underlying this partial inhibition remain obscure; however, compounds offering 100% inhibition of both mediators or indeed all mediators would offer a potential clinical advantage.

In summary, as laboratory and clinical experience with both cromolyn sodium and novel antiallergic agents increases, a clearer understanding of the mechanism of action of this class of compounds undoubtedly will emerge. When this time approaches, many problems that concern us today will, hopefully, have fallen away and, perhaps, the design of and search for antiallergic drugs will be considerably less confusing and frustrating than they have been for the past decade.

REFERENCES

(1) L. W. Chakrin and R. D. Krell, *J. Pharm. Sci.*, **69**, 236 (1980).

(2) "Intal—Cromolyn Sodium, A Monograph," Fisons Corp., Bedford, Mass., 1973.

(3) L. W. Chakrin and R. D. Krell, *J. Pharmacol. Exp. Ther.*, **207**, 756 (1978).

(4) K. Ishizaka and T. Ishizaka, *Arch. Pathol. Lab. Med.*, **100**, 289 (1976).

(5) L. M. Stotland and N. N. Share, *Can. J. Physiol. Pharmacol.*, **52**, 1119 (1974).

(6) J. B. Farmer, I. M. Richards, P. Sheard, and A. M. Woods, *Br. J. Pharmacol.*, **55**, 57 (1975).

(7) P. Armitage, H. Herxheimer, and L. Rosa, *ibid.*, **7**, 625 (1952).

(8) L. Diamond and M. O'Donnell, *J. Appl. Physiol.*, **43**, 942 (1977).

(9) R. Patterson, *Prog. Allergy*, **13**, 332 (1969).

(10) R. Patterson and J. F. Kelly, *Ann. Rev. Med.*, **25**, 53 (1974).

(11) R. D. Krell, L. W. Chakrin, and J. R. Wardell, Jr., "Immunopharmacology," Spectrum, New York, N.Y., 1975, pp. 125–148.

(12) R. D. Krell, *Life Sci.*, **19**, 1777 (1976).

(13) R. Patterson, K. E. Harris, I. M. Suszko, and M. Roberts, *J. Lab. Clin. Invest.*, **57**, 586 (1976).

(14) R. D. Krell and L. W. Chakrin, *J. Allergy Clin. Immunol.*, **58**, 664 (1976).

(15) R. Patterson, *Yale J. Biol. Med.*, **40**, 495 (1968).

(16) T. J. Sullivan, K. L. Parker, W. Stenson, and C. W. Parker, *J. Immunol.*, **114**, 1473 (1975).

(17) L. M. Lichtenstein and E. Gillespie, *Nature*, **244**, 287 (1973).

(18) P. Sheard, P. G. Killingback, and A. M. J. N. Blair, *Nature*, **216**, 283 (1967).

(19) L. W. Chakrin, R. D. Krell, J. Mengel, D. Young, C. Zaher, and J. R. Wardell, Jr., *Agents Actions*, **4**, 297 (1974).

(20) R. P. Orange, W. G. Austen, and K. F. Austen, *J. Exp. Med.*, **134**, 136S (1971).

(21) R. D. Krell and L. W. Chakrin, *Int. Arch. Allergy Appl. Immunol.*, **56**, 39 (1978).

(22) E. S. K. Assem and J. L. Mongar, *ibid.*, **38**, 68 (1970).

(23) M. Kaliner, R. P. Orange, and F. K. Austen, *J. Exp. Med.*, **136**, 556 (1972).

(24) J. L. Walker, *Adv. Biosci.*, **9**, 235 (1973).

(25) R. P. Orange, *J. Immunol.*, **114**, 182 (1975).

(26) B. Wuethrich, P. Radielovic, and M. Debelic, *Int. J. Clin. Pharm.*, **16**, 424 (1978).

(27) R. D. Krell and L. W. Chakrin, *Eur. J. Pharm.*, **44**, 25 (1977).

ACKNOWLEDGMENTS

Presented as part of the Symposium on New Approaches to the Treatment of Asthma, APhA Academy of Pharmaceutical Sciences, Anaheim meeting, April 1979.

Antiasthmatic Drug Therapy and Calcium Ions: Review of Pathogenesis and Role of Calcium

ELLIOTT MIDDLETON, Jr.

Received May 18, 1979, from the *School of Medicine, State University of New York at Buffalo, Buffalo, NY 14203.* Accepted for publication July 27, 1979.

Abstract □ This paper presents the calcium-dependent pathophysiological features of allergic and nonallergic asthma. Various theories concerning the role of free calcium ions in the pathogenesis of asthma are discussed.

Keyphrases □ Antiallergic drugs—calcium antagonists, inhibition of calcium influx and intracellular calcium movements, inhibition of mediator release □ Calcium-ion dependency—asthma pathophysiology, cyclic adenosine and guanosine monophosphate levels, mediator release, smooth muscle contraction–excitation, mast cell and mucous cell secretion, vagus nerve activation □ Mediator release—antigen dependency, calcium dependency, antiallergic drugs □ Asthma—review of current theories, clinical evaluation, symposium, antiallergic drugs

The principal pathogenetic features of asthma are ultimately calcium-related phenomena: smooth muscle contraction, mast cell chemical mediator secretion, mucous gland secretion, and vagal cholinergic reflex activity. In these cell types, the availability of free calcium ions for excitation–contraction coupling, stimulus–secretion coupling, and nerve impulse conduction determines significantly the smooth muscle contractility, mast cell mediator secretion, mucous gland secretion, and vagus nerve activity. Increased free calcium-ion concentrations might account for heightened smooth muscle contractility and increased mucous gland secretion and perhaps also for an increased mast cell mediator secretion rate and vagal nerve activity.

If the critical pathogenetic pathways in asthma are ultimately related to free calcium-ion availability in smooth muscle, mast cells, mucous glands, and vagus nerve, it follows that effective asthma drug therapy must reduce calcium availability to the essential contractile, secretory, and vagus nerve functions. This concept places altered transmembrane and intracellular calcium movements at the level of final common pathway for the

0022-3549/80/0200-0243$01.00/0

© 1980, American Pharmaceutical Association

pathogenesis of asthma, regardless of the proximate stimulus (*e.g.*, allergy, infection, or exercise) and may be considered the *calcium hypothesis of asthma*.

DISCUSSION

Asthma is a clinically heterogeneous disorder. Examples of heterogeneity are noted in nonuniform heredity, variable airways reactivity and physiological abnormalities (large *versus* small airway involvement), immunologic features (allergic *versus* nonallergic asthma and the unique nonimmunologic form of aspirin-induced asthma), and variable therapeutic response with different drug classes. Furthermore, many asthma precipitants are recognized clinically including allergy, certain viral infections, exercise, cold air, respiratory irritants, aspirin (~10% of asthma patients), and emotional state, especially fear and anger. Does each of these aspects of clinical heterogeneity and variable precipitants represent different biochemical pathways? Perhaps in a proximate sense they do, yet the final expression of the pathological changes in asthma must finally be related to the translocation of calcium ions to activate the smooth muscle contractile system, the mast cell and mucous gland secretory systems, and nerve impulse initiation and conduction in vagal fibers.

It is reasonable to consider the interaction between an excitatory agent and specific tissue receptors (*e.g.*, antigen with mast cells or histamine or acetylcholine with smooth muscle) as the stimulus that activates some calcium membrane transport or membrane releasing system, or both. Following activation, the transport (or releasing) system promotes calcium-ion flow from intra- and/or extracellular storage sites into the cytoplasmic matrix. The result is an increase in free calcium ions near the contractile proteins in smooth muscle, the secretory apparatus in mast cells and mucous glands, and the nerve impulse initiation and conduction systems. The interaction of the calcium ions with the relevant specific sites then initiates a response.

When the excitatory agent is removed from the vicinity of the smooth muscle, mast cell, mucous gland, or nerve cell, calcium-ion flow into the cytoplasm is reduced and the cellular response is decreased. With smooth muscle, a gradual reduction in the calcium-ion level is accompanied by a gradual dissociation of calcium from the contractile elements and by a gradual decrease in the capacity of the contractile machinery to maintain increased tension. Removal of free cytoplasmic calcium is considered to be accomplished by a metabolically dependent calcium pump located in the plasma membrane and/or the membranes of intracellular organelles. The pump transports calcium from the cytoplasm to sequestration sites or to the external medium.

Based on these considerations, it is inferred that both the rate of inward flow or the release of calcium ions (from extra- or intracellular calcium stores) initiated by the excitatory agent and the simultaneous removal rate of free cytoplasmic calcium by the energy-linked calcium pump are important in determining the final cytosol calcium concentration. Since the calcium ions enter into a reversible interaction with specific contractile and secretory proteins, depending on cell type, their concentration in the cytoplasm determines the extent to which they elicit a response. It might be assumed, therefore, that the free calcium concentration in the cytoplasmic matrix and, therefore, the rate at which the calcium transport system delivers calcium to the cytoplasmic matrix would be important in the magnitude of the response.

The application of physiological techniques has uncovered energy-dependent calcium-concentrating systems in mitochondrial and microsomal preparations from smooth muscle. Therefore, it can be inferred that these structures possess a calcium-sequestering function, which, in the case of smooth muscle, induces relaxation. Conversely, these structures possibly may function as sites from which activator calcium can be mobilized for contraction. Comparable regulatory mechanisms may exist in mast cells and mucous glands.

The mechanisms by which excitatory agents mobilize calcium from different storage sites apparently vary from tissue to tissue. Vascular and nonvascular smooth muscle studies suggested that membrane depolarization is associated predominantly with the mobilization of extracellular or loosely bound calcium. It is difficult, however, to generalize about the manner in which an excitatory agent mobilizes calcium or about the calcium sources in smooth muscles, mast cells, mucous glands, or nerve tissues.

The manner in which the calcium transport system is coupled to the excitatory agent–receptor complex is not clear, and it is difficult to propose a model that accounts for graded increases in the activation of a calcium transport system that presumably occurs with incremental increases in the excitatory agent concentration (*e.g.*, antigen with mast cells or histamine or acetylcholine with smooth muscle).

Any exciting agent stimulating the relevant cell types in the lung in asthma (smooth muscle, mucous glands, mast cells, and nerve) may involve an induced shift of calcium ions from storage sites to the cytoplasmic matrix; this shift is carried out by a finite number of cellular transport sites which exist in cellular membranes. These calcium transport sites normally function at a low activity level unless activated by two independent cellular reactions. One of these reactions involves calcium transport site activation in an unknown fashion by the interaction of the excitatory agent with specific tissue receptors. The second involves reversible complex formation between calcium in storage depots and the activated calcium transport sites.

The entry rate of free calcium into the cytoplasmic matrix and, ultimately, the cellular response are dependent on the fraction of the total number of calcium transport sites participating in each type of reaction. Calcium transport site activation may be accomplished by a complex interaction between the excitatory agent and specific tissue receptors, which seem to be linked to the transport sites. The increase in calcium conductance that results from transport site activation is usually a graded response. It follows that calcium-ion transport by activated sites must be preceded by the formation of a reversible complex between the calcium transport sites and the calcium ions that are to be transported into the cytoplasmic matrix and, eventually, that are to interact with contractile, secretory, and nerve excitation systems.

Many recognized calcium-dependent processes are important in asthma pathogenesis: excitation–contraction coupling in smooth muscle, stimulus–secretion coupling in secretory cell types including mast cells and mucous glands, and microtubule function and calcium effects on certain enzyme systems such as adenyl cyclase, guanyl cyclase, and phosphodiesterase activator protein.

Currently utilized drugs presumably indirectly affect calcium movement and disposition by acting on the adenyl cyclase–cyclic adenosine monophosphate system (β-agonists) and, perhaps, through phosphodiesterase inhibition (theophylline); cromolyn sodium, however, may have some calcium-antagonistic properties, *i.e.*, inhibition of calcium uptake by activated mast cells. The relationship of the antiasthmatic effects of corticosteroids to calcium movement is obscure. The possibility of developing compounds with calcium-antagonistic properties selective for cells involved in asthma pathogenesis is attractive, and such compounds are being studied.

Calcium antagonists can be defined as compounds that affect the translocation of calcium ions across cell membranes and within cells; certain calcium antagonists appear to act by affecting transmembrane calcium translocations while others affect intracellular calcium movements. Their mechanism of action is complex and ill defined. Some calcium antagonists have been studied for their effects on myocardial and vascular smooth muscle responses to various agonists (and are in use therapeutically, *e.g.*, verapamil), a few have been studied for their effect on nonvascular smooth muscle, and several have been studied for their ability to block specific secretory processes (*e.g.*, catecholamine secretion from the adrenal medulla), but few investigations of calcium antagonists on mediator secretion from mast cells or basophils have been undertaken.

IMMUNOLOGICAL–CHEMICAL MEDIATOR THEORY: REGULATION OF CHEMICAL MEDIATOR RELEASE

Immunoglobulin E-sensitized mast cells and basophils can be triggered to release chemical mediators upon stimulation with a specific antigen, and the release process has an absolute requirement for calcium ions (*cf.*, 1–7). Indeed, calcium microinjection into mast cells provokes local granule extrusion (8). It was believed that the chemical mediators acted locally and directly on smooth muscle, blood vessels, and mucous glands and attracted eosinophils and other inflammatory cells to produce the characteristic pathological changes of asthma in the lung. Not all causes of asthma have an identifiable immunological basis, however, so it is safe to say that allergy is a sufficient, but not an exclusive, explanation for asthma.

The discovery of immunoglobulin E (*cf.*, 9) established the immunological basis of immediate-type hypersensitivity reactions and spurred research on the mechanisms by which chemical mediators are released from mast cells and basophils. It is now well recognized that immunoglobulin E antibody is firmly bound to special receptors in the cell membrane of mast cells and basophils and that interaction of the bound antibody with specific antigen initiates a series of intracellular biochemical reactions, which culminate in the noncytolytic secretion of

preformed mediators and the generation and release of several mediators that exist as precursors (1–7).

The essential role of immunoglobulin E receptors in mediator release from rat mast cells was demonstrated by Ishizaka and Ishizaka (10), who found that antibodies specifically directed against immunoglobulin E receptors caused histamine release and immediate skin reactions in normal rats. This important observation indicates that membrane perturbation, presumably involving the spatial relationships of the immunoglobulin E receptor and subtended functional units, is sufficient to initiate the biochemical sequence required for mediator release.

The list of mast cell- and basophil-derived mediators includes: (*a*) preformed mediators such as histamine, eosinophil chemotactic factor of anaphylaxis, eosinophil chemotactic factor oligopeptides, neutrophil chemotactic factor, heparin, chymase, lung kallikrein of anaphylaxis, superoxide radical, and superoxide dismutase; and (*b*) newly generated mediators such as slow-reacting substance of anaphylaxis, platelet-activating factor(s), lipid chemotactic factors, and prostaglandins. The source, nature, properties, and metabolism of these substances have been reviewed extensively (1–6, 11). Some or all of these substances may participate in the pathogenesis of the acute and chronic aspects of lung pathology and altered physiology in various types of asthma, acting through direct and reflex mechanisms. It is also pertinent that histamine and slow-reacting substance of anaphylaxis require calcium for stimulation of smooth muscle contraction.

Extensive studies (1–6) of immunoglobulin E-dependent immunological release of chemical mediators from mast cells and basophils have provided a partial picture of the biochemical events involved and, thus, the various loci for potential pharmacological intervention. Interaction of specific antigen with mast cell-bound immunoglobulin E antibody presumably results in membrane perturbation associated with extracellular calcium-ion transport into the cell. This initial step is accompanied by the activation of a proesterase, which is converted to an active chymotrypsin-like serine esterase, which subsequently engages in further autocatalytic activation. Possibly, calcium-ion influx is essential for initial activation and autocatalytic activation of the proesterase to the esterase. The active esterase decays quite rapidly.

A subsequent energy-dependent step can be inhibited by 2-deoxyglucose. Precisely what function in the mediator release sequence is subserved by the energy requirement is not certain, but it may be related to the function of a "contractile protein" since dense bands of microfilaments have been observed around mast cell granules, especially during degranulation. Microfilament function is probably involved in mediator release, and the function of microfilaments can be affected by cytochalasins A and B. Studies with these agents indicated that histamine release and slow-reacting substance of anaphylaxis generation and release can be dissociated. Although cytochalasins A and B enhance histamine release, they inhibit the concomitant formation of slow-reacting substance of anaphylaxis. An interesting study (12) indicated a significant difference in the cytochalasin sensitivity of histamine release in a comparison of asthmatic and normal basophils.

Several studies indicated an important function of microtubules in mediator release from mast cells and basophils. Colchicine, a compound that binds to the microtubular subunit protein, thereby preventing microtubule assembly and function, inhibits histamine release. Heavy water (D_2O), on the other hand, favors microtubule subunit aggregation and facilitates histamine release.

The metabolic regulation of chemical mediator release has been investigated intensively. One aspect of metabolic control of mediator release that is quite well worked out in several *in vitro* systems involves the effects of cyclic adenosine monophosphate, cyclic guanosine monophosphate, and agents that affect their intracellular concentration. Numerous studies (1–6) indicated that agents that increase cyclic adenosine monophosphate levels (*e.g.*, β-agonists, histamine, prostaglandin E, and cholera toxin) tend to decrease chemical mediator release, while agents that decrease cyclic adenosine monophosphate levels (*e.g.*, α-adrenergic agonists) tend to increase chemical mediator release. On the other hand, compounds that increase cyclic guanosine monophosphate levels (*e.g.*, cholinergic agents and, perhaps, α-adrenergic compounds) tend to increase chemical mediator release. Indeed, recent evidence appears to establish cyclic guanosine monophosphate as an important mediator of immunological mediator release in human lung (13).

The effect of each adrenergic or cholinergic agonist can be blocked by antagonists such as propranolol, phentolamine, and atropine, substantiating the β- and α-adrenergic and muscarinic cholinergic nature of the receptor systems involved. Thus, chemical mediator release can be modulated by products of the sympathetic and parasympathetic nervous system and other substances acting through specific receptor systems and affecting the intracellular levels of cyclic adenosine monophosphate and cyclic guanosine monophosphate. Calcium plays a critical role in control of intracellular cyclic nucleotide concentrations through its ability to inhibit adenyl cyclase (14), stimulate guanyl cyclase (15), and regulate the phosphodiesterase regulator protein (16).

NEUROGENIC THEORY

The neurogenic theory of asthma pathogenesis is grounded in the observation that stimulation of subepithelial airway receptors by released chemical mediators or other irritants results in a vagally mediated cholinergic bronchoconstrictor reflex, which can be markedly attenuated or abolished by atropine or experimental manipulations that attenuate vagal afferent and efferent impulse transmission. Atropine acts competitively to inhibit the calcium-dependent effects of released acetylcholine on smooth muscle and mucous glands. The asthma-worsening effect of viral respiratory infections that denude the epithelium is thought to be due to sensitization of these rapidly adapting subepithelial sensory receptors; in normal subjects with "colds," a transient (1–6 weeks) hyperirritability of the airways to inhaled histamine or citric acid aerosols has been demonstrated (17). Much evidence supports the neurogenic theory, and it has been reviewed extensively (18–21).

Mediators immunologically released from intraluminal airway mast cells or basophiloid cells (22) may stimulate vagal sensory endings and increase bronchial epithelial permeability to macromolecular antigens, allowing them to gain access to submucosal mast cells where further mediator release might occur. Although cholinergic agents increase immunological mediator release *in vitro*, there is no evidence that cholinergic stimulation *in vivo* does the same thing.

β-ADRENERGIC BLOCKADE THEORY

Another major theory concerned with asthma pathogenesis is that of β-adrenergic blockade proposed by Szentivanyi (23). Briefly, this theory states that there is diminished responsiveness of β-adrenergic receptors to stimulation by β-adrenergic agents. This theory implies that β-receptor-mediated relaxation of smooth muscle and β-receptor-mediated functions of other cells and organ systems might be reduced. It also implies a diminished accumulation of cyclic adenosine monophosphate following stimulation with β-agonists because of the linked relationship of β-receptors and adenyl cyclase to convert adenosine triphosphate to cyclic adenosine monophosphate. Adenyl cyclase is a membrane-bound, magnesium-dependent enzyme and is inhibited by calcium ions. Although not totally conclusive, considerable evidence supports this theory and the data have been reviewed extensively (23–27). An interesting recent supportive observation also was reported: salbutamol inhalation increased plasma and urinary cyclic adenosine monophosphate levels in both normal and asthmatic subjects, but the increase in asthmatics was significantly less (28).

A major objection to the β-adrenergic blockade theory arose when several investigators (29–32) noted that asthmatic patients or normal individuals given β-adrenergic drugs developed subsensitivity of leukocytes to stimulation by isoproterenol as determined by cyclic adenosine monophosphate accumulation. These studies indicated that the apparent state of "β-adrenergic blockade" was, in fact, drug induced. However, Busse (33) later studied asthmatic patients who had not received β-adrenergic drugs for 2 weeks and determined that the isoproterenol-induced inhibition of polymorphonuclear leukocyte lysosomal enzyme release was reduced as well as the isoproterenol-stimulated accumulation of cyclic adenosine monophosphate in these cells. These data are generally consistent with the β-adrenergic blockade theory. Bush *et al.* (34) also found a decreased isoproterenol polymorphonuclear leukocyte response in normal individuals during rhinovirus infection, indicating that the viral infection produces dysfunction of the β-receptor–adenyl cyclase system in nonpulmonary tissues. Thus, viral infections appear to have metabolic effects on cell function and direct airway effects. Precisely what aspect of enzymatic control of cyclic adenosine monophosphate synthesis (or degradation) is affected by the virus or the products of viral infection remains to be determined.

Recent experiments on asthmatic and nonasthmatic dogs (sensitive to *Ascaris suum*) compared trachael ring smooth muscle responsiveness to antigen, methacholine, and isoproterenol; isoproterenol induced consistently greater relaxation in nonasthmatic rings following standardized precontraction with methacholine (35). The finding of isoproterenol hyporesponsiveness in natively asthmatic dogs, under conditions excluding possible receptor densensitization due to prior exposure to β-agonists, is consistent with the β-adrenergic blockade theory but does

not exclude the importance of vagal mechanisms.

In summary, solid evidence apparently substantiates the possibility of β-adrenergic receptor–adenyl cyclase dysfunction in certain cells of asthmatic patients (and dogs). However, the studies do not shed any light on the locus of the biochemical–pharmacological defect. Since the β-receptor–adenyl cyclase system is considered to be composed of a recognition or discriminator (receptor) unit, a transducer unit, and a catalytic unit, dysfunction could occur at any (or all) of these levels.

It is now well recognized that guanosine triphosphate is an important regulator of adenyl cyclase in many hormone-stimulated systems (36). The data indicate that guanylyl nucleotide regulation of adenyl cyclase activity is not only ubiquitous but also independent of the receptor coupled to the enzyme. It is also known that the guanosine triphosphate concentration is under the control of a phosphatase (36–38). Thus, it is possible that cells of asthmatics may have abnormal function of this regulator site or possibly increased phosphatase activity, which would indirectly depress adenyl cyclase function. There are no data to support these ideas, but it is interesting to consider the therapeutic possibilities of agents that might act at the guanosine triphosphate regulator site to increase adenyl cyclase responsiveness to β-agonists or that might inhibit the phosphatase.

REDUCED FUNCTION OF NONADRENERGIC INHIBITORY SYSTEM THEORY

Another theory is reduced function of the nonadrenergic inhibitory system in the lungs of asthmatic patients (39). Human airways, from the midtrachea to the distal bronchi, have been studied *in vitro* for inhibitory nerves (40). Electrical field stimulation of the tissues demonstrated cholinergic excitatory nerves inhibitable by atropine. In the presence of atropine, field stimulation of the tissues relaxed smooth muscle, even when it was contracted by histamine. This relaxation was neither blocked nor modified by adrenergic antagonists. Nerve stimulation involvement in the field stimulation–induced relaxation was established when tetrodotoxin blocked the effect.

This airway relaxation system shares some characteristics of the nonadrenergic inhibitory system in the GI tract and of the comparable system reported in guinea pig tracheal smooth muscle. Moreover, no evidence of adrenergic inhibitory fibers in human bronchial muscle could be found by either pharmacological or histochemical techniques. The nonadrenergic inhibitory system may be the principal inhibitory system of human airway smooth muscle, and a defect in this system might be a possible explanation for the hyperreactivity of the airways noted in asthma patients.

The asthma theories reviewed are not mutually exclusive. Nevertheless, in each case, the final common denominator is considered to be the increased availability of free (activator) calcium ions for excitation–contraction coupling in smooth muscle, stimulus–secretion coupling in mast cells and mucous glands, and nerve impulse initiation and conduction in the vagus.

AIRWAY HYPERIRRITABILITY

Any asthma theory must explain the disease hallmark, namely, hyperirritability of the airways from various stimuli. Hyperirritability has been demonstrated in clinical investigations with various substances that are chemically unrelated but require calcium for their smooth muscle stimulating activity. These agents include acetylcholine, methacholine, histamine, slow-reacting substance of anaphylaxis, prostaglandin $F_{2\alpha}$, and bradykinin.

Airway hyperreactivity suggests that the airway smooth muscle is partially depolarized, *i.e.*, reactive to low doses of various chemically diverse substances or stimuli. The basic cause of the hyperreactivity (or postulated state of partial depolarization) is unknown but certainly suggests an increased permeability of smooth muscle cell membrane to calcium ions, which activate the contractile machinery. The single piece of evidence that possibly supports increased permeability (or partial depolarization) was provided by Simonsson *et al.* (41), who demonstrated that airway smooth muscle from a patient with chronic bronchitis responded *in vitro* to very low doses of carbachol and bradykinin as compared to normal bronchial smooth muscle. Whether such findings might obtain in asthma remains to be determined.

BIOLOGICAL PROPERTIES OF CALCIUM AND CALCIUM ANTAGONISTS

Calcium plays a critical and central role in many biological events at both the intra- and extracellular levels (42, 43). However, calcium distribution across the cell membrane is far from equilibrium; if the testing membrane potential (~−60 mv) were equal to the calcium equilibrium potential, then the intracellular calcium activity should be some 100-fold greater than the extracellular activity. This is clearly not so. Although accurate measurements of free ionized intracellular calcium concentrations have not been made in many systems, the consensus of evidence firmly indicates that the free intracellular calcium concentration is less than 10^{-7} *M* (44–46).

The very large driving force for calcium entry indicates that specific mechanisms must exist for intracellular calcium removal. Subsequent to entry, calcium may be removed through complexation with cytoplasmic constituents (including the internal membrane surface) or by sequestration into the intracellular structures, mitochondria (47, 48), and sarcoplasmic reticulum (49, 50) of smooth muscle, for example.

Although there can be no doubt as to the importance of calcium uptake by the active transport processes mediated by mitochondria and sarcoplasmic reticulum, the cell must, to maintain its total calcium reasonably constant, ultimately transport calcium to the external medium. Two major processes for intracellular calcium removal have been described. In one process, calcium extrusion is directly coupled to adenosine triphosphate hydrolysis; in the second process, calcium extrusion is coupled to an influx of sodium ions.

CALCIUM ENTRY PROCESS

Given the existence of the several mechanisms that operate to maintain a low intracellular free calcium concentration, there must also exist mechanisms that increase intracellular calcium concentrations and couple membrane excitation to intracellular calcium-modulated events. This calcium may be derived from intracellular stores or from extracellular sources. Although initial emphasis was placed by Hodgkin and Huxley (51) on Na^+ and K^+ as the current carrying species during squid axon excitation, there is now substantial evidence for this and many other tissues that calcium entry through "specific calcium channels" also contributes to the total membrane current (44, 46, 52, 53).

Of particular importance, pharmacological differentiation of the Na^+, K^+, and calcium channels is possible with selective antagonists. Tetrodotoxin and tetraethylammonium are well known for their actions on Na^+ and K^+ channels, respectively (54), and the inorganic ions Mg^{2+}, Mn^{2+}, Ni^{2+}, Co^{2+}, and La^{3+} and the organic agents verapamil, methoxyverapamil, and nifedipine have gained prominence as calcium channel antagonists (46, 55, 56). There is an obvious analogy between this differentiation of ion channels and the differentiation of pharmacological receptors through specific antagonist action.

There is not considerable evidence that the calcium entry process similar to that seen in the squid axon occurs in several excitable tissues and that a calcium channel is utilized distinct from that carrying the early sodium current. The basis for the differentiation of such a process rests on the following properties:

1. Membrane currents and potential changes in Na^+-free solution are basically identical to those seen in Na^+-containing media in the presence of tetrodotoxin or in preprations where the Na^+ channel has been inactivated by prior depolarization.
2. The calcium current is insensitive to tetrodotoxin and tetraethylammonium but is sensitive to antagonism by Mg^{2+}, Mn^{2+}, Co^{2+}, La^{3+}, verapamil, methoxyverapamil, and nifedipine.
3. Both Sr^{2+} and Ba^{2+} can substitute for calcium.
4. The threshold voltage- and time-dependent activation and inactivation parameters and gating currents are quite distinct from those determined for the early sodium current.

As judged by these criteria, calcium channels mediating calcium translocation have been described in various preparations, from protozoan to mammalian (57), although complete ionic, electrophysiological, and pharmacological characterization is not available in many instances.

CALCIUM CHANNEL ANTAGONISTS

The di- and trivalent cations Mn^{2+}, Ni^{2+}, Co^{2+}, and La^{3+} and the organic molecules verapamil, methoxyverapamil, and nifedipine are defined as calcium channel antagonists, and their actions serve as one important component of calcium channels. However, neither the sites nor the mechanisms of action of these antagonists have been defined precisely.

Certain data also suggest that the channels may possess gating mechanisms involving phosphatidylinositol breakdown as the initial event

required to open calcium channels following agonist–receptor interaction (58, 59). Phenoxybenzamine, an α-adrenergic blocker with antagonistic effects against several chemically unrelated agonists, was the only one of a number of compounds tested found to block phosphatidylinositol breakdown and to inhibit smooth muscle contraction (60). The nature and controlling mechanisms of calcium gates/channels is far from completely understood, but they are of central importance to the calcium hypothesis of asthma.

Various organic calcium antagonists have been developed which block entry of calcium ions from the outside to the inside of the cell and which can modify the effects of various calcium-dependent agonists (61). Some examples are verapamil, methoxyverapamil, nifedipine, benzyclane, prenylamine, and cinnarizine. These compounds have been studied mainly for their effect on myocardial cell function as well as on coronary and other vascular smooth muscles. Verapamil and methoxyverapamil have potent effects on excitation–contraction coupling in several vascular smooth muscle preparations and also in guinea pig ileal smooth muscle (57). Verapamil and methoxyverapamil are also quite potent inhibitors of stimulus–secretion coupling in several systems (57).

Other calcium antagonists such as the aminoindenes (62, 63) and mecinarone (66) appear not to block the calcium entry process but rather to affect the intracellular sites of calcium action. This was demonstrated in studies of cholinergically stimulated release of catecholamines from the adrenal medulla (62) and also with histamine- and carbachol-induced contraction of different smooth muscle preparations (63). Ketotifen, an effective antiasthmatic drug (64), inhibits calcium 45 uptake by rat mast cells (65) and also possesses antihistaminic activity (H_1). Compounds with such properties are of interest for both theoretical and practical considerations in drug design, *i.e.*, in the search for compounds that affect the essential intracellular calcium movements required for contractile and secretory phenomena. The effects of certain calcium antagonists on rat mast cell mediator release will be described later.

It is plausible that the calcium channel organization is basically similar to that suggested for the sodium channel by Hille (54). An important component of Hille's model is the channel cation coordination site, which constitutes a rate-limiting selectivity filter. The energetics of cation interaction at this site determine whether a cation is a permeant or nonpermeant species. In the case of the sodium channel, Na^+ binds the least well and is the most permeant. The geometry and ligand characteristics of this proposed site determine the selectivity between monovalent and divalent cations (67, 68) and, in the case of the calcium channel, divalent cations with ionic radii similar to those of calcium may be expected to interact with this site and to serve as substitutes for or antagonists of calcium permeation.

Little quantitative data exist for ion interactions at the calcium channel. However, for antagonism, it is clear that $M^{3+} > M^{2+}$ (69) and that among divalent cations the order of permeation is $Ba^{2+} > Sr^{2+} > Ca^{2+} \gg Mg^{2+}$; Ni^{2+} and Co^{2+} serve as antagonists, and Mn^{2+} acts as both an antagonist and permeant species (70). These findings suggest that the ionic radius and hydration energy may be important factors in determining cation interaction and permeation in the calcium channel. Many of these effects of di- and trivalent cations are likely related to a general adsorption to negatively charged membrane sites. Finally, the lanthanide series of cations are calcium channel antagonists, probably by virtue of their rather general ability to substitute for calcium at calcium-binding sites (71, 72).

The organic calcium antagonists verapamil, methoxyverapamil, and, to a lesser extent, nifedipine have achieved prominence recently and are classified, largely on the basis of electrophysiological evidence in cardiac preparations, as specific calcium channel antagonists (55, 56, 73–75). At 10^{-6}–10^{-8} *M*, these agents produce a selective antagonism of the slow calcium current with minimal effect on the fast sodium current and thus produce electromechanical decoupling of the heart in the activity sequence nifedipine > methoxyverapamil > verapamil (56, 74, 76–79). The effects of these agents are overcome by increased extracellular calcium.

Because of the high activity and apparent selectivity of action of these agents, their activity in other calcium-utilizing systems can be viewed as evidence that basically similar slow calcium channels are operative in secretory and mechanical processes (57). A complete characterization of the calcium current is not available for many of these processes. However, the similar activities of these antagonists in cardiac and smooth muscle and secretory systems certainly indicate a common basis of action. In a few preparations, widely different activities have been noted according to the stimulus employed; this finding is probably indicative of different modes of calcium translocation. Thus, in rabbit mesenteric artery, verapamil is ~1000 times more active against K^+ than against norepinephrine-induced contractions, suggesting that norepinephrine and K^+ employ principally intracellular and extracellular calcium sources, respectively (80). In contrast, K^+ and muscarinic receptor-induced contractions of guinea pig ileal longitudinal smooth muscle appear, as judged by antagonist activities, to employ identical calcium translocation mechanisms.

Where kinetic evidence is available, verapamil, methoxyverapamil, and nifedipine appear to act noncompetitively against agonists and competitively against calcium. An exceptional situation apparently exists in cardiac tissue where the effects of these agents can be overcome by increasing extracellular calcium and by catecholamines (55, 56, 74) so that a competitive relationship appears to exist also between β-agonists and the calcium antagonists (81, 82). A likely explanation is that the action of catecholamines at the cardiac β-receptor is to increase the available calcium channels, perhaps by a cyclic adenosine monophosphate-dependent mechanism (83). Nifedipine, which shows no apparent structural similarity to verapamil or methoxyverapamil, appears to behave similarly.

There is no question but that verapamil, methoxyverapamil, and nifedipine act as potent antagonists of the slow inward calcium current. There is also evidence that these agents may have important effects on other ionic processes. Verapamil and methoxyverapamil, at the fairly high concentrations used in the squid axon (2×10^{-4} *M*), do reduce the fast Na^+ current; in cardiac fibers, these agents also appear to have some effect on both the Na^+ and K^+ currents (84, 85). However, some part of their effects on K^+ currents probably is due to the dependence of the latter on calcium entry (86).

Whether nifedipine, which is generally assumed to be a calcium channel antagonist acting similarly to verapamil and methoxyverapamil but of higher activity, shares all of the actions of verapamil and methoxyverapamil is not fully established. However, nifedipine does exert a greater depressant effect on the guinea pig atria with increasing stimulus frequency, suggesting that it also modifies the kinetics of the calcium restitution processes (87). That compounds of such differing structure as verapamil and nifedipine exert similar effects on both the calcium permeation and restitution processes is perhaps suggestive that both processes are controlled through interaction at a common site.

EXCITATION–CONTRACTION COUPLING IN SMOOTH MUSCLE

The source of calcium utilized in smooth muscle excitation–contraction coupling remains ill-defined since, unlike fast skeletal muscle, the smaller less well-developed sarcoplasmic reticulum and slower and more sustained contractile responses of smooth muscle do not impose the dominant demands for an intracellular calcium mobilization–sequestration system that is seen in skeletal muscle. Hence, in smooth muscle, intracellular and extracellular sources of calcium may be utilized in excitation–contraction coupling; the relative usage depends on the tissue, the experimental conditions, and the stimulus (88–93).

Sarcoplasmic reticulum does exist in smooth muscle, and studies (94–96) showed that the amounts vary significantly, being greatest in rabbit main pulmonary artery and least in portal anterior mesenteric vein and taenia coli. A correlation exists between the sarcoplasmic reticulum volume and the ability to sustain contraction in the absence of extracellular calcium. Furthermore, the central and peripheral tubules of sarcoplasmic reticulum are continuous with each other, and the peripheral tubules enjoy a close relationship with the surface membrane in some tissues, suggesting that this arrangement may provide the structural basis for calcium release by the invasion of the action potential (94–98). Calcium-sequestering subcellular fractions have been isolated from several smooth muscles (89, 99–101). However, the quantitative contribution of sarcoplasmic reticulum calcium to excitation–contraction coupling is not established, and it is also clear that there is no obligatory association between electrical and mechanical events for some smooth muscles (94, 97, 102–105). Agonist and antagonist actions are identical or very similar to polarized and depolarized tissues, and excitation–contraction coupling can therefore be independent membrane potential changes.

Attempts to define intracellular or extracellular calcium pools as the source of calcium used in smooth muscle excitation–contraction coupling simply on the basis of dependence of responses on extracellular calcium is of questionable value. Thus, Keatinge (104) showed, for norepinephrine-induced contractions of sheep carotid artery, that a 30-min incubation in calcium-free media left sufficient extracellular calcium to sustain excitation–contraction coupling. Furthermore, in any experiments in which calcium is altered, significant "stabilizing" or "labilizing" actions

occur at the cell membrane level and may profoundly alter membrane excitability (105–108).

Finally, even where tissues do show extreme dependence on extracellular calcium, this does not imply equivalent utilization of extracellular calcium. Studies on guinea pig ileal longitudinal and rat vas deferens, both of which are extremely dependent on extracellular calcium, are illustrative of this fact (109, 110). Comparison of the action of calcium antagonists including La^{3+}, methoxyverapamil, nifedipine, 2-diethylaminoethyl-2,2-diphenyl valerate, and local anesthetics revealed significant differences in their activities in these two tissues. It was concluded: "that although qualitative similarities are apparent with respect to the dependence of agonist-induced activity in both the guinea pig ileum and rat vas deferens it is clear that calcium translocation processes in these two tissues, as examined by the use of a variety of calcium antagonists" are both tissue and agonist selective (110). The calcium ionophore A-23187 produces contractile responses in the guinea pig ileal but not in the rat vas deferens smooth muscle (111). Clearly, differences in calcium utilization are involved in these two smooth muscles which, however, show an equal apparent dependence on extracellular calcium.

It has been suggested that the lanthanide cations may provide a method for distinguishing between intracellular and extracellular calcium use (112–116) on the basis that the similar ionic radius of La^{3+} and calcium and the higher charge density of La^{3+} will enable it to replace calcium influx and efflux. If these assumptions are correct (53, 96, 117), then the use of La^{3+} may permit a qualitative diagnosis of intracellular *versus* extracellular calcium use. Moreover, because La^{3+} is presumed to displace the superficial, rapidly exchangeable calcium and prevent calcium flux, it can be used to "trap" intracellular calcium and, hence, to obtain a quantitative estimate of any change in intracellular calcium occurring during excitation–contraction coupling. Thus, in guinea pig ileal longitudinal muscle, phasic and tonic components of the acetylcholine- and K^+-induced responses are equally (and highly) sensitive to La^{3+}, suggesting that a similar or identical calcium source is involved (53). In rat uterus, acetylcholine responses are significantly more resistant than K^+ responses (115). In rabbit aorta, La^{3+} abolishes the slow phase of the norepinephrine response but leaves the fast phase unchanged (114, 115), suggesting that extracellular and intracellular calcium components are utilized, respectively.

The "lanthanum method" has also been used to determine quantitatively extracellular calcium uptake during excitation–contraction coupling. Thus, in rabbit aorta, Van Breemen *et al.* (114) showed that there were parallel changes in tension development and intracellular calcium content during K^+ or Li^+ stimulation but only after the complicating effects of extracellular calcium exchange had been eliminated by La^{3+} treatment.

Although progress is being made in the delineation of the relative use of intracellular and extracellular calcium in smooth muscle excitation–contraction coupling, the mechanisms by which drug–receptor interactions are linked to calcium mobilization are poorly understood. Several mechanisms must be considered:

1. Coupling of membrane potential changes to calcium release from sarcoplasmic reticulum. This may be regarded as some equivalent of skeletal muscle excitation–contraction coupling and may also include an influx of "trigger" calcium that initiates an intracellular calcium release.
2. Direct entry of calcium through action potentials that totally or partially use calcium as the current-carrying species.
3. Mobilization of calcium (intracellular or extracellular) by potential-independent changes, *i.e.*, the pharmacomechanical coupling of Somlyo and Somlyo (94, 97).
4. Influx of calcium through intracellular sodium–extracellular calcium exchange processes.

These processes are not mutually exclusive, and it is entirely possible that their combinations are used in a given smooth muscle. Additionally, it is obviously necessary to consider the roles of cyclic adenosine monophosphate and cyclic guanosine monophosphate because of their well-described relationships to receptor processes, notably to adrenergic (α and β) and cholinergic (muscarinic) processes (*cf.*, 118–121), and because of the mutual interrelationship of calcium and the cyclic nucleotides in many systems (122, 123). Increasing evidence suggests that the cyclic nucleotides may, through their activation of protein kinase, control membrane phosphorylation and that this may serve to control ion permeabilities (124, 125).

Finally, a comment must be made on the possible role of calcium as a component of membrane receptors such that agonist displacement of the calcium serves to labilize the membrane and initiate excitatory events and enhanced calcium binding serves to stabilize the membrane. Some implications of this proposal were considered previously (53, 108, 126).

As noted previously, calcium entry through specific calcium channels is one source of calcium for excitation–contraction coupling in smooth muscle. The guinea pig ileal longitudinal smooth muscle represents one such system (53, 126–128). This tissue is very sensitive to extracellular calcium for both K^+ and acetylcholine responses, and both responses are equally sensitive to La^{3+}, methoxyverapamil, and nifedipine.

Calcium 45 uptake has been measured by the "lanthanum procedure" and is equally sensitive to the calcium antagonists as the mechanical responses. A 1:1 relationship was found for calcium uptake and mechanical response and, in a series of muscarinic agonists and partial agonists, decreased calcium uptake was associated with partial agonists. The guinea pig ileal longitudinal smooth muscle is sensitive to stimulants including K^+, acetylcholine, and histamine, and the responses are all equally sensitive to the calcium antagonists methoxyverapamil and nifedipine. This finding suggests that several stimulants serve to activate a common pool of calcium channels.

Much of the reviewed material with respect to smooth muscle physiology and pharmacology may be relevant to calcium-ion involvement in chemical mediator releasing systems.

CALCIUM AND CHEMICAL MEDIATOR RELEASE

Lichtenstein's studies (129) with the basophil system indicated that the release reaction can be divided into two stages: first, an antigen-dependent, calcium-independent stage, and second, an antigen-independent, calcium-dependent stage. The agents affecting histamine release *via* increased accumulation of cyclic adenosine monophosphate appear to act only in the first stage whereas agents affecting microtubule function (colchicine and deuterium oxide) act in the calcium-dependent second stage.

Further investigation (130) of the mechanism of mediator release from chopped human lung revealed an additional edetic (ethylenediaminetetraacetic) acid-inhibitable, calcium-dependent step beyond the initial immunological stimulation of (presumed) increased permeability of the cell membrane to calcium and ingress of calcium ions. The experiments of Kaliner and Austen (130) also disclosed that isoproterenol increased cyclic adenosine monophosphate concentrations and prevented the observed calcium reversal of edetic acid inhibition of mediator release, suggesting that the second calcium-dependent step might relate to the action of a contractile protein susceptible to inhibition by phosphorylation by the cyclic adenosine monophosphate-dependent protein kinase system (130). In this connection, it is important to recall that calcium plays a critical role in the control of intracellular cyclic adenosine monophosphate and cyclic guanosine monophosphate concentrations through its ability to inhibit adenyl cyclase (14), stimulate guanyl cyclase (15), and control the phosphodiesterase regulator protein (16).

The most familiar pathophysiological stimulus to the mast cell is the antigen–antibody reaction, which leads to the release of histamine and other substances. In 1958, Mongar and Schild (7) observed that calcium was essential for this response. When it became apparent that calcium influx also mediated the effect of the physiological stimulus to adrenal chromaffin cell catecholamine release, Douglas (131) suggested that the role of calcium in immunological mast cell activation might be similar, namely, to act as a coupling agent between stimulus and response. Later, Douglas (132) cited the mast cell as an example of a class of secretory cells using calcium influx to activate exocytosis. Indeed, the microinjection of calcium ions into mast cells caused granule exocytosis (8).

The calcium inophore A-23187 also stimulates mediator release in rat mast cells (133) and human basophils (134). Comparisons of immunological and ionophore-induced mediator release from basophils have been made. Ionophore and antigen-stimulated histamine release in human basophils differ in several respects (134): the kinetics and total quantity of histamine released are different, depending on the concentrations of antigen or ionophore (with low antigen concentrations producing a slower rate and less total histamine release but low ionophore concentrations producing delayed onset but rapid and essentially complete histamine release); antigen-desensitized basophils release histamine with ionophore; cyclic adenosine monophosphate or agents that stimulate increased cyclic adenosine monophosphate concentrations do not reduce ionophore-induced histamine release as with antigen-induced release; the microtubule-active agents colchicine and deuterium oxide inhibit and augment ionophore-induced release, respectively, but quantitatively less so as compared to antigen-stimulated release; and the calcium antagonist lanthanum inhibits both processes, but the ionophore effect is slightly more sensitive. Thus, ionophore and antigen appear to share certain as-

pects of the codependent biochemical mechanisms involved in histamine release but clearly differ in some respects (134). The specific calcium channel(s) involved, their topography in relationship to the cell surface immunological event, and the mechanisms of opening and closing the calcium channels involved in immunological or ionophore-stimulated mediator release remain unknown.

The calcium antagonist lanthanum produces significant inhibition of histamine release not only in basophils but also in mast cells (135) following immunological challenge. Based on a series of experiments, Foreman and Mongar (*cf.*, 136) concluded that mast cell activation (by several different mechanisms) is accompanied by calcium uptake that is temporally and quantitatively related to histamine release, suggesting that the trigger to histamine release from mast cells is an increased mast cell membrane permeability to calcium and an influx of calcium ions to the intracellular compartment. In the stimulated cell, it is supposed that the equilibrium between "open" and "closed" calcium channels is shifted in favor of open, allowing increased influx of calcium, the magnitude of which determines the degree of histamine secretion. In their model, Foreman and Mongar (136) considered that cyclic adenosine monophosphate has an effect that closes calcium channels and reduces calcium influx.

The recent experiments of Payne (137) on histamine release in the rat mast cell system concerned the effects of quercitin, dantrolene sodium, and 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate, all calcium antagonists (137). Quercitin and a small number of other flavones of plant origin recently were shown to inhibit histamine release by immunological and nonimmunological stimuli (but no ionophore) (138). Flavones are chemically related to cromolyn sodium; it was suggested that they act similarly by interfering with the normal path of calcium entry into stimulated mast cells. Like quercitin, dantrolene sodium (130) and 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate (140) have been reported to interfere with calcium translocations. Dantrolene sodium is a peripherally acting smooth muscle relaxant thought to prevent intracellular mobilization of calcium. 8-(*N*,*N*-Diethylamino)-octyl-3,4,5-trimethoxybenzoate is an aliphatic reserpine analog, which has also been suggested to act as an intracellular calcium antagonist based on its inhibitory effects on smooth muscle contraction (140).

Payne's results (137) indicated that histamine release by compound 48/80 from peritoneal and pleural rat mast cells was inhibited in a concentration-dependent manner by quercitin, which also inhibited dextran-induced histamine release. Dantrolene sodium inhibited histamine release by either compound 48/80 or dextran whereas it produced only weak inhibition of histamine release induced by the calcium ionophore A-23187. On the other hand, histamine release by dextran and ionophore A-23187 was strongly inhibited by 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate. The results were interpreted to suggest that quercitin, dantrolene sodium, and 8-(*N*,*N*-diethylamino)-octyl-3,4,5-trimethoxybenzoate may act at different sites to inhibit calcium translocations required for histamine release. Also, Barrett-Bee (141) found that the calcium antagonist cinnarizine inhibited antigen-induced histamine release (50%) in the rat mast cell system.

SUMMARY

Asthma may be considered to be a calcium-dependent disease by virtue of the dependency of excitation–contraction coupling in smooth muscle, stimulus–secretion coupling in mast cells and mucous glands, and vagal nerve activity on the availability of increased cytoplasmic calcium-ion concentrations. The nature and regulation of the calcium channels or pathways involved in various cell types are unknown. Whether the increased responsiveness of the relevant cells in asthma is characterized by increased ease of calcium influx, release from intracellular storage sites, or decreased calcium sequestration mechanisms in the plasma membrane or intracellular organelles is a subject for future research.

Effective antiasthmatic therapy must act finally to reduce availability of cytoplasmic calcium to excitation–contraction, stimulus–secretion, and nerve activation systems. Many effective drugs presumably act in this fashion, but the mechanisms are poorly understood.

It is possible that new calcium antagonists could be synthesized that would inhibit calcium influx or intracellular calcium movements in airway smooth muscle, mast cells, mucous glands, and vagus nerve and that might prove of value in asthma treatment.

REFERENCES

(1) K. F. Austen and R. P. Orange, *Am. Rev. Resp. Dis.*, **112**, 423 (1975).

(2) K. F. Austen, in "Immunological Diseases," 3rd ed., M. Santer, Ed., Little, Brown, Boston, Mass., 1978, p. 183.

(3) T. J. Sullivan and C. W. Parker, *Am. J. Pathol.*, **85**, 437 (1976).

(4) M. Kaliner and K. F. Austen, *Biochem. Pharmacol.*, **23**, 763 (1974).

(5) R. G. Coffey and E. Middleton, Jr., in "Comprehensive Immunology, Part III: Immunopharmacology," J. W. Hadden, R. G. Coffey, and F. Spreafico, Eds., Plenum, New York, N.Y., 1977, p. 203.

(6) M. Plaut and L. M. Lichtenstein, in "Allergy: Principles and Practice," E. Middleton, Jr., C. E. Reed, and E. F. Ellis, Eds., Mosby, St. Louis, Mo., 1978, p. 115.

(7) J. L. Mongar and H. O. Schild, *J. Physiol.*, **140**, 272 (1958).

(8) T. Kanno, D. E. Cochrane, and W. W. Douglas, *Can. J. Physiol. Pharmacol.*, **51**, 1001 (1973).

(9) K. Ishizaka and T. Ishizaka, in "Allergy: Principles and Practice," E. Middleton, Jr., C. E. Reed, and E. F. Ellis, Eds., Mosby, St. Louis, Mo., 1978, p. 52.

(10) T. Ishizaka and K. Ishizaka, *J. Immunol.*, **120**, 800 (1978).

(11) R. A. Lewis and K. F. Austen, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **36**, 2676 (1977).

(12) H. R. Colten and K. H. Gabbay, *J. Clin. Invest.*, **51**, 1972 (1972).

(13) M. Kaliner, *J. Allergy Clin. Immunol.*, **60**, 204 (1977).

(14) G. A. Robison, R. W. Butcher, and E. W. Suterland, "Cyclic AMP," Academic, New York, N.Y., 1971, p. 81.

(15) G. Schultz, in "Asthma: Physiology, Immunopharmacology and Treatment," K. F. Austen and L. M. Lichtenstein, Eds., Academic, New York, N.Y., 1978, p. 77.

(16) C. O. Brostorm, Y. Huang, B. M. Breckenridge, and D. J. Wolff, *Proc. Natl. Acad. Sci. USA*, **72**, 64 (1975).

(17) D. W. Empey, W. A. Laitinen, L. Jacobs, W. M. Gold, and J. A. Nadel, *Am. Rev. Resp. Dis.*, **113**, 131 (1976).

(18) J. A. Nadel, in "Asthma: Physiology, Immunopharmacology and Treatment," K. F. Austen and L. M. Lichtenstein, Eds., Academic, New York, N.Y., 1973, p. 29.

(19) J. A. Nadel, *Am. Rev. Resp. Dis.*, **115**, 117 (1977).

(20) W. M. Gold, *Postgrad. Med. J., Suppl. 7*, **51**, 53 (1975).

(21) W. M. Gold, in "Allergy: Principles and Practice," E. Middleton, Jr., C. E. Reed, and E. F. Ellis, Eds., Mosby, St. Louis, Mo., 1978, p. 499.

(22) R. Patterson, J. C. McKenna, I. M. Suszko, N. H. Solliday, J. J. Pruzansky, M. Roberts, and T. J. Kehoe, *J. Clin. Invest.*, **59**, 217 (1977).

(23) A. Szentivanyi, *J. Allergy*, **42**, 203 (1968).

(24) C. E. Reed and R. G. Townley, in "Allergy: Principles and Practice," E. Middleton, Jr., C. E. Reed, and E. F. Ellis, Eds., Mosby, St. Louis, Mo., 1978, p. 659.

(25) C. W. Parker, in "Asthma: Physiology, Immunopharmacology and Treatment," K. F. Austen and L. M. Lichtenstein, Eds., Academic, New York, N.Y., 1973, p. 185.

(26) E. Middleton, Jr., *Adv. Intern. Med.*, **18**, 177 (1972).

(27) R. G. Coffey and E. Middleton, Jr., in "Comprehensive Immunology, Part III: Immunopharmacology," J. W. Hadden, R. G. Coffey, and F. Spreafico, Eds., Plenum, New York, N.Y., 1977, p. 203.

(28) K. Raij, K. Alanko, A. Muittari, and M. Harkonen, *Scand. J. Resp. Dis.*, **57**, 223 (1976).

(29) M. E. Connolly and J. K. Greenacre, *J. Clin. Invest.*, **58**, 1307 (1976).

(30) H. S. Nelson, J. W. Black, L. B. Branch, B. Pfuetze, H. Spauling, R. Summers, and D. Wood, *J. Allery Clin. Immunol.*, **55**, 299 (1975).

(31) H. G. Morris, S. A. Rusnak, J. C. Selner, and J. Barnes, *Chest, June Suppl.*, **73**, 973 (1978).

(32) A. Kalisker, H. E. Nelson, and E. Middleton, Jr., *J. Allergy Clin. Immunol.*, **60**, 259 (1977).

(33) W. W. Busse, *Am. Rev. Resp. Dis.*, **115**, 783 (1977).

(34) R. K. Bush, W. W. Busse, D. K. Flaherty, D. Warshauer, E. C. Dick, and C. E. Reed, *J. Allergy Clin. Immunol.*, **61**, 80 (1978).

(35) G. A. Rinard, A. R. Rubinfeld, L. L. Brunton, and S. E. Mayer, *Proc. Natl. Acad. Sci. USA*, **76**, 1472 (1979).

(36) M. Rodbell, M. C. Lin, Y. Salmon, C. Londos, J. P. Harwood, B. R. Martin, M. Rendell, and M. Berman, *Acta Endocrinol., Suppl. 191*, **77**, 11 (1974).

(37) T. Pfeuffer and E. J. M. Helmreich, *J. Biol. Chem.*, **250**, 867 (1975).

(38) D. Cassel and Z. Selinger, *Biochim. Biophys. Acta*, **452**, 538 (1976).

(39) J. Richardson and T. Bouchard, *J. Allergy Clin. Immunol*, **56**, 473 (1975).
(40) J. Richard and J. Beland, *J. Appl. Physiol.*, **41**, 764 (1976).
(41) B. G. Simonsson, R. Andersson, N. P. Bergh, B. E. Skoogh, and N. Svedmyr, in "Bronchitis III," N. G. M. Orie and R. Van Der Lende, Eds., Charles C Thomas, Springfield, Ill., 1970, p. 334.
(42) C. J. Duncan, "Symposium of the Society for Experimental Biology, XXX," Cambridge University Press, Cambridge, England, 1976.
(43) R. H. Kretsinger, *Chem. Rev.*, **18**, 29 (1976).
(44) P. F. Baker, *Progr. Biophys. Mol. Biol.*, **24**, 177 (1972).
(45) M. P. Blaustein, *Rev. Physiol. Biochem. Pharmacol.*, **70**, 33 (1974).
(46) H. Rueter, *Progr. Biophys. Mol. Biol.*, **26**, 1 (1973).
(47) A. L. Lehninger, *Suppl. III, Circ. Res.*, **1974**, 83.
(48) E. Carafoli and M. Crampton, "Symposium of the Society for Experimental Biology, XXX," Cambridge University Press, Cambridge, England, 1976.
(49) D. H. MacLennan and P. C. Holland, *Ann. Rev. Biophys. Bioeng.*, **4**, 377 (1975).
(50) E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, "Calcium Transport in Contraction and Secretion," North-Holland Amsterdam, The Netherlands, 1975.
(51) A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)*, **117**, 500 (1952).
(52) S. Hagiwara, in "Dynamic Properties of Lipid Membranes and Bilayers," vol. 3, G. Eisenman, Ed., Dekker, New York, N.Y., 1975.
(53) D. J. Triggle and C. R. Triggle, "Chemical Pharmacology of the Synapse," Academic, New York, N.Y., 1976, chap. IV.
(54) B. Hille, *Progr. Biophys. Mol. Biol.*, **21**, 1 (1970).
(55) A. Fleckenstein, in "Calcium and the Heart," P. Harris and L. H. Opie, Eds., Academic, New York, N.Y., 1971, p. 135.
(56) A. Fleckenstein, *Arzneim.-Forsch.*, **22**, 2019 (1972).
(57) L. Rosenberger and D. J. Triggle, in "Calcium in Drug Action," G. B. Weiss, Ed., Plenum, New York, N.Y., 1978, p. 3.
(58) S. S. Jafferji and R. H. Michell, *Biochem. J.*, **154**, 653 (1976).
(59) *Ibid.*, **160**, 397 (1976).
(60) *Ibid.*, **160**, 163 (1976).
(61) A. Fleckenstein, *Annu. Rev. Pharmacol, Toxicol.*, **17**, 149 (1977).
(62) M. F. Piascik, R. G. Rahwan, and D. T. Witiak, *J. Pharmacol. Exp. Ther.*, **205**, 155 (1978).
(63) R. G. Rahwan, M. M. Faust, and D. T. Witiak, *ibid.*, **201**, 126 (1977).
(64) J. P. Girard and M. Cuevas, *Allergol..Immunopathol., Suppl. V*, **1977**, 13.
(65) U. Martin and D. Roemer, *ibid.*, **1977**, 5.
(66) B. Pourrias and F. Friedrich, *Eur. J. Pharmacol.*, **49**, 203 (1978).
(67) J. M. Diamond and E. M. Wright, *Annu. Rev. Physiol.*, **31**, 581 (1969).
(68) R. J. P. Williams, *Q. Rev. Chem. Soc.*, **24**, 331 (1970).
(69) P. F. Baker and H. G. Glitsch, *Phil. Trans. R. Soc. London Ser. B.*, **270**, 389 (1975).
(70) R. Ochi, *J. Physiol. (London)*, **263**, 139 (1976).
(71) E. Nieboer, *Struc. Bonding*, **22**, 1 (1975).
(72) R. B. Mikkelsen, in "Biological Membranes," D. Chapman and D. F. H. Wallach, Eds., Academic, New York, N.Y., 1976, p. 153.
(73) A. Fleckenstein, *Adv. Cardiol.*, **12**, 183 (1974).
(74) A. Fleckenstein, in "New Therapy of Ischemic Heart Disease," W. Lochner, Ed., Springer, Berlin, Germany, 1976, p. 56.
(75) A. Fleckenstein, H. Tritthart, H. J. Doring, and K. Y. Byon, *Arzneim.-Forsch.*, **22**, 22 (1972).
(76) M. Kohlardt, B. Bauer, H. Krause, and A. Fleckenstein, *Pfluegers Arch.*, **335**, 309 (1972).
(77) *Ibid.*, **338**, 115 (1973).
(78) M. Kohlardt, A. Herdey, and M. Kubler, *ibid.*, **344**, 149 (1973).
(79) H. Tritthart, R. Volkman, R. Weiss, and A. Fleckenstein, *Naunyn-Schmeidebergs Arch. Pharmacol.*, **280**, 239 (1973).
(80) H. J. Schuman, B. D. Gorlitz, and J. Wager, *ibid.*, **289**, 409 (1975).
(81) B. D. Gorlitz, J. Wagner, and H. Schuman, *ibid.*, **283**, 311 (1975).
(82) M. Endoh, J. Wagner, and H. J. Schuman, *ibid.*, **287**, 61 (1975).
(83) H. Reuter and H. Scholz, *J. Physiol. (London)*, **264**, 17 (1977).
(84) P. F. Cranefield, R. S. Aronson, and A. L. Wit, *Circ. Res.*, **34**, 204 (1974).
(85) R. S. Kass and R. W. Tsien, *J. Gen. Physiol.*, **66**, 169 (1975).
(86) J. B. Bassingthwaighte, C. H. Fry, and J. A. S. McGuigan, *J. Physiol. (London)*, **262**, 15 (1976).
(87) K. Baumann, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **294**, 161 (1976).
(88) E. E. Daniel, in "Muscle," Pual, Danile, and Monckton, Eds., Oxford University Press, New York, N.Y., 1965.
(89) L. Hurwitz and A. Suria, *Annu. Rev. Pharmacol.*, **11**, 303 (1971).
(90) D. J. Triggle, "Neurotransmitter-Receptor Interactions," Academic, New York, N.Y., 1971.
(91) D. J. Triggle, *Prog. Surf. Mem. Sci.*, **5**, 267 (1972).
(92) T. Tomita, *Prog. Biophys. Mol. Biol.*, **30**, 185 (1975).
(93) E. E. Daniel and R. A. Janis, *Pharmacol. Ther. B.*, **1**, 695 (1975).
(94) A. V. Somlyo and A. P. Somlyo, *Science*, **174**, 1955 (1971).
(95) C. E. Devine, A. V. Somlyo, and A. P. Somlyo, *J. Cell Biol.*, **52**, 690 (1972).
(96) C. E. Devine, A. V. Somlyo, and A. P. Somlyo, *Phil. Trans. Roy. Soc. London B*, **265**, 17 (1973).
(97) A. V. Somlyo and A. P. Somlyo, *J. Pharmacol. Exp. Ther.*, **159**, 129 (1968).
(98) A. P. Somlyo and A. V. Somlyo, in "Methods in Pharmacology," vol. 3, E. E. Daniel and D. M. Paton, Eds., Plenum, New York, N.Y., 1975, pp. 3–45.
(99) D. F. Fitzpatrick, E. J. Landon, G. Debbes, and L. Hurwitz, *Science*, **176**, 305 (1972).
(100) M. L. Hess and G. D. Ford, *J. Mol. Cell Cardiol.*, **6**, 275 (1974).
(101) R. C. Webb and R. C. Bhalla, *ibid.*, **8**, 145 (1976).
(102) K. A. P. Edman and H. O. Schild, *J. Physiol.*, **169**, 404 (1963).
(103) J. Axelsson, in "Smooth Muscle," E. Bulbring, A. F. Brading, A. W. Jones, and T. Tomita, Eds., Williams & Wilkens, Baltimore, Md., 1970.
(104) W. R. Keatinge, *J. Physiol.*, **224**, 21 (1972).
(105) D. J. Triggle and C. R. Triggle, "Chemical Pharmacology of the Synpase," Academic, New York, N.Y., 1976.
(106) B. Frenkenhaeuser and A. L. Hodgkin, *J. Physiol. (London)*, **137**, 218 (1957).
(107) A. M. Shanes, *Pharmacol. Rev.*, **10**, 59 (1958).
(108) D. J. Triggle, *Annu. Rev. Pharmacol.*, **12**, 185 (1972).
(109) C. R. Triggle and D. J. Triggle, *J. Physiol.*, **254**, 39 (1976).
(110) C. R. Triggle, V. C. Swamy, and D. J. Triggle, *Can. J. Physiol. Pharmacol.*, in press.
(111) V. C. Swamy, M. Ticku, C. R. Triggle, and D. J. Triggle, *ibid.*, **53**, 1108 (1975).
(112) M. Takata, W. F. Pickard, J. Y. Lettvin, and J. W. Moore, *J. Gen. Physiol.*, **50**, 461 (1966).
(113) C. Van Breemen, *Arch. Int. Physiol. Biochim.*, **77**, 710 (1969).
(114) C. Van Breemen, B. R. Farinas, P. Gerba, and E. McNaughton, *Circ. Res.*, **30**, 44 (1972).
(115) C. Van Breemen, B. R. Farinas, R. Casteels, P. Gerba, R. Wuytack, and R. Deth, *Phil. Trans. Roy. Soc. B*, **265**, 57 (1973).
(116) G. B. Weiss, *Annu. Rev. Pharmacol.*, **14**, 343 (1974).
(117) B. J. Hodgson, A. M. Kidwai, and E. E. Daniel, *Can. J. Physiol. Pharmacol.*, **50**, 730 (1972).
(118) R. G. G. Andersson, *Acta Physiol. Scand. Suppl.*, **1972**, 382.
(119) H. P. Bar, *Adv. Cyclic Nucleotides Res.*, **4**, 174 (1974).
(120) T. P. Lee, J. F. Kuo, and P. Greengard, *Proc. Natl. Acad. Sci. USA*, **69**, 3817 (1972).
(121) G. Schultz and J. G. Hardman, *Adv. Cyclic Nucleotide Res.*, **5**, 339 (1975).
(122) H. Rasmussen, *Science*, **170**, 404 (1971).
(123) M. J. Berridge, *Adv. Cyclic Nucleotide Res.*, **6**, 1 (1975).
(124) P. Greengard, *ibid.*, **5**, 582 (1975).
(125) P. Greengard, *Nature*, **260**, 101 (1976).
(126) K. J. Chang and D. J. Triggle, in "Membranes in Metabolic Regulation," M. Mehlmann, Ed., Academic, New York, N.Y., 1973.
(127) M. Ticku and D. J. Triggle, *Gen. Pharmacol.*, **7**, 133 (1976).
(128) L. Rosenberg, M. Ticku, and D. J. Triggle, *Can. J. Physiol. Pharmacol.*, **57**, 333 (1979).
(129) L. M. Lichtenstein, *J. Immunol.*, **107**, 1122 (1971).
(130) M. Kaliner and K. F. Austen, *J. Exp. Med.*, **138**, 1077 (1973).

(131) W. W. Douglas, in "Calcium Transport in Contraction and Secretion," E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, Eds., American Elsevier, New York, N.Y., 1975, p. 167.
(132) W. W. Douglas, *Br. J. Pharmacol.*, **34**, 451 (1968).
(133) J. C. Foreman, J. L. Mongar, and B. D. Gomperts, *Nature*, **245**, 249 (1973).
(134) L. M. Lichtenstein, *J. Immunol.*, **114**, 1692 (1975).
(135) J. C. Foreman and J. L. Mongar, *Br. J. Pharmacol.*, **48**, 527 (1973).
(136) J. C. Foreman and J. L. Mongar, in "Calcium Transport in Contraction and Secretion," E. Carafoli, F. Clementi, W. Drabikowski, and A. Margreth, Eds., American Elsevier, New York, N.Y., 1975, p. 175.
(137) A. Payne, Ph.D. thesis, London University, London, England, 1978, chap. 8.
(138) C. M. S. Fewtrell and B. D. Gomperts, *Biochim. Biophys. Acta*, **469**, 52 (1977).
(139) K. G. Morgan and S. H. Bryant, *J. Pharmacol. Exp. Ther.*, **201**, 138 (1977).
(140) M. H. Malagodi and C. Y. Chiou, *Eur. J. Pharmacol.*, **27**, 25 (1974).
(141) K. J. Barrett-Bee, *Biochem. Soc. Trans.*, **5**, 965 (1977).

ACKNOWLEDGMENTS

Presented as part of the Symposium on New Approaches to the Treatment of Asthma at the APhA Academy of Pharmaceutical Sciences, Anaheim meeting, April 1979.

The author gratefully acknowledges many informative and stimulating conversations with Dr. David Triggle regarding the role of calcium in smooth muscle functions.

Clinical Evaluation of Antiallergic Agents

RICHARD R. ROSENTHAL

Received May 4, 1979, from the *School of Medicine, Johns Hopkins University, and the Good Samaritan Hospital, Baltimore, MD 21239.* Accepted for publication September 10, 1979.

Abstract ☐ Testing methods used to detect antiallergic activity are described for several pharmacological classes of drugs. The pharmacodynamics of each drug determine the type of testing required to detect antiallergic or antiasthmatic activity.

Keyphrases ☐ Pharmacokinetics—clinical testing in mildly asthmatic human volunteers, dose–response curve of antiallergic drugs ☐ Antiallergic drugs—pharmacokinetics, various clinical testing procedures in humans, inhibition of mediator release, cyclic adenosine monophosphate levels, potential asthma inhibitors ☐ β-Adrenoceptor agonists—antiallergic drug testing, bronchiolar smooth muscle, cyclic adenosine monophosphate levels, effects on mediator release ☐ Asthma—review of current theories, clinical evaluation, symposium, antiallergic drugs

Hypersensitivity involves an allergic reaction that is an immunological event characterized by the release of a chemical mediator, histamine or slow-reacting substance of anaphylaxis, in response to exposure to a foreign antigen. Foreign (environmental) antigens are usually complex protein mixtures such as cat dander or ragweed pollen. Initial antigen exposure results in the elaboration of antibody of the immunoglobulin E class, which, as circulating specific immunoglobulin E antibody, is in equilibrium with cell-fixed immunoglobulin E on circulating basophils or tissue mast cells. When a subsequent environmental antigenic insult occurs, the antigen combines with the cell-fixed immunoglobulin E antibody, whereupon histamine and other chemical mediators are released to act directly on target tissue. Accordingly, the characteristic allergic reactions seen in asthma, rhinitis, urticaria, and even systemic anaphylaxis reflect the anatomic sites where histamine and other chemical mediators are released as well as their respective tissue responses.

DISCUSSION

Histamine release induced by antigen can be modified by drugs that act on various stages within the release mechanism. For example, the β-agonists, isoproterenol, ephedrine, metaproterenol, and terbutaline, activate adenyl cyclase to increase intracellular levels of cyclic adenosine monophosphate, an enhancement that prevents histamine release. Because phosphodiesterase catalyzes the conversion of cyclic adenosine monophosphate to 5′-adenosine monophosphate, inhibition of this enzyme by drugs such as the xanthines (theophylline) arrests the breakdown of cyclic adenosine monophosphate, thereby preventing histamine release.

Cromolyn sodium is the prototype of a new class of compounds that act presumably by preventing mediator release, although the exact mechanism of action is unknown. The anti-inflammatory steroids appear to stabilize the mast cell membrane and also enhance β-receptor sensitivity. The prostaglandins not only influence intracellular cyclic adenosine monophosphate but also may prevent mediator release, whereas prostaglandin synthetase inhibitors may regulate allergic hypersensitivity reactions in either direction. At the cellular level, α-agonists (phenylephrine) and cholinergic agonists (acetylcholine) may increase intracellular cyclic guanosine monophosphate by stimulating guanyl cyclase and enhancing histamine release. On the basis of experimental evidence, α-blockers and cholinergic antagonists may have some usefulness as antiallergic agents.

The classical antihistamines, exemplified by diphenhydramine, compete with histamine at H_1-receptor sites to allay allergic reactions. This group has a new member with different pharmacological attributes, the H_2-receptor antagonist cimetidine, which blocks gastric acid secretion. Although this kind of blockade has not previously been associated with allergy treatment, preliminary studies suggest that H_1- and H_2-blockers in combination may be effective against urticaria (1–3). Drugs that alter the immune response also may be considered antiallergic. Ragweed extract or denatured antigens can raise the protective immunoglobulin G antibody titers and reduce the severity of an allergic reaction.

A number of clinical testing procedures can be used to evaluate new antiallergic medications. The inhalation challenge method elicits an asthmatic attack under laboratory conditions, enabling a test drug to be evaluated (4). In this technique, mildly asthmatic volunteers with near normal pulmonary function inhale graded doses of antagonists such as antigen, methacholine, or histamine, using a specialized inhalation dosing apparatus, the inhalation dosimeter. Spirometry and specific airway conductance are monitored, and the dose–response curves to the inhaled antagonists indicate patient sensitivity.

The provocation dose, defined as the amount of antagonist causing a 20% fall in forced expiratory volume in 1 sec, is interpolated from the dose–response curve and used as a reproducible index of patient sensitivity to the inhalant.

A change in the dose–response curve or a shift of the provocation dose toward a higher antigen requirement indicates an alteration of antigen sensitivity. By this procedure, antiasthma agents can be evaluated for

0022-3549/ 80/ 0200-0251$01.00/ 0
© 1980, American Pharmaceutical Association