

REVIEW ARTICLE

ADRENERGIC AND CHOLINERGIC REGULATION OF IMMEDIATE TYPE ALLERGIC REACTIONS

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SYNOPSIS

Both adrenergic and cholinergic stimulation are implicated in immediate type hypersensitivity. The mechanism of action is discussed.

INTRODUCTION

Asthma and other causes of airways obstruction have several important triggers such as allergy, infection, emotion, exercise and air pollution. Thus, these respiratory disorders may broadly be classified as allergic and non-allergic. The nature of non-allergic reactions are poorly understood. These are often considered a result of structural changes and therefore are irreversible. In contrast, certain allergic reactions are readily reversible with appropriate therapy. Allergic asthma is an immunological result of altered sensitivity in the lung to foreign protein, and the term allergy has been summarized (1) as the 'acquired', 'specificity', and 'altered capacity to react'. 'Acquired' means a prior adequate antigenic or allergic exposure for immunological stimulation. 'Specificity' describes the physico-chemical relationship between allergens and the corresponding antibodies. 'Altered capacity to react' describes the different response elicited by an agent after the production of its antibodies. This reaction may be decreased (immunity) or increased (hypersensitivity) for example, to a toxin or infective agent. The essential qualities of this altered capacity to react are its accelerated and amplified nature and the smallness of immunological dosage that is able to elicit it.

In many patients it is possible to identify a causative antigen and to demonstrate the presence of the corresponding antibody (extrinsic asthma). Their sputum and sometimes their blood contains an increased number of eosinophils. Other patients may have similar clinical features and may respond to similar treatment, but presence of antigen and antibody cannot be demonstrated (intrinsic asthma). Extrinsic type is associated with history of eczema, hay fever, urticaria, and other allergic manifestations, often with family history of allergy, and commonly occurs in children and young people. Protein sensitivity reaction of 'immediate type' is frequently obtained. Intrinsic type usually occurs in adults over 40 with no previous history of asthma, eczema, hay fever or urticaria, and no family history of allergic disorders. Intrinsic asthma is frequently associated with infection within the lungs and upper respiratory tract and polypi in the nose, and protein sensitivity reaction of 'immediate type' does not occur.

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Coombs and Gell (2) classified all allergic reactions into four main groups. Type 1, the immediate allergy, is reversible and mediated by allergen (antigen) reacting with the target cells (mast cells, basophils) sensitized by non-precipitating heat-labile reaginic (IgE) antibody. This type of reaction causes atopic allergy (asthma, hay fever, eczema, urticaria and anaphylactic reactions) that develops rapidly and is maximum at about 10 to 15 minutes, and lasts for about one and a half to two hours (3). The skin reaction consists of itching, erythema and wealing, and is accompanied by a local infiltration of eosinophils. Nasal, conjunctival and bronchial reactions to clinical exposure or provocation tests are analogous to skin reaction tests in speed of appearance and duration. IgG (precipitating, heat-stable) antibody is now known also to participate in Type 1 allergic reactions (4).

Type 2, cytotoxic allergy, is mediated by cytotoxic auto-antibodies that are directed against antigens on cells or tissues, or antigens formed there in combination with another antigen or hapten. This allergic reaction is involved in drug allergy, homograft rejection and haemolytic disease of the neonates.

Type 3, Arthus immune-complex reactions (polyarteritis nodosa, serum sickness, etc.), is mediated by precipitating heat-stable antibodies such as IgG and IgM, and are implicated in diseases such as cutaneous vasculitis, nephritis and extrinsic non-atopic asthma, allergic alveolitis and allergic broncho-pulmonary aspergillosis. The immune-complex formed in combination with antigen fixes the β_2C component of complement and activates it enzymatically. The enzymatic aggregates are chemotactic for polymorphonuclear neutrophil cells that are essential for type 3 reactions. These cells are destroyed after ingestion of the enzymatic aggregates and liberate their own enzymes, the lysosomes, which proceed to digest the extracellular tissues (5). Type 3 allergic reactions may cause irreversible tissue damage. They appear more slowly than Type 1 reactions and become evident 4 to 5 hours after the test, are maximal at 7 to 8 hours, and resolve within 24 to 36 hours. The late asthmatic reactions may be accompanied by fever, myalgia and malaise, and polymorphonuclear neutrophil leucocytes.

Type 4 delayed allergy is initiated by thymus dependent lymphocytes with the ability to respond specifically to allergen. Free antibody is not an absolute necessity. Tissue necrosis may occur at the site of intense reactions which usually take 24 hours or more to reach the peak. This type of reaction is responsible for tuberculin type allergy, contact dermatitis and antimicrobial immunity.

IMMUNOLOGICAL TARGET CELLS

The mast cell is the primary target cell in immediate type of tissue reaction. Uvnas and associates (6) showed that histamine and serotonin are stored within the granules in this cell. Mast cells have been found in the human bronchiole in both mucosa and submucosa (7). Salvato (8) demonstrated a significant decrease in lung mast cells during an acute asthmatic attack in man. Other target cells include basophil in man and rat (9) and platelet in the rabbit (10).

The antibody known to participate in Type 1 allergy is a non-precipitating heat-stable immunoglobulin, IgE, produced by lymphoid tissues (11). Coca and Cooke (12) introduced it as atopic reaginic antibody to describe their patients with hay fever and asthma. The antibody is a gamma 1 glycoprotein having two light and two heavy chains (molecular weight, 190,000). The concentration of the protein in normal human serum is in the range of 0.1 to 0.4 $\mu\text{g/ml}$. However, the serum of some atopic patients and those with parasitic infestations contain significantly elevated levels of IgE (11). The antibody has the ability to

sensitize homologous tissues including human lung. It has a high affinity for mast cells and basophil granulocytes, whereas an atypical myeloma protein (E) with antigenic determinants identical to IgE binds only to mast cells. In the IgE-mediated reaction, the IgE receptor serves as an anchor for the specific IgE antibody molecule. Once IgE antibody binds to the receptor, the cell-bound IgE molecules permit antigen to bridge adjacent to receptor molecules, which in turn initiates the process of chemical mediator release. Because no immunoglobulin other than IgE binds to IgE receptors with high affinity under the physiological condition, triggering of the mediator release through IgE receptor is mediated only by IgE antibodies.

Little is known about chemical or other features of allergens that may favour IgE formation, though there is suggestion that it may be due to presence of lysine-sugar determinants in the molecule (13). Two types of lymphocytes are involved in the antibody response (14). One type is the B lymphocytes, which are precursors of antibody forming cells and bear immunoglobulin on their cell surfaces. Another type of lymphocytes, called thymus-derived lymphocytes or T cells, regulate the differentiation of B lymphocytes to antibody-forming cells. The majority of B cells bear IgM and are not committed for the production of a certain immunoglobulin isotype. B cells have been found in fetal liver and spleen. In mice and rats, all B cells in fetal liver bear IgM. However, within 24 to 48 hours after birth lymphocytes bearing IgE are detectable in the neonatal spleen. IgE-bearing B cells are precursors of IgE-forming cells and appear to be committed for the formation of IgE after differentiation to plasma cells. The differentiation of virgin B cells to the IgE-bearing B cells requires neither antigen nor T cells.

It is now known that IgG, a precipitating heat-stable antibody, can also initiate reaginic type hypersensitivity reactions (4). This antibody has also been found together with IgE antibody in some atopic patients (1). However, it binds only weakly to homologous target cells and is thus less effective than IgE antibody in provoking acute immediate reactions. It has been demonstrated in rats that IgE and IgG antibodies cause the release of chemical mediators through different cellular and humoral mechanisms (9).

CHEMICAL MEDIATORS OF ANAPHYLAXIS

An immunological reaction is termed anaphylactic when antigen reacts with cell-bound antibody, in response to which some cells release pharmacologically active substances (chemical mediators). Unlike anaphylaxis, however, cytotoxic reactions occur, as already mentioned, as a result of union between circulating antibody and antigen situated in the cell membrane or closely to it. Activated complement participates in this reaction, its role apparently being to damage the cell membrane at multiple discrete sites.

The chemical mediators, that are released in human allergy* or anaphylaxis of the guinea-pig, include histamine, serotonin (5-HT), kinins, PGE_2 , $\text{PGF}_2\alpha$, adrenaline, noradrenaline and slow reacting substance of anaphylaxis (SRS-A) (15-17). Some studies suggest that PGE_1 (18), an eosinophil chemotactic factor (ECF-A) (19) and a platelet activating factor (PAF) (20) are also released in human allergic responses. Histamine, SRS-A and $\text{PGF}_2\alpha$ cause bronchoconstriction in man. PGE_1 is a bronchodilator, adrenaline and noradrenaline (which have also slight bronchoconstrictor effect via receptors) and PGE_2 are equivocators. ECF-A may account for the accumulation of eosinophils at the sites of allergic tissue injury. Therefore, the biological effects of most of these chemical agents in human allergic diseases include increased vascular permeability, induction of bronchial smooth mus-

cle contraction, stimulation of parasympathetic afferent nerve endings, increased mucous gland secretion, selective attraction of eosinophils, and activation of a platelet release reaction.

RELEASE MECHANISM OF CHEMICAL MEDIATORS

The sequence of events to the immediate anaphylactic response in the target cells may be summarized as follows: antibody-receptor binding, antigen-antibody union, activation of enzymes, influx of Ca^{2+} ions, release of the chemical mediators of anaphylaxis and effects of these agents on tissues to produce pharmacological responses.

Although the morbid patterns of anaphylaxis amongst different species show a marked variation, the union of antigen with cell-bound antibody is associated with the release of histamine and SRS-A by a biochemical mechanism many features of which are common to at least four experimental systems: human and guinea pig lungs, rat peritoneal mast cells, rabbit basophils and human leucocytes.

Histamine release in anaphylaxis is a process requiring energy, and is dependent on calcium ions and temperature. It is also influenced by pH and ionic milieu, and inhibited by anoxia (oxygen lack) and numerous enzyme inhibitors including 2-deoxyglucose, iodoacetate and metallic chelates. The IgE-mediated immunological release of histamine and SRS-A from human leucocytes and lungs is an antigen-dependent and calcium-independent first step and a second non-antigen requiring calcium dependent release step (21, 22). Antigen-induced degranulation of mast cells or basophils is not a cytotoxic event, since the degranulated cells retain their motility (23). Degranulation is associated with communication of the granule with the extracellular space permitting ionic exchange. Considerable evidence has accumulated to suggest that the union of antigen with antibody results in activation of certain membrane bound serine esterase(s), although no such enzyme has yet been positively identified. It may be either a protease or phospholipase A_2 or both (11, 24, 25). Bridging of IgE receptors on rat mast cells also activates methyltransferase and induces the stimulation of phospholipid methylation. The methylation of phospholipids is followed by an increase in Ca^{2+} influx and then histamine release (11). Thus this process may be involved in the early triggering events and may set the stages for Ca^{2+} influx and subsequent histamine release. Selective calcium channel blocking drugs or antagonists may possibly be used in the treatment of bronchial asthma.

The role that the dense microfilament system of the human mast cell plays in the immunological release of chemical mediators has still not been clearly elucidated (7,26). Cytochalasin B, a fungal metabolite which interferes with the function of microfilaments, markedly enhanced the release of histamine from IgE-sensitized human peripheral leucocytes, whereas the compound inhibited the release of histamine from rat peritoneal mast cells. Moreover, cytochalasin B when added to sensitized human lung fragments enhanced the antigen-induced release of histamine but inhibited the release of SRS-A. This apparent dissociation of histamine and SRS-A release may indicate different roles for the microfilament system in the release of histamine and in the formation and release of SRS-A in human lung. Alternatively, it may reflect the other diverse metabolic effects of cytochalasin B.

Adrenergic control

Probably the first evidence for the existence of two distinct

tly different sets of effects in the sympathetic system was offered by Dale (27) in 1906. He demonstrated that ergot prevents the excitatory action of adrenaline but has no effect on its inhibitory action. Later, Cannon and Rosenblueth (28) postulated the existence of sympathin-E (excitatory) and sympathin-I (inhibitory) as mediator substance to explain these phenomena.

In 1948, Ahlquist (29) introduced the concept of two different receptors for adrenaline (epinephrine) at the effector sites, and designated them as α — and β — adrenergic receptors. These two receptors have now been divided into α -1, α -2, β -1 and β -2, respectively (30, 31). Chronotropic and inotropic cardiac responses and lipolysis are examples of β -1 effects. Smooth muscle relaxation including dilatation of the bronchi, inhibition of mitosis, and eosinopenia are some of the effects of β -2. The α -1 adrenoceptors, when activated, mediate bronchial and cardiac contraction, whereas the α -2 receptors cause platelet aggregation, and inhibition of cholinergic neurons and lipolysis. Apparently β -2 responses are chiefly impaired in asthmatics. In the presence of antagonists, the α -adrenoceptors intensify the respective cardiac and bronchodilating effects of β -1 and β -2 stimulators. In asthmatics, however, α -1 and β -2 stimulators have complementary effects on the airways.

Various representatives of the ergot alkaloids, haloalkylamines (dibenzamine, phenoxybenzamine), benzodioxans and imidazolines are α -adrenergic receptor antagonists. Among the catecholamines, norepinephrine stimulates mainly α -receptors, isoproterenol acts almost exclusively on β -receptors, while epinephrine possesses dual action. Epinephrine and norepinephrine are approximately equipotent activators of β -1 receptors which are preferentially inhibited by practolol. Epinephrine is more potent than norepinephrine in activating β -2 receptors that are inhibited by butoxamine. Various β -receptor blockers including propranolol, alprenol and pindolol do not distinguish between β -1 and β -2 receptors.

Subsequently, Sutherland and colleagues (32) proposed the second messenger theory, according to which the intracellular messenger is cyclic 3', 5'-adenosine monophosphate (cyclic-AMP) and the extracellular hormone or nerve impulse is the first messenger. Thus, the cyclic nucleotide acts as a mediator of some actions of catecholamines. Alpha-adrenergic receptor activation elicits a response by a sequence of largely unidentified steps, while β -adrenergic response includes accumulation of cyclic AMP within the cells. Therefore, intracellular cyclic AMP levels indicate the occupancy and function of β -adrenoreceptors. The cyclic nucleotide has been implicated in the regulation of hormone synthesis, enzyme activation and inhibition, DNA, RNA and protein synthesis, cell growth, differentiation and aggregation, muscle contraction and relaxation, sensory and neural excitation, and cellular permeability and secretion (33-37).

The cellular level of cyclic AMP depends on the activity of enzymes regulating its synthesis and degradation, as well as those factors responsible for its release into the extracellular fluid. The cyclic nucleotide is formed from the parent molecule adenosine 5'-triphosphate (5'-ATP) by the action of membrane-bound adenylate cyclase, and is degraded by cyclic AMP phosphodiesterase. Beta-adrenergic hormone-receptors cause most of their physiological effects by activating adenylate cyclase and thereby increasing intracellular levels of cyclic AMP. Other stimulators of the enzyme include prostaglandins E_1 and E_2 and prostacyclin. There are indications that the activation of α — adrenoceptors leads to inhibition of adenylate cyclase and/or to an increase in intracellular free calcium mediated by increased hydrolysis of phosphatidylinositol (31). Various agents like methylxanthines (theophylline, aminophylline, caffeine), isoquinolines (e.g.

papaverine) and disodium cromoglycate (DSCG) inhibit the activity of phosphodiesterase (38). Thus, the factors that affect the activity of adenylate cyclase and phosphodiesterase would influence the degree and duration of the physiological effects of cyclic AMP.

Later, another second messenger cyclic nucleotide, cyclic 3', 5'-guanosine monophosphate (cyclic GMP), has been implicated in β -adrenergic and particularly to cholinergic stimulation (33-37). There is an antagonism between cyclic AMP and cyclic GMP in many systems.

Goldberg and colleagues (39) offered a 'bi-directional' model of control embodied in the 'dualism' or Yin-Yang hypothesis, based on an analogy from the classical Chinese precept which states that for every point of stimulation (say, of a nerve or in the practice of acupuncture) there is a corresponding point of inhibition and suppression. Thus in a bi-directional system, stimulation or inhibition of a process is achieved by a simultaneous decrease in concentrations of cyclic AMP and an increase in cyclic GMP or vice versa. Therefore, factors influencing the activity of second messengers would affect the expression of α and β -adrenergic and/or cholinergic stimulation.

In 1968, Szentivanyi (40) proposed that asthma might be influenced or modulated by the status of the β -adrenergic receptor system, and unresponsiveness or depressed activity of the β -receptor might be an important factor in the genesis of bronchial asthma. He derived this hypothesis in part from the animal model of the pertussis-sensitized mouse. Mice given pertussis vaccine become hypersensitive to histamine and other mediators of anaphylaxis. This sensitivity to histamine is associated with diminished response to β -stimulation and enhanced response to α -stimulation. The concept that irritability of the airways is due to β -blockade received support from the observation of McNiell (41) that the β -blocking drug, propranolol, caused acute bronchoconstriction in asthmatic subjects. Propranolol has, however, no effect on the airway responses of normal person to histamine or cholinergic aerosols (42). Moreover, asthmatic subjects have a decreased eosinopenic response. Reed and associates (43) demonstrated that β -adrenergic blockade decreased the anticipated response with epinephrine. However, from various observations it may be concluded that adrenergic system, particularly β -receptor is involved in atopic extrinsic asthma and not in intrinsic hypersensitivity.

An early, if not the first, step in the chain of biochemical reactions set off by β -adrenergic stimulation is activation of the membrane-bound adenylate cyclase. Increased activity of this enzyme leads to increased intracellular cyclic AMP, some of which might leave the cell. One effect then of β -stimulation is increased blood levels and urinary excretion of cyclic AMP. Epinephrine caused a two-fold increase in urinary excretion of cyclic AMP in normal subjects without any increase in asthmatic patients (44). Glucagon produced a four-fold increase in both groups, suggesting that only some β -responses are affected by the abnormality.

In some studies, cyclic AMP levels in response to catecholamines were higher in asthmatic than in normal individuals (45, 46), and the cyclic nucleotide levels in lymphocytes and leucocytes from both asthmatic and normal subjects were increased in a dose-dependent manner when exposed to isoproterenol (47). However, the response of cells to the drug from asthmatic individuals was slightly less than from normal control subjects. Similarly, basal cyclic AMP levels were lower in the cells of asthmatics compared with normal individuals. Further studies in this direction have shown that leucocyte adenylate cyclase from patients with active extrinsic bronchial asthma exhibited a diminished response to

isoprenaline (isoproterenol) (48). On the other hand, patients in remission had a significant increase in cyclic AMP levels when their cells were subjected to stimulation with isoproterenol. A significant difference in the control (basal) values of leucocyte adenylate cyclase activity between the patients with active asthma and those in remission suggests that in the active phase of asthma, β -adrenergic receptors are maximally activated by circulating endogenous catecholamines and further stimulation with sympathomimetic amines becomes increasingly difficult. In this situation, the minor α -adrenoreceptor stimulating effect of adrenaline may become a predominant factor leading to the reversal of adrenaline action that is commonly observed in status asthmaticus.

Many studies have shown that antigen-induced histamine release from basophils and mast cells may be inhibited by increasing intracellular cyclic AMP levels. Lichtenstein and Margolis (49) and Middleton and Finke (50) attributed the inhibition of antigen-induced and IgE-mediated release of histamine and SRS-A from human peripheral leucocytes by β -adrenergic agents and methylxanthines to the capacity of these agents to increase cellular levels of cyclic AMP. These observations were later confirmed by direct measurements of leucocyte cyclic AMP (51). Similar observations were subsequently made using human lung tissues (52-54). Whereas β -adrenergic stimulation increases the level of cyclic AMP and inhibits mediator release, α -adrenergic stimulation decreases tissue levels of cyclic AMP and enhances the release of both mediators. This inverse association between tissue levels of cyclic AMP and the degree of mediator release suggests that the changes in cyclic AMP in the lung fragments reflect those occurring in the subpopulation of relevant target cells. Furthermore, the observed effects with adrenergic agents indicate that these target cells possess both α - and β -adrenergic receptors, and that β -adrenergic agents and theophylline may inhibit the IgE-mediated skin reactions through their effects on the skin mast cells rather than on the peripheral vasculature.

Dibutyl cyclic AMP and agents that raise intracellular cyclic AMP levels such as isoprenaline relaxed the guinea-pig tracheal chain (55). The effect of isoprenaline, but not of dibutyl cyclic AMP, was blocked by propranolol. Isolated strips of human bronchi responded similarly (56). Dibutyl cyclic AMP and theophylline produced dose-dependent relaxation of the strips independent of β -receptor blockade. This study also demonstrated that human bronchi possess a sparse population of α -adrenoreceptors. Murad and Kimura (57) have examined the effect of several hormones and drugs on cyclic AMP and cyclic GMP levels in guinea-pig tracheal rings. Their results suggest that the agents (e.g. PGE₁, epinephrine) which relax tracheal smooth muscle produced a dose-dependent increase in cyclic AMP levels, whereas agents that contract the muscle increased the levels of cyclic GMP. The effect of epinephrine was antagonized by propranolol and unaltered by the α -blocker, phenoxybenzamine. Combination of epinephrine and theophylline caused greater relaxation of the smooth muscle.

Foreman and associates (58) proposed that dibutyl cyclic AMP, theophylline and DSCG inhibit the antigen-antibody union induced mediator release by preventing the entry of calcium ions into the mast cells, by raising intracellular cyclic AMP levels. This concept is based on the experiments using a calcium ionophore, A23187, that induces a non-cytolytic release of histamine and SRS-A from rat mast cells, and human leucocytes and lung tissue. The hypothesis is supported by the findings that DSCG inhibited the fall of cyclic AMP levels in rat peritoneal mast cells following antigen-antibody stimulus (59), and the drug

increased the cyclic AMP content in the actively sensitized guinea-pig lung (60). Moreover, phospholipase A₂ induced uptake of Ca²⁺ ions was inhibited in a dose-related manner by dibutyl cyclic AMP, DSCG and theophylline (59).

Recently, Ishizaka (11) has obtained evidence that cyclic AMP regulates phospholipid methylation. Preincubation of mast cells with theophylline resulted in an increase in intracellular cyclic AMP. When these cells were challenged by divalent antireceptor antibody, phospholipid methylation, calcium ion uptake and subsequent histamine release were all inhibited. Dibutyl cyclic AMP also inhibited all of the three reactions in a similar dose-response manner. However, it is not yet known whether cyclic AMP directly inhibits methyltransferase or activation of cyclic AMP-dependent enzyme(s) is responsible for the inhibition of phospholipid methylation.

Cholinergic control

Bronchial hypersensitivity to inhaled cholinergic agents such as methacholine and acetylcholine is common in patients with bronchial asthma (61). Atropine, the muscarinic receptor blocking agent, by injection or more effectively by inhalation, relieves the airways obstruction and greatly reduces the bronchoconstriction in this situation (62-65). This drug has been used in the control of asthmatic syndrome since the 18th Century. The vagus nerve containing the muscarinic receptors is involved in the maintenance of bronchomotor tone and in promoting bronchoconstriction in asthmatic episodes (66). Cholinergic stimulation of human lung fragments enhances the antigen-induced release of both histamine and SRS-A in vitro, and this is not attributed to a decrease in cellular levels of cyclic AMP (67). Atropine also blocks this in vitro cholinergic effect. These findings suggest that the target cells in human lung also possess cholinergic receptors of the muscarinic type.

Cholinergic stimulation of certain tissues results in an increase in cyclic GMP content and in smooth muscle, membrane depolarization and a subsequent influx of calcium ions may be the primary events in stimulating cyclic GMP synthesizing enzyme, guanylate cyclase (33-37, 39). Derivatives of cyclic GMP (e.g. 8-bromo cyclic GMP) have been shown to produce a dose-related enhancement of the immunological release of chemical mediators from human lung (67). Cyclic GMP also induces the degranulation of rat peritoneal mast cells (68). It has recently been reported that IgE-sensitized rat mast cells produced a sharp increase in intracellular cyclic GMP levels a few seconds after antigen challenge and declined thereafter (69, 70). Although cholinergic innervation of mast cells has not been demonstrated, the relationship of mast cells to nerves is well recognized (71), and there is some evidence to suggest that an antidromic reflex in skin causes mast cell degranulation (72). Thus, in mast cells, as in airways, the balance between cholinergic and β -adrenergic as well as between α and β -stimulation may play a critical homeostatic role. The neuronal mechanisms involved in the respiratory regulation of airways calibre and maintenance of homeostasis may influence the mediator release.

The role of calmodulin

Because of the importance of calcium in cellular reactions, the second messenger theory has been expanded to include both cyclic nucleotides and intracellular calcium ions. Meyer and colleagues (73), working on calcium-activated muscle phosphorylase kinase, were the first to suggest that Ca²⁺ may not act in its free form, but requires a binding protein to regulate its action. Several calcium-binding proteins (calcioproteins) have been identified, of

which the most widely distributed in the animal kingdom is calmodulin (74). The protein is present in many cytoplasmic structures, especially membranes, actin-containing filaments and microtubules.

Calmodulin is a heat-stable acidic protein of 16,700 daltons with a highly flexible tertiary structure. The molecule has four high affinity and specific binding sites for Ca²⁺, and it plays a role of multifunctional intracellular receptor for the divalent cation. Binding with Ca²⁺ gives calmodulin the helical conformation which makes it biologically active. It mediates a wide range of essential processes regulated by Ca²⁺ including those involved in cyclic nucleotide metabolism, smooth muscle contraction, neurotransmitter release, intracellular motility (microtubules and microfilaments) and calcium transport. Thus calmodulin, calcium and cyclic AMP are inextricably linked and probably autoregulatory, and calmodulin plays a pivotal role in concerted cellular regulation by Ca²⁺ and cyclic AMP. Calmodulin stimulates both adenylate cyclase and phosphodiesterase, and in addition activates the calcium pump which returns a raised intracellular calcium to the resting level. Therefore, both calmodulin and cyclic AMP may regulate Ca²⁺-influx independently.

The effect of calmodulin on cyclic GMP metabolism is less well documented. Although calmodulin seems to play an essential role in regulating the metabolism of cyclic nucleotides, this still remains unclear.

The role of the products of arachidonic acid cascade

Arachidonic acid, a polyunsaturated fatty acid, occurs in membrane and other subcellular phospholipids of all tissues in the body (for reviews, ref. 75-79). The fatty acid is released from cell membranes by the action of a cellular enzyme, phospholipase A₂ which may be activated by changes in chemical environment including hormonal, nervous or mechanical stimulation, and immunological challenge. Free arachidonic acid thus liberated is converted by two main enzyme pathways to biologically active products.

"Fatty acid cyclo-oxygenase" or "prostaglandin synthetase" catalyzes the formation of prostaglandins (PGs) and thromboxanes (TXs) whereas "lipoygenase" is responsible for the generation of leukotrienes (LTs). All these products of arachidonic acid cascade possess a range of biological activities. PGE₂ and PGI₂ (prostacyclin) are vascular and bronchodilators, while PGF₂ α , TXA₂ and leukotrienes induce vascular and bronchoconstriction.

Human lung tissue in vitro releases PGE₂, PGF₂ α and their metabolites following immunological challenge (18, 80, 81). It has yet to be established whether TXA₂ and prostacyclin that are generated in animal lung (82, 83) are also formed in human lung. Appearance of PGs during human lung anaphylaxis has a close and parallel relationship to histamine release. Histamine and several other mediators of anaphylaxis may induce PG generation. Histamine acts through an H-1 receptor to cause bronchial smooth muscle constriction and PG generation. Recently, it has been suggested that anaphylaxis of human lung generates a novel mediator, prostaglandin-generating factor of anaphylaxis (PGF-A), which can induce PG synthesis (84). Moreover, SRS-A has lately been identified as leukotrienes (LTC₄, LTD₄ and LTE₄) (79).

PGE₁ (product of dihomono- γ -linolenic acid) causes bronchodilatation with an increase in specific airway conductance in asthmatic subjects (for review, ref. 85). In normal subjects, inhalation of PGF₂ α produces reproducible fall in specific airway conductance which may be reversed by isoprenaline. Asthmatic patients exhibit a striking hyperreactivity to inhaled PGF₂ α and develop bronchoconstriction with doses approximately 150 to 1000 times less than normal subjects. Furthermore, serum

levels of $\text{PGF}_2 \alpha$ or its stable metabolite 13,14-dihydro-15-keto $\text{PGF}_2 \alpha$ were higher in some asthmatic patients than in healthy control subjects, and increased amount of PG degradation products were found in the urine of asthmatic patients. Although a definite role for prostaglandins in the lung has yet to be defined, it seems that they act as autoregulatory agents. Their role is thought to be secondary and may be related to physiological attempts to restore the bronchi to a more normal state. Prostaglandins may be involved in the maintenance of normal bronchial tone and thereby adjust local ventilation perfusion ratio in the lungs. An imbalance between dilator and constrictor agents may thus result in bronchoconstriction.

Prostaglandins E_1 and E_2 have been shown to inhibit the immunological release of histamine and SRS-A in human leucocytes and lung (86, 87). The effect also caused an increase in cellular cyclic AMP through the PG receptors. Moreover, β -adrenergic stimulation which inhibits the anaphylactic release of histamine and SRS-A also suppresses the anaphylactic release of PGE and $\text{PGF}_2 \alpha$ in human lung tissue in vitro. These effects of adrenergic agents are also blocked by propranolol indicating that inhibition of PG release is also mediated by the β -adrenoreceptors. Since any event that increases the intracellular levels of cyclic AMP prevents the release of histamine and SRS-A, it is suggested that this is also the mechanism by which β -adrenoreceptor agonists inhibit PG release.

Although $\text{PGF}_2 \alpha$ increases the cyclic GMP content of human lung in a dose-related manner (81), there is a small cholinergic component of the effects of $\text{PGF}_2 \alpha$ in man. Atropine has no effect on the fall in specific airway conductance induced by $\text{PGF}_2 \alpha$ in normal subjects, and it reduces the effects of $\text{PGF}_2 \alpha$ to a very limited extent in asthmatic patients. Moreover, the constriction of the lungs induced by $\text{PGF}_2 \alpha$ in vitro was unaffected by atropine, and α - and β -adrenergic blockade. Thus the $\text{PGF}_2 \alpha$ -induced constriction may be due to a direct action on smooth muscle cells, possibly via PG receptors.

Corticosteroids, which are therapeutically used in asthma, are now known to inhibit phospholipase A_2 activity (88). Therefore, these compounds limit the availability of free arachidonic acid for subsequent metabolism to prostaglandins and SRS-A.

Although PGE₁ and PGE₂ may have therapeutic role in reversible airways obstruction, irritation of the upper respiratory tract and other side effects mitigate against their introduction in clinical practice. However, selective PGE analogues and $\text{PGF}_2 \alpha$ antagonists may be drugs of choice in the future.

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