dietary salt on bronchial reactivity to histamine in asthma. *Br. Med. J.* 297:454, 1988.
144. Johnson, K. J., et al. In vivo damage of rat lungs by oxygen metabolites. *J. Clin. Invest.* 67:983, 1981.
145. Jones, K. A., et al. Halothane decreases cytosolic ionized calcium concentration ($[Ca^{2+}]_i$) during acetylcholine-induced contraction of canine trachealis muscle. *Anesthesiology* 75:A639, 1991.
146. Juniper, E. F., et al. Airway responsiveness to histamine and methacholine: Relationship to minimum treatment to control symptoms of asthma. *Thorax* 36:575, 1981.
147. Kaibuchi, K., Takai, Y., and Nishizuka, Y. Cooperative roles of various membrane phospholipids in the activation of calcium activated phospholipid-dependent protein kinase. *J. Biol. Chem.* 256:7146, 1981.
148. Kamm, K. E., and Stull, J. T. Myosin phosphorylation, force, and maximum shortening velocity in neurally stimulated tracheal smooth muscle. *Am. J. Physiol.* 249:C238, 1985.

149. Kanazawa, H ., et al. The role of free radicals in airway obstruction in asthmatic patients. *Chest* 100:1319, 1991.
150. Katsumata, U., et al. Reactive oxygen exposure produces airway hyperresponsiveness. *Am. Rev. Respir. Dis.* 137:285, 1988.
151. Kelly, C. A., et al. Numbers and activity of cells obtained at bronchoalveolar lavage in asthma and their relationship to airway responsiveness. *Thorax* 43:684, 1988.
152. Kivity, S., et al. The combined effect of nifedipine and sodium cromoglycate on the airway response to inhaled hypertonic saline in patients with bronchial asthma. *Eur. Respir. J.* 2:513, 1989.
153. Kivity, S., et al. Combined effect of inhaled verapamil and sodium cromoglycate on the airway response to hypertonic saline. *Ann. Allergy* 64:163, 1990.
154. Kirkpatrick, C. T. Excitation and contraction in bovine tracheal smooth muscle. *J. Physiol* (*Lond.*) 244:263, 1975.
155. Kirkpatrick, C. T., Jenkinson, H. A., and Cameron, A. R. Interaction between drugs and potassium-rich solutions in producing contraction in bovine tracheal smooth muscle: Studies in normal and calcium depleted tissues. *Clin. Exp. Pharmacol. Physiol.* 2:559, 1975.
156. Kirkpatrick, M. B., Sanders, R. V., and Bass, J. B., Jr. Physiologic effects and serum lidocaine concentrations after inhalation of lidocaine from a compressed gas-powered jet nebulizer. *Am. Rev. Respir. Dis.* 136:1987, 1987.
157. Kneussl, M., et al. Nifedipine protects acetylcholine induced bronchoconstriction: The role of mediator release (abstract). *Am. Rev. Respir. Dis.* 127:108, 1983.
158. Koppermann, G., and Kaukel, G. The influence of nifedipine, a calcium channel blocker, on allergen-induced bronchoconstriction. *Prog. Respir. Res.* 19:271, 1985.
159. Kotlikoff, M. I. Calcium currents in isolated canine airway smooth muscle cells. *Am. J. Physiol.* 254:C793, 1988.
160. Kotlikoff, M. I., Murray, R. K., and Reynolds, E. E. Histamine induced Ca^{2+} release and phorbol antagonism in cultured airway smooth muscle cells. *Am. J. Physiol.* 253:C561, 1987.
161. Kramer, K., et al. A disbalance between β-adrenergic and muscarinic responses caused by hydrogen peroxide in rat airways in vitro. *Biochem. Biophys. Res. Commun.* 145:357, 1987.
162. Kramer, K., et al. Influence of lipid peroxidation on β-adrenoreceptors. *FEBS Lett.* 198:80, 1986.
163. Krebs, E. G., and Beavo, J. A. Phosphorylation-dephosphorylation of enzymes. *Annu. Rev. Biochem.* 48:923, 1979.
164. Kurasawa, M., et al. Superoxide anion formation from activated peritoneal mast cells in vitro measured by MCLA-dependence luminescence. *Immunol. Allergy Pract.* 235:17, 1991.
165. Kuthan, H., and Ullrich, V. Oxidase and oxygen function of the microsomal cytochrome P450 monoxygenase system. *Eur. J. Biochem.* 126:583, 1982.
165a. Lansing, M. W., et al. Lipid mediators contribute to oxygen-radical-induced airway responses in sheep. *Am. Rev. Respir. Dis.* 144:1291, 1991.
166. Lee, V. Y., et al. Verapamil inhibits mediator release from human lung in vitro. *Thorax* 38:386, 1983.
167. Lemoine, H., Pohl, V., and Teng, K. J. Serotonin (5-HT) stimulates phosphatidylinositol (PI) hydrolysis only through the R-state of allosterically regulated 5-HT_2 receptors in calf tracheal smooth muscle. *Naunyn Schmiedebergs Arch. Pharmacol.* 377(Suppl.):R103, 1988.
168. Lever, A. M., et al. Nifedipine enhances the bronchodilator effect of salbutamol. *Thorax* 39:576, 1984.
169. Lewis, M. S., et al. Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. *J. Clin. Invest.* 82:2045, 1988.
170. Lichey, J., et al. Effect of diltiazem on methacholine-induced bronchoconstriction in extrinsic asthmatics. *Respiration* 50:44, 1986.
171. Lidji, M., et al. Therapy with nifedipine in asthma: A randomized double-blind crossover trial. *Isr. J. Med. Sci.* 24:1, 1988.
172. Litosch, I., Wallis, C., and Fain, J. N. 5-Hydroxytryptamine stimulated inositol phosphate production in a cell-free system from blowfly salivary glands: Evidence for a role of GTP in coupling receptor activation to phosphoinositide breakdown. *J. Biol. Chem.* 260:5464, 1985.
173. Loesberg, C., Henricks, P. A. J., and Nijkamp, F. P. Inverse relationship between superoxide anion production of guinea pig alveolar macrophages and tracheal β-adrenergic receptor function; influence of dietary polyunsaturated fatty acids. *Int. J. Immunopharmacol.* 11:165, 1989.
174. Lofdahl, C. G., et al. Effects of nifedipine or atenolol on ventilatory capacity and hemodynamics in asthmatic patients: Interaction with terbutaline. *Curr. Ther. Res.* 36:282, 1984.
175. Magnussen, H., et al. Influence of diltiazem on bronchoconstriction induced by cold air breathing during exercise. *Thorax* 39:579, 1984.
176. Malik, S., et al. Effects of sublingual nifedipine on inhaled histamine and methacholine-induced bronchoconstriction in atopic subjects (abstract). *Thorax* 37:230, 1982.
177. Malmgren, R., et al. Lowered glutathione peroxidase activity in asthmatic patients with food and aspirin intolerance. *Allergy* 41:43, 1986.
178. Mannioni, P. F., et al. Free radicals as endogenous histamine releasers. *Agents Actions* 23:129, 1989.
179. Mansour, S., and Daniel, E. E. Responsiveness of isolated tracheal smooth muscle from normal and sensitized guinea pigs. *Can. J. Physiol. Pharmacol.* 65:1942, 1987.
180. Mapp, C., et al. Protective effect of antiasthma drugs on late asthmatic reactions and increased airway responsiveness induced by toluene diisocyanate in sensitized subjects. *Am. Rev. Respir. Dis.* 136:1403, 1987.
181. Marin, J. M., et al. Nifedipine in exercise-induced asthma. *Allergol. Immunopathol.* (*Madr.*) 14:37, 1986.
182. Marthan, R., et al. Extracellular calcium and human isolated airway muscle: Ionophore A23187 induced contraction. *Respir. Physiol.* 71:157, 1988.
183. Marthan, R., et al. Calcium channel currents in isolated smooth muscle cells from human bronchus. *J. Appl. Physiol.* 66:1706, 1989.
184. Massey, K. L., et al. Duration of protection of calcium channel blockers against exercise-induced bronchospasm: Comparison of oral diltiazem and inhaled gallopamil. *Eur. J. Clin. Pharmacol.* 34:555, 1988.
185. Massey, K. L., et al. Dose response of inhaled gallopamil (D600), a calcium channel blocker, in attenuating airway reactivity to methacholine and exercise. *J. Allergy Clin. Immunol.* 81:912, 1988.
186. Matthews, J. I., et al. Nifedipine does not alter methacholine-induced bronchial reactivity. *Ann. Allergy* 43:462, 1984.
187. McDonald, R. J., Berger, E. M., and Repine, J. M. Alveolar macrophage antioxidants prevent hydrogen peroxide-mediated lung damage. *Am. Rev. Respir. Dis.* 143:1088, 1991.
188. McIntyre, E., et al. Inhaled verapamil in histamine-induced bronchoconstriction. *J. Allergy Clin. Immunol.* 71:375, 1983.
189. Meisheri, K. D., and van Breemen, C. Effects of β-adrenergic stimulation on calcium movements in rabbit aortic smooth muscle: Relationship with cyclic AMP. *J. Physiol.* 331:429, 1982.
190. Meltzer, S., et al. Superoxide generation and its modulation by adenosine in neutrophils of subjects with asthma. *J. Allergy Clin. Immunol.* 83:960, 1989.
191. Meurs, H., et al. Evidence for a direct relationship between phosphoinositide metabolism and airway smooth muscle contraction induced by muscarinic agonists. *Eur. J. Pharmacol.* 156:271, 1988.
192. Miadonna, A., et al. Effect of verapamil on allergen-induced asthma in patients with respiratory allergy. *Ann. Allergy* 51:201, 1983.
193. Middleton, E., Jr. Antiasthmatic drug therapy and calcium ions: Review of pathogenesis and role of calcium. *J. Pharm. Sci.* 69:243, 1980.
194. Miller, J. R., Silver, P. J., and Stull, S. T. The role of myosin light chain kinase phosphorylation in beta-adrenergic relaxation of tracheal smooth muscle. *Mol. Pharmacol.* 24:235, 1983.
195. Miller, W. C., and Awe, R. Effect of nebulized lidocaine on reactive airways. *Am. Rev. Respir. Dis.* 111:739, 1975.
196. Miller-Hance, W. C., et al. Biochemical events associated with activation of smooth muscle contraction. *J. Biol. Chem.* 263:13979, 1988.
197. Mita, H., et al. Increased activity of 5-lipooxygenase in polymorphonuclear leukocytes from asthmatic patients. *Life Sci.* 37:907, 1985.
197a. Miura, M., et al. Role of potassium channels in bronchodilator responses in human airways. *Am. Rev. Respir. Dis.* 146:132, 1992.
198. Molho, M., et al. Nifedipine in asthma. Dose-related effect on resting bronchial tone. *Chest* 91:667, 1987.
199. Moscato, G., et al. Effect of nifedipine on hyperreactive bronchial responses to methacholine. *Ann. Allergy* 56:145, 1986.
200. Moscato, G., et al. Protective effect of nifedipine upon specific bronchial responses to isocyanates. *G. Ital. Med. Lav.* 5:247, 1983.
201. Murray, R. K., et al. Mechanism of phorbol ester inhibition of histamine-induced IP3 formation in cultured airway smooth muscle. *Am. J. Physiol.* 257:L209, 1989.
202. Nagai, P. K., and Walsh, M. P. Inhibition of smooth muscle actin-activated myosin Mg2 + −ATPase activity by caldesmon. *J. Biol. Chem.* 259: 13656, 1984.
203. Nair, N., et al. Safety of nifedipine in subjects with bronchial asthma and COPD. *Chest* 86:515, 1984.
204. Nakagawara, M., et al. Inhibition of superoxide production and calcium

mobilization in human neutrophils by halothane, enflurane, and isoflurane. *Anesthesiology* 64:4, 1986.
205. Neyens, H. J., et al. Altered leukocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. *Am. Rev. Respir. Dis.* 130:744, 1984.
206. Nishida, Y., Suzuki, S., and Miyamoto, T. Biphasic contraction of isolated guinea pig tracheal rings by superoxide radical. *Inflammation* 9:333, 1985.
207. Nishikawa, M., Hidaka, H., and Adelstein, R. S. Phosphorylation of smooth muscle heavy meromyosin by calcium activated phospholipid-dependent protein kinase. *J. Biol. Chem.* 258:14069, 1983.
208. Nishizuka, Y. Studies and perspectives of protein kinase C. *Science* 233: 305, 1986.
209. Ogilvy, C. S., Dubois, A. B., and Douglas, J. S. Effects of ascorbic acid and indomethacin on the airways of healthy male subjects with and without induced bronchoconstriction. *J. Allergy Clin. Immunol.* 67:363, 1981.
210. Olive, S. R., et al. Comparison of PY 108-068, a new calcium antagonist, with nifedipine in exercise-induced asthma. *Chest* 90:208, 1986.
211. Owen, S., et al. A randomised double blind placebo controlled crossover study of a potassium channel activator in morning dipping (abstract). *Thorax* 44:852, 1989.
212. Ozenne, G., et al. Nifedipine in chronic bronchial asthma: A randomized double-blind crossover trial against placebo. *Eur. J. Respir. Dis.* 67:238, 1985.
213. Park, S., and Rasmussen, H. Activation of tracheal smooth muscle contraction: Synergism between Ca^{2+} and activators of protein kinase C. *Proc. Natl. Acad. Sci. USA* 82:8835, 1985.
214. Parker, J. C., et al. Prevention of free radical-mediated vascular permeability increases in lung using superoxide dismutase. *Chest* 83:52S, 1983.
215. Parrish, R. W., and Davies, B. H. Allergen bronchial challenge and effects of lodoxamide and nifedipine (abstract). *J. Allergy Clin. Immunol.* 71: 152, 1983.
216. Patakas, D., et al. Nifedipine treatment of patients with bronchial asthma. *J. Allergy Clin. Immunol.* 79:959, 1987.
217. Patakas, D., et al. Nifedipine in bronchial asthma. *J. Allergy Clin. Immunol.* 72:269, 1983.
218. Patel, K. R. The effect of calcium antagonist, nifedipine, in exercise-induced asthma. *Clin. Allergy* 11:429, 1981.
219. Patel, K. R. Calcium antagonists in exercise-induced asthma. *Br. Med. J.* 282:932, 1981.
220. Patel, K. R. The effect of verapamil on histamine and methacholine-induced bronchoconstriction. *Clin. Allergy* 11:441, 1981.
221. Patel, K. R. Sodium cromoglycate and verapamil alone and in combination in exercise induced asthma. *Br. Med. J.* 286:606, 1983.
222. Patel, K. R., and Al-Shamma, M. R. Effect of nifedipine on histamine reactivity in asthma. *Br. Med. J.* 284:1916, 1982.
223. Patel, K. R., et al. The effect of inhaled verapamil on allergen-induced bronchoconstriction. *Clin. Allergy* 13:119, 1983.
224. Patel, K. R., and Kerr, J. W. Calcium antagonists in experimental asthma. *Clin. Allergy* 12(Suppl.):15, 1982.
225. Patel, K. R., and Peers, E. Felodipine, a new calcium antagonist, modifies exercise-induced asthma. *Am. Rev. Respir. Dis.* 138:54, 1988.
226. Patel, K. R., and Tullett, W. M. Comparison of two calcium antagonists, verapamil and gallopamil (D-600), in exercise-induced asthma. *Eur. J. Respir. Dis.* 67:269, 1985.
227. Paterson, J. W., Woolcock, A. J., and Shenfield, G. M. Bronchodilator drugs. *Am. Rev. Respir. Dis.* 120:1149, 1979.
228. Pato, M. D., and Adelstein, R. S. Dephosphorylation of the 20,000 dalton light chain of myosin by two different phosphatases from smooth muscle. *J. Biol. Chem.* 255:6535, 1980.
229. Perpina, M., et al. Different ability of verapamil to inhibit agonist-induced contraction of lung parenchymal strips from control and sensitized guinea pig. *Respiration* 50:174, 1986.
230. Perpina, M., et al. Nifedipine decreases sensitivity and reactivity to methacholine in mild asthmatics. *Respiration* 51:49, 1987.
231. Pfaffinger, P. J., et al. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature* 317:536, 1985.
232. Phipps, R. J., et al. The effect of 0.5 ppm ozone on glycoprotein secretion, ion and water fluxes in sheep trachea. *J. Appl. Physiol.* 60:918, 1986.
233. Popa, V. T., et al. The effect of inhaled verapamil on resting bronchial tone and airway contractions induced by histamine and acetylcholine in normal and asthmatic subjects. *Am. Rev. Respir. Dis.* 130:1006, 1984.
234. Postma, D. S., et al. Association between nonspecific bronchial hyperreactivity and superoxide anion production by polymorphonuclear leukocytes in chronic airflow obstruction. *Am. Rev. Respir. Dis.* 137:57, 1988.
235. Raeburn, D., et al. Agonist-induced contractile responses of human bronchial muscle in vitro: Effects of Ca2+ removal, La3+ and PY108068. *Eur. J. Pharmacol.* 121:251, 1986.
236. Raeburn, D., et al. Concentration-related differences in the effects of the Ca^{2+} agonist BAY K8644 in human bronchus in vitro. *Gen. Pharmacol.* 19:399, 1988.
237. Raeburn, D., and Rodger, I. W. Lack of effect of leukotriene D_4 on calcium-uptake in airway smooth muscle. *Br. J. Pharmacol.* 83:499, 1984.
238. Rafferty, P., et al. Effects of verapamil and sodium cromoglycate on bronchoconstriction induced by isocapnic hyperventilation. *Clin. Allergy* 15:531, 1985.
239. Rafferty, P., and Holgate, S. T. Terfenadine (Seldane) is a potent and selective histamine H1 receptor antagonist in asthmatic airways. *Am. Rev. Respir. Dis.* 135:181, 1987.
240. Rafferty, P., et al. Inhibition of exercise-induced asthma by nifedipine: A dose-response study. *Br. J. Clin. Pharmacol.* 24:479, 1987.
241. Rang, H. P., and Dale, M. M. *Pharmacology* (2nd ed.). London: Churchill-Livingstone, 1991.
242. Rasmussen, H., et al. TPA-induced contraction of isolated rabbit vascular smooth muscle. *Biochem. Biophys. Res. Commun.* 122:776, 1984.
243. Rasmussen, H., Kelley, G., and Douglas, J. S. Interactions between Ca^{2+} and cAMP messenger system in regulation of airway smooth muscle contraction. *J. Physiol.* 258:L279, 1990.
244. Rhoden, K. J., and Barnes, P. J. Effect of hydrogen peroxide in guinea-pig tracheal smooth muscle in vitro: Role of cyclo-oxygenase and airway epithelium. *Br. J. Pharmacol.* 98:325, 1989.
245. Ringqvist, T. Effect of verapamil in obstructive airways disease. *Eur. J. Clin. Pharmacol.* 7:61, 1974.
246. Riska, H., et al. Effects of captopril on blood pressure and respiratory function compared to verapamil in patients with hypertension and asthma. *J. Cardiovasc. Pharmacol.* 15:57, 1990.
247. Riska, H., et al. Comparison of the effects of an angiotensin converting enzyme inhibitor and a calcium channel blocker on blood pressure and respiratory function in patients with hypertension and asthma. *J. Cardiovasc. Pharmacol.* 10(Suppl. 10):S79, 1987.
248. Roberts, J. A., et al. In vitro and in vivo effect of verapamil on human airway responsiveness to leukotriene D_4. *Thorax* 41:12, 1986.
249. Roberts, J. A., et al. Effect of verapamil and sodium cromoglycate on leukotriene D_4 induced bronchoconstriction in patients with asthma. *Thorax* 41:753, 1986.
250. Roberts, J. A., and Thomson, N. C. Effect of verapamil on bronchodilator response to beta receptor agonists in asthma (abstract). *Thorax* 39:171, 1984.
251. Rodger, I. W. Excitation-contraction coupling in airway smooth muscle. *Br. J. Clin. Pharmacol.* 20:255S, 1985.
252. Rodger, I. W. Calcium channels. *Am. Rev. Respir. Dis.* 136:S15, 1987.
253. Rolla, G., et al. Magnesium attenuates methacholine-induced bronchoconstriction in asthmatics. *Magnesium* 6:201, 1987.
254. Rolla, G., et al. Nifedipine inhibits deep-inspiration-induced bronchoconstriction in asthmatics. *Lancet* 1:1305, 1982.
255. Russell, J. A. Tracheal smooth muscle. *Clin. Chest Med.* 7:189, 1986.
256. Russi, E. W., et al. Comparative modification of antigen-induced bronchoconstriction by the calcium antagonists, nifedipine and verapamil. *Chest* 88:74, 1985.
257. Ryan, G., et al. Effect of beclomethasone dipropionate on bronchial responsiveness to histamine in controlled nonsteroid-dependent asthma. *J. Allergy Clin. Immunol.* 75:25, 1985.
258. Sands, H., and Mascali, J. Effects of cAMP and protein kinase on the calcium uptake of various smooth muscle organelles. *Arch. Int. Pharmacodyn. Ther.* 236:180, 1978.
259. Sands, H., Meyer, T. A., and Rickenberg, H. V. Adenosine 3′,5′-monophosphate-dependent protein kinase of bovine tracheal smooth muscle. *Biochim. Biophys. Acta* 302:267, 1973.
260. Sanguinetti, M. C., and Kass, R. S. Voltage dependent block of calcium channel current in the calf cardiac Purkinje fiber by dihydropyridine calcium channel antagonists. *Circ. Res.* 55:336, 1984.
261. Sanz, C., et al. Different ability of trifluoperazine to inhibit agonist-induced contraction of lung parenchymal strips from control and sensitized guinea pigs. *J. Pharm. Pharmacol.* 40:120, 1988.
262. Scarpa, M., et al. Superoxide ion as active intermediate in the autooxidation of ascorbate by molecular oxygen. *J. Biol. Chem.* 258:6695, 1983.
263. Schwartzstein, R. S., and Fanta, C. H. Orally administered nifedipine in

chronic stable asthma. Comparison with an orally administered sympathomimetic. *Am. Rev. Respir. Dis.* 134:262, 1986.
264. Sedwick, J. B., Geiger, K. M., and Busse, W. W. Superoxide generation by hypodense eosinophils from patients with asthma. *Am. Rev. Respir. Dis.* 142:120, 1990.
265. Sellers, J. R., Pato, M. D., and Adelstein, R. S. Reversible phosphorylation of smooth muscle myosin, heavy meromyosin and platelet myosin. *J. Biol. Chem.* 256:13137, 1981.
266. Serhan, C., et al. Phosphatidate and oxidized fatty acids are calcium ionophores. *J. Biol. Chem.* 256:2736, 1981.
267. Shah, J., and Pant, H. C. Potassium channel blockers inhibit inositol triphosphate induced calcium release in microsomal fractions isolated from rat brain. *Biochem. J.* 250:617, 1988.
268. Sharma, S. K., and Pande, J. N. Effect of verapamil inhalation on bronchial asthma. *J. Asthma* 27:31, 1990.
269. Sharma, S. K., et al. The effect of nifedipine on exercise-induced asthma. *J. Asthma* 23:15, 1986.
270. Sharma, S. K., et al. Immediate effect of verapamil on pulmonary functions in bronchial asthma. *J. Asthma* 24:179, 1987.
271. Silver, P. J., and Stull, J. T. Regulation of myosin light chain and phosphorylase phosphorylation in tracheal smooth muscle. *J. Biol. Chem.* 257:6145, 1982.
272. Skobelloff, E. M., et al. Intravenous magnesium sulfate for the treatment of acute asthma in the emergency department. *JAMA* 262:1210, 1989.
273. Skoner, D. P., Gentile, D. J., and Evans, R. Decreased activity of the platelet Na^+, K^+ adenosine triphosphatase enzyme (ATPase) in allergic subjects. *J. Lab. Clin. Med.* 115:535, 1990.
274. Skoner, D. P., Gentile, D., and Evans, R. W. A circulating inhibition of the platelet Na^+, K^+ adenosine triphosphatase (ATPase) enzyme in allergy. *J. Allergy Clin. Immunol.* 87:476, 1991.
275. Sly, P. D., et al. Does nifedipine affect the diurnal variation of asthma in children? *Pediatr. Pulmonol.* 2:206, 1986.
276. Small, R. C. Electrical slow waves and tone of guinea pig isolated trachealis muscle: Effects of drugs and temperature changes. *Br. J. Pharmacol.* 77:45, 1982.
277. Small, R. C., et al. Airway Smooth Muscle: Electrophysiological Properties and Behavior. In D. K. Agrawal and R. G. Townley (eds.), *Airway Smooth Muscle: Modulation of Receptors and Response*. Boston: CRC Press, 1990. P. 69.
278. Small, R. C., and Foster, R. W. Electrophysiologic behavior of normal and sensitized airway smooth muscle. *Am. Rev. Respir. Dis.* 136:S7, 1987.
279. Smith, S. R., et al. Effect of nifedipine on serum theophylline concentrations and asthma control. *Thorax* 42:794, 1987.
280. So, S. Y., et al. Effect of calcium antagonists on allergen-induced asthma. *Clin. Allergy* 12:595, 1982.
281. Sobieszek, A. Vertebrate Smooth Muscle Myosin. Enzymatic and Structural Properties. In N. L. Stephens (ed.), *The Biochemistry of Smooth Muscle*. Baltimore: University Park Press, 1977. P. 413.
282. Sobue, K., et al. Control of actin-myosin interaction of gizzard smooth muscle by calmodulin and caldesmon-linked flip-flop mechanism. *Biomed. Res.* 3:188, 1982.
283. Solway, J., and Fanta, C. H. Differential inhibition of bronchoconstriction by the calcium channel blockers, verapamil and nifedipine. *Am. Rev. Respir. Dis.* 132:666, 1985.
284. Somlyo, A. V., et al. Inositol trisphosphate-induced calcium release and contraction in vascular smooth muscle. *Proc. Natl. Acad. Sci. USA* 82: 5231, 1987.
285. Souhrada, J. F., and Souhrada, M. Significance of the sodium pump for airway smooth muscle. *Eur. J. Respir. Dis.* 64:196, 1983.
286. Souhrada, M., and Souhrada, J. F. Re-assessment of electrophysiological and contractile characteristics of sensitized airway smooth muscle. *Respir. Physiol.* 46:17, 1981.
287. Souhrada, M., Souhrada, J. F. Potentiation of Na^+-electrogenic pump of airway smooth muscle by sensitization. *Respir. Physiol.* 47:69, 1982.
288. Souhrada, M., and Souhrada, J. F. Immunologically induced alterations of airway smooth muscle cell membrane. *Science* 225:723, 1984.
289. Souhrada, M., and Souhrada, J. F. Alterations of airway smooth muscle cell membrane by sensitization. *Pediatr. Pulmonol.* 1:207, 1985.
290. Souhrada, M., and Souhrada, J. F. A transient calcium influx into airway smooth muscle cells induced by immunization. *Respir. Physiol.* 67:323, 1987.
291. Souhrada, M., and Souhrada, J. F. Sodium and calcium influx induced by phorbol esters in airway smooth muscle cells. *Am. Rev. Respir. Dis.* 139:927, 1989.
292. Sparrow, M. P., et al. Effect of calmodulin, Ca2+, and cAMP protein kinase on skinned tracheal smooth muscle. *Am. J. Physiol.* 246:C308, 1984.
293. Spedini, C., and Lombardi, C. Long-term treatment with oral nifedipine plus theophylline in the management of chronic bronchial asthma. *Eur. J. Clin. Pharmacol.* 31:105, 1986.
294. Squire, J. Muscle regulation: A decade of the steric blocking model. *Nature* 291:614, 1981.
295. Stephens, N. L., Seow, C. Y., and Kong, S. K. Mechanical properties of sensitized airway smooth muscle: Shortening capacity. *Am. Rev. Respir. Dis.* 143:S13, 1991.
296. Stewart, R. M., et al. Hydrogen peroxide contracts airway smooth muscle: A possible endogenous mechanism. *Respir. Physiol.* 45:333, 1981.
297. Stone, J., et al. Selenium status of patients with asthma. *Clin. Sci.* 77: 495, 1989.
298. Stryer, L., and Bourne, H. R. G proteins: A family of signal transducers. *Annu. Rev. Cell Biol.* 2:391, 1986.
299. Styrt, B., Rocklin, R. E., and Klempner, M. S. Characterization of the neutrophil respiratory burst in atopy. *J. Allergy Clin. Immunol.* 81:20, 1988.
300. Suematsu, E., et al. Inositol 1,4,5-trisphosphate releases Ca^{2+} from intracellular store sites in skinned single cells of porcine coronary artery. *Biochem. Biophys. Res. Commun.* 120:481, 1984.
301. Svedmyr, K., et al. Nifedipine—a calcium channel blocker—in asthmatic patients. Interaction with terbutaline. *Allergy* 39:17, 1984.
302. Takemura, H., and Putney, J. W., Jr. Capacitative calcium entry in parotid acinar cells. *Biochem. J.* 258:409, 1989.
303. Takuwa, Y., Takuwa, N., and Rassmussen, H. Carbachol induces a rapid and sustained hydrolysis of phosphotidylinositide in bovine tracheal smooth muscle measurements of the mass of polyphosphoinositides, 1,2-diacylglycerol and phosphatidic acid. *J. Biol. Chem.* 261:14670, 1986.
304. Tate, R. M., et al. Oxygen-radical-mediated permeability edema and vascoconstriction in isolated perfused rabbit lungs. *Am. Rev. Respir. Dis.* 126:802, 1982.
305. Taylor, D. A., Bowman, B. F., and Stull, J. T. Cytoplasmic Ca^{2+} is a primary determinant for myosin phosphorylation in smooth muscle cells. *J. Biol. Chem.* 264:6207, 1989.
306. Taylor, D. A., and Stull, J. T. Calcium dependence of myosin light chain phosphorylation in smooth muscle cells. *J. Biol. Chem.* 263:14456, 1988.
307. Test, S. T., and Weiss, S. J. Quantitative and temporal characteristics of the extracellular H_2O_2 pool generated by human neutrophils. *J. Biol. Chem.* 259:399, 1984.
308. Tomioka, S., et al. Effect of nifedipine on dose-response curves to acetylcholine and histamine measured during quiet breathing. *Respiration* 50:185, 1986.
309. Torphy, T. J. Biochemical regulation of airway smooth muscle tone: Current knowledge and therapeutic implications. *Rev. Clin. Basic Pharmacol.* 6:61, 1987.
310. Torphy, T. J., et al. Cyclic nucleotide-dependent protein kinase in airway smooth muscle. *J. Biol. Chem.* 257:11609, 1982.
311. Torphy, T. J., and Gerthoeffer, W. T. Biochemical Mechanisms of Smooth Muscle Contraction and Relaxation. In: M. A. Hollinger (ed.), *Current Topics in Pulmonary Pharmacology and Toxicology*, Vol. 1. New York: Elsevier Science, 1986. P. 23.
312. Triggle, D. J. Cellular calcium metabolism: Activation and antagonism. *J. Asthma* 21:375, 1984.
313. Triggle, D. J. Calcium ions and respiratory smooth muscle function. *Br. J. Clin. Pharmacol.* 20:213s, 1985.
314. Triggle, D. J., and Janis, R. A. Nitrendipine: Binding Sites and Mechanism of Action. In A. Scriabine, et al. (eds.), *Nitrendipine*. Baltimore: Urban & Schwarzenberg, 1984. P. 34.
315. Tsuda, T., et al. Bronchodilating effect of inhaled or orally administered calcium channel blocking agents on methacholine-induced bronchoconstriction. *Can. J. Anaesth.* 37:S166, 1990.
316. Twort, C. H. C., and van Breemen, C. Human airway smooth muscle in cell culture: Control on the intracellular calcium store. *Pulmon. Pharmacol.* 2:45, 1989.
317. Verdiani, P., et al. Effect of nisoldipine (BAY k 5552) on methacholine-induced bronchoconstriction in patients with nonspecific bronchial hyperreactivity. *Ann. Allergy* 63:498, 1989.
318. Vincenc, K. S., et al. Comparison of in vivo and in vitro responses to histamine in human airways. *Am. Rev. Respir. Dis.* 128:875, 1983.

319. Wagner, P. D., et al. Protection against pulmonary O_2 toxicity by N-acetylcysteine. *Eur. Respir. J.* 2:116, 1989.
320. Walsh, M. P. Calcium dependent mechanisms of regulation of smooth muscle. *Biochem. Cell Biol.* 69:771, 1991.
321. Walsh, M. P., et al. Is smooth muscle myosin a substrate for cAMP-dependent protein kinase? *FEBS Lett.* 126:107, 1981.
322. Walters, E. H., et al. Effects of calcium channel blockade on histamine induced bronchoconstriction in mild asthma. *Thorax* 39:572, 1984.
323. Watson, K. V., et al. Magnesium sulfate: Rational for its use in pre-eclampsia. *Proc. Natl. Acad. Sci. USA* 83:1075, 1986.
324. Weiss, E. B. Leukotriene-associated toxic oxygen metabolites induce airway hyperreactivity. *Chest* 5:709, 1986.
325. Weiss, E. B. Toxic oxygen products alter calcium homeostasis in an asthma model. *J. Allergy Clin. Immunol.* 75:692, 1985.
326. Weiss, E. B., and Bellino, J. R. Superoxide anion generation during airway anaphylaxis. *Int. Arch. Allergy Appl. Immunol.* 80:211, 1986.
327. Weiss, E. B., Hargraves, W. A., and Viswanath, S. G. The inhibitory action of lidocaine in anaphylaxis. *Am. Rev. Respir. Dis.* 117:859, 1978.
328. Weiss, E. B., Mullick, P. C., and Barbero, L. J. Leukotriene effect in airways smooth muscle: Calcium dependency and verapamil inhibition. *Prostaglandins Leukot. Med.* 12:53, 1983.
329. Weiss, E. B., and Viswanath, S. G. Calcium hypersensitivity in airways smooth muscle: Isometric tension responses following anaphylaxis. *Respiration* 38:266, 1979.
330. Welling, A., et al. Beta-adrenergic receptor stimulates L-type calcium current in adult smooth muscle cells. *Blood Vessels* 28:154, 1991.
331. Whicker, S. D., Armour, C. L., and Black, J. L. Responsiveness of bronchial smooth muscle from asthmatic patients to relaxant and contractile agonists. *Pulmonol. Pharmacol.* 1:25, 1988.
331a. Williams, R. J. P. Calcium fluxes in cells: new views on their significance. *Cell Calcium* 13:273, 1992.
332. Williams, D. O., et al. Effects of nifedipine on bronchomotor tone and histamine reactivity in asthma. *Br. Med. J.* 283:348, 1981.
333. Yamakage, M., and Namiki, A. Effect of halothane on canine tracheal smooth muscle; role of cytosolic Ca^{2+}, cAMP and protein kinase C. *Anesthesiology* 75:A354, 1991.
334. Yamamoto, S. Enzymes in the Arachidonic Acid Cascade. In C. Pace-Ascieak and E. Granstrom (eds.), *Prostaglandins and Related Substances*. Amsterdam: Elsevier Science, 1983. P. 171.
335. Zoratti, E. M., et al. The effect of platelet activating factor on the generation of superoxide anion in human eosinophils and neutrophils. *J. Allergy Clin. Immunol.* 88:749, 1991.
336. Zorychta, E., and Richardson, J. B. Control of smooth muscle in human airways. *Bull. Eur. Physiopathol. Respir.* 16:581, 1980.
337. Small, R. C., et al. Potassium channel activators and bronchial asthma. *Clin. Exp. Allergy* 22:11, 1992.
338. Kai, T., et al. Lidocaine-induced relaxation of airway smooth muscle and intracellular Ca^{2+}. *Anesthesiology* 77:No. 3A, Sept. 1992.