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COMMENTARY

CYCLIC NUCLEOTIDES AND MODULATION OF EFFECTOR SYSTEMS OF INFLAMMATION*

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DIVERSE effector systems of inflammation such as the secretion of chemical mediators from mast cells and basophils, the destruction of target cells by lymphocytes, and the release of lysosomal enzymes from polymorphonuclear leukocytes (PMN) are modulated by agents affecting the intracellular concentrations of cyclic nucleotides. The immunologic release of chemical mediators from human lung tissue will be analyzed in detail and the data derived interpolated with studies of other effector systems to implicate the cyclic nucleotides in the modulation of not only immediate hypersensitivity reactions but also cellular hypersensitivity and immune complex reactions.

IgE-dependent release of chemical mediators from human lung tissue

Human lung tissue,[1] nasal polyps[2] and leukocytes[3] from allergic individuals release chemical mediators *in vitro* upon contact with specific antigen. These tissues[2,4,5] and cells[6] obtained from non-allergic individuals can be passively sensitized with serum from allergic patients so that subsequent introduction of antigen induces the secretion of histamine, slow-reacting substance of anaphylaxis (SRS-A) and eosinophil chemotactic factor of anaphylaxis (ECF-A) from lung and polyps, and histamine and ECF-A[7] from leukocytes. The responsible immunoglobulin is of the IgE class[8] and appears to have the unique ability to attach selectively to mast cells and basophils[9] by its Fc piece[10] so that antigen can induce the bridging of adjacent molecules. Since antigen-treated basophils exclude supravital dyes and retain normal microscopic appearance, motility, and incorporated-^{42}K while releasing histamine, the IgE-dependent secretory phenomenon does not appear to be cytotoxic.[11]

The biochemical reactions which are initiated by the union of antigen with tissue-fixed IgE antibody and result in the release of histamine, SRS-A and ECF-A from human lung tissue have recently been partially sequenced and appear to proceed as follows: a calcium-dependent activation of a serine esterase from its diisopropyl-fluorophosphate (DFP)-resistant proesterase form to its active DFP-sensitive form; an energy-dependent step utilizing glycolysis or oxidative phosphorylation; a second calcium-requiring step inhibited by ethylenediaminetetraacetate (EDTA) but not by simple deprivation of calcium; and a step inhibited by increased concentrations of cyclic 3′,5′-adenosine monophosphate (cyclic AMP).[12]

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The evidence that increased concentrations of cyclic AMP in the mixed cell population of intact lung fragments reflect commensurate changes within the subpopulation of target cells responding to the immunologic challenge with mediator release includes: the kinetic relationship between increases in cyclic AMP and inhibition of mediator release after stimulation with the beta-adrenergic agonist isoproterenol[13], prostaglandin E_1[14] or cholera toxin;[2] the parallel rank order of potency of isoproterenol $\cong$ epinephrine > norepinephrine > PGE_1 > $PGF_{2\alpha}$ (range of concentrations = nM to μM) in terms of increasing cyclic AMP levels and inhibiting mediator release;[14] the synergism expressed between stimulators of adenylate cyclase, whether catecholamines or prostaglandins, and inhibitors of phosphodiesterase upon both phenomena;[14] and the capacity of exogenous dibutyryl cyclic AMP to suppress mediator release.[15] That each of these effects is manifested upon the release of three chemically distinct mediators and involves interaction with pharmacologically discrete receptor sites supports the contention that the cyclic AMP accumulations in whole human lung fragments indeed reflect similar changes within the target cells responsible for the release of these mediators. Studies with isolated populations of rat peritoneal mast cells also reveal that an increase in cyclic AMP induced by phosphodiesterase inhibitors and PGE_1 or by the addition of dibutyryl cyclic AMP results in suppression of histamine release. Mediator release from purified rat mast cells with rabbit anti-rat Fab is inhibited by elevating the cellular level of cyclic AMP.[16]

Enhancement of the immunologic release of mediators in association with depletion of the levels of cyclic AMP in lung fragments is observed after alpha-adrenergic stimulation with norepinephrine or phenylephrine employed either alone or in combination with the beta-adrenergic antagonist propranolol,[17,18] after interaction with dilute concentrations of PGE_1 or $PGF_{2\alpha}$,[14] and after introduction of the phosphodiesterase-activating agent imidazole.[19] These data indicate an inverse relationship between cyclic AMP concentrations and the antigen-activated, IgE-dependent release of mediators.

The cholinomimetic agents, acetylcholine and carbamylcholine, in pM concentrations, enhance the immunologic release of mediators without producing a measurable change in the tissue levels of cyclic AMP.[17] Pretreatment with atropine prevents this effect, indicating involvement of muscarinic receptor sites in the target tissue. Neither alpha-adrenergic nor cholinergic stimulation alone releases mediators, and the mechanisms by which immunologic release is enhanced appear to be discrete. There is a consistent association of alpha-adrenergic but not cholinergic enhancement with depletion of cyclic AMP, combinations of alpha-adrenergic and cholinergic agents yield additive enhancement, and atropine selectively prevents cholinergic enhancement. The finding that exogenous 8-bromo-cyclic 3′,5′-guanosine monophosphate (8-bromo-cyclic GMP) produces a dose-related enhancement of the immunologic release of histamine, SRS-A[17] and ECF-A* and the observations of others[19–21] that cholinergic stimulation selectively increases the intracellular concentrations of cyclic GMP suggest that increased levels of this nucleotide may mediate cholinergic enhancement of mediator release.

Sensitized tissue challenged in the presence of EDTA yields no mediators but gives full release after being washed and resuspended in calcium-containing buffer without antigen. The capacity of cyclic AMP and cyclic GMP to modulate mediator release

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after cation reversal of inhibition by EDTA of the antigen-activated secretory pathway suggests that both these nucleotides act late in the reaction sequence.[12] When incremental concentrations of carbamylcholine are superimposed upon a constant dose of isoproterenol, the capacity of isoproterenol to increase cyclic AMP concentrations and suppress the immunologic release of SRS-A is unaffected, while the inhibition of histamine release is progressively reversed.[18] These findings with regard to histamine document the opposing functional effects of the two cyclic nucleotides, as has been recently found in several other systems.[22,23] The failure of cholinergic stimulation to reverse the suppression of SRS-A release may relate to the necessity for formation of SRS-A before release, thereby providing additional steps which may be influenced by cyclic AMP.

Clinical implications

The immunologic release of histamine, SRS-A and ECF-A from human lung tissue has been implicated in allergic bronchial asthma.[1,24] Histamine is capable of increasing vascular permeability, inducing bronchial smooth muscle contractions and increasing exocrine gland secretion; SRS-A produces prolonged smooth muscle contraction, with human bronchial smooth muscle being exquisitely sensitive, and increases vascular permeability; and ECF-A selectively attracts eosinophils. While histamine and ECF-A derive from the mast cell or basophil[7,25] where they are granule-associated,[26] the cellular origin of SRS-A remains obscure. All 3 molecules are less than 1000 daltons in molecular size,[27,28] and although the chemical structures of SRS-A and ECF-A have not been elucidated, SRS-A appears to be an acidic lipid[28] and ECF-A a peptide.[27]

Pulmonary function tests reveal abnormalities at both central (greater than 2 mm dia) and peripheral airways (less than 2 mm, includes bronchiolar and alveolar airways) in patients with bronchial asthma.[29] While the central airway obstruction is attributed to reflex cholinergic discharges initiated by an action of mediators on epithelial irritant receptor sites,[30] the peripheral effects may reflect the direct action of chemical mediators. In this regard, it is noteworthy that the intravenous administration of SRS-A to unanesthetized guinea pigs elicits a decrease in lung compliance, reflecting peripheral airway obstruction, while the administration of histamine is followed by an increase in central airway resistance,[31] an effect previously demonstrated to be mediated by reflex cholinergic discharges.[30] Agents such as isoproterenol, epinephrine and aminophylline, currently used to ameliorate bronchial asthma, increase cyclic AMP indiscriminately and could act directly to influence both mediator release and bronchial smooth muscle tone. Disodium cromoglycate inhibits the immunologic release of mediators *in vitro* without increasing cyclic AMP[24] and prevents aerosol allergen-induced bronchospasm in allergic asthmatics[32] without a direct action on bronchial smooth muscle. Thus, prevention of the immunologic mediator release *per se* appears to be sufficient to alleviate the acute allergic symptom complex.

The proposal of Szentivanyi[33] that the adrenergic receptors of the tissues involved in the acute allergic symptom complex are imbalanced has been followed by the development of evidence for a more generalized defect in beta-adrenergic receptors. The responses of asthmatics to beta-adrenergic stimuli in terms of the anticipated eosinopenia,[34] cardiac rhythm changes[35] and hyperglycemia[36] are impaired and their lymphocytes exhibit a dampened cyclic AMP response to isoproterenol.[37] The

findings that the immunologic release of the chemical mediators of the allergic reaction in human lung fragments is augmented not only by beta-adrenergic blockade and alpha-adrenergic stimulation but also by cholinergic stimulation (see Fig. 1) further extend and emphasize the possible effects of pharmacologic responsiveness on the allergic reaction. In view of the opposing actions of elevations of cyclic AMP and cyclic GMP and the bidirectional effects of the level of cyclic AMP, it is not yet possible to hold a single pharmacologic receptor abnormality accountable for the underlying defect of asthma.

Lymphocyte-mediated cytotoxicity (LMC)

Lymphocytes from recipient rats of one strain sensitized by an allograft of a second strain produce cytolysis of cells bearing the antigenic determinants of the second or donor strain.[38] Intimate contact between viable attacking (recipient) lymphocytes and target (donor) cell populations is a prerequisite for the cytolytic reaction.[39] The reaction is immunologically specific in that the attacking cells must be from a sensitized animal and the target cells must bear antigenic determinants in common with the allograft used to achieve sensitization.[38,39] Studies of purified lymphocytes from spleens of sensitized rats indicate that a subpopulation of thymus-derived (T lymphocytes) cells attack chromium-labeled target thymocytes, as the selective deletion of immunoglobulin-bearing splenic lymphocytes (B lymphocytes) has no inhibitory effect upon the reaction.[40] This lymphocyte-mediated cytotoxicity is modulated by agents influencing the intracellular concentrations of cyclic nucleotides.[40,41]

Agents which activate adenylate cyclase, such as PGE_1, isoproterenol and cholera toxin,[40] produce an accumulation of cyclic AMP within the attacking cell population in association with attenuation of the capacity of these cells to induce cytolysis; both effects are dose dependent. The attenuation of LMC and increases in lymphocyte cyclic AMP exhibit the same time course. PGE_1 induces an immediate increase in cyclic AMP and suppression of LMC, while cholera toxin has no effect until 3 hr after its introduction, when increases in cyclic AMP occur simultaneously with inhibition of LMC. The combination of agents which activate adenylate cyclase, such as isoproterenol or PGE_1, with competitive inhibitors of phosphodiesterase, such as aminophylline, results in synergistic actions upon both phenomena. Further evidence that cyclic AMP levels influence LMC derives from the capacity of dibutyryl cyclic AMP to suppress LMC.[41,42]

The influence of cyclic AMP is again bidirectional, as revealed by the effect of imidazole, which reduces lymphocyte cyclic AMP with concomitant augmentation of LMC.[40] Cholinergic enhancement with carbamylcholine was not associated with alterations in cyclic AMP levels and was again attributed to an elevation of cyclic GMP, both because of the capacity of 8-bromo-cyclic GMP to augment LMC[40] and the finding of increased cyclic GMP in lymphocytes after cholinergic stimulation.[43] Cholinergic enhancement was prevented by atropine. The finding that the capacity of pharmacologic agents to influence LMC is dependent upon preincubation of the agent with the attacking cells and does not require alterations in the cyclic nucleotide levels throughout the 4-hr period in which attacking and target cells interact suggests that cyclic nucleotide modulation occurs early in the cytolytic process.[40] This hypothesis is consistent with the observation that the attacking cells can be destroyed 15 min after the introduction of target cells without suppressing the cytolytic events.[44]

Polymorphonuclear leukocyte (PMN) release of lysosomal enzymes

Whereas the two systems already described involve immunologic activation, the release of lysosomal enzymes from PMN has been studied in relation to other types of initiating factors as well. During the phagocytosis of particles or immune complexes[45] and after stimulation with chemotactic factors, such as the C3a and C5a fragments derived from the activation of the complement system,[46] PMN selectively extrude lysosomal but not cytoplasmic enzymes.[47] PMN treated with cytochalasin B respond to the stimulus of phagocytosis by intense secretion of lysosomal enzymes without actual phagocytosis, facilitating the study of the effect of cyclic nucleotides upon enzyme release.[48]

The selective secretion of lysosomal enzymes is inhibited by agents which increase the intracellular levels of cyclic AMP, such as isoproterenol, epinephrine and histamine.[49] A cyclic AMP-dependent protein kinase recovered from PMN phosphorylates substrates such as histone,[50] suggesting that the action of cyclic AMP upon the release of lysosomal enzymes involves pathways comparable to those delineated in other tissues.[51] Carbamylcholine augmentation of the release of lysosomal enzymes was prevented by atropine and reproduced by the introduction of exogenous cyclic GMP.[50] Although these studies have not been correlated with direct measurements of intracellular cyclic nucleotides, the findings are again consistent with the exertion by cyclic AMP and cyclic GMP of opposing effects on a membrane-activated cell, in this case the PMN.

Concluding comments

As early as 1936, it was observed that epinephrine suppressed the capacity of intradermally administered antigen to elicit a wheal and flare reaction in an allergic individual,[52] and these data were extended to include isoproterenol in 1951.[53] The suppression by epinephrine of histamine release consequent to anaphylactic shock in the isolated lung of the guinea pig was noted in 1937.[54] That these observations related to increased levels of cyclic AMP was suggested in 1968, based upon the capacity of beta-adrenergic agents to suppress the immunologic release of histamine from human peripheral leukocytes and act synergistically with methylxanthines, and the reproduction of the effects with dibutyryl-cyclic AMP.[55] The association by direct measurement of elevated-cyclic AMP levels in mixed cell systems[56] or tissue fragments [13] with suppression of the immunologic release of chemical mediators followed and has been extended to a homogeneous mast cell population.[16] Subsequent studies with human lung fragments have revealed the bidirectional influence of cyclic AMP as well as the opposing roles of cyclic AMP and cyclic GMP in modulation of the IgE-dependent, antigen-induced release of three chemically and functionally distinct mediators (Fig. 1). Comparable studies of other effector cells of inflammation have revealed a similar influence of cyclic nucleotides (Table 1). The isolation from the PMN of a cyclic AMP-dependent protein kinase capable of inducing the phosphorylation of suitable substrates[50] suggests that the influence of cyclic AMP upon the cells involved in the inflammatory reaction proceeds through the pathways delineated in other tissues.[51] In contrast to the role of cyclic AMP as the *intracellular mediator* of hormonal stimulation in a variety of cells, the effect of cyclic nucleotides on the effector cells of inflammation is limited to *modulation* of the physiologic consequences of their specific activation. Further, elevations of cyclic AMP inhibit rather than

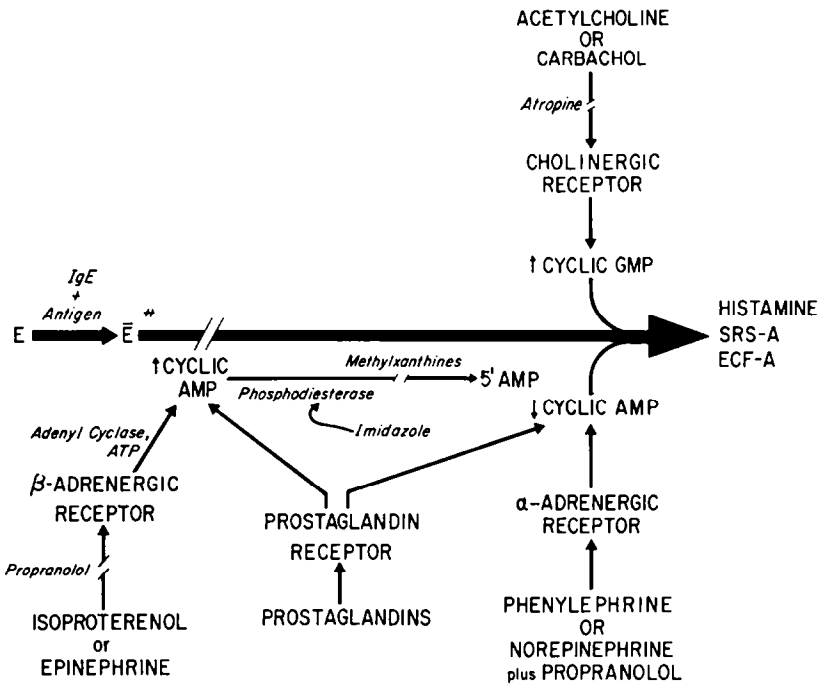

FIG. 1. Pharmacologic modulation of the antigen-induced, IgE-dependent secretion of chemical mediators from human lung tissue. E to Ē depicts the series of events which precede the modulation.

TABLE 1. MODULATION BY CYCLIC NUCLEOTIDES OF THREE DISTINCT EFFECTOR SYSTEMS OF INFLAMMATION*

Agents	Antigen-induced, IgE-dependent secretion of chemical mediators from human lung tissue	Lysosomal enzyme release from polymorphonuclear leukocytes	Lymphocyte mediated cytotoxicity
Effect of agents increasing cyclic AMP levels			
1. Dibutyryl cyclic AMP	I	I	I
2. Phosphodiesterase inhibitors	I	I	I
3. Beta-adrenergic agents	I	I	I
4. Prostaglandin E_1	I	I	I
5. Beta-adrenergic agents + propranolol	NE	NE	ND
6. Cholera toxin	I	ND	I
Effect of agents decreasing cyclic AMP levels			
1. Alpha-adrenergic agents	E	NE	ND
2. Imidazole	E	ND	E
3. Prostaglandin $F_{2\alpha}$	E	ND	ND
Effect of cholinergic agents			
1. Acetylcholine or carbamylcholine	E	E	E
2. Carbamylcholine + atropine	NE	NE	NE
3. 8-bromo-cyclic GMP	E	E	E

* Abbreviations: I — inhibitory; E — enhancing; NE — no effect; ND — not done.

augment the inflammatory effects of mast cells, polymorphonuclear leukocytes and lymphocytes.

Partial delineation of the biochemical events involved in the immunologic release of mediators from human lung tissue has demonstrated that the effects of cyclic nucleotides are preceded by several other steps[12] and perhaps permits the following speculation (Fig. 2). The interaction of antigen with tissue fixed IgE antibody results in the transportation of extracellular calcium to the site of a proesterase, which is subsequently converted to an active chymotrypsin-like serine esterase; the esterase acts upon its substrate (perhaps removing an inhibitory protein); the subsequent energy requirement and the second calcium-dependent step may relate to the action of a "contractile protein", which is susceptible to inhibition by phosphorylation by the cyclic AMP-dependent protein kinase system; the mediator granule then is moved to the cell membrane whereby sequential exocytosis[57] release takes place. The stage depicted as "contractile protein" in Fig. 2 may reflect a complex set of interactions between calcium and a cyclic AMP-activated protein kinase substrate and the consequences of this reaction in terms of the participation of other factors. The similarities of specific lysosomal enzyme release from polymorphonuclear leukocytes and of target cell killing by sensitized T lymphocytes to IgE-dependent, antigen-induced mediator release are most evident in terms of modulation by the cyclic nucleotides (Table 1), but can be extended to include requirements for energy[58] and calcium ions [58,59] and, in the case of the polymorphonuclear leukocytes, involvement of an activatable esterase.[60] It may well be that specific membrane perturbation, immunologic and otherwise, leads to effector cell functions which involve a comparable sequence of steps and controls but appears specific to a cell type because of its unique features or the nature of assay.

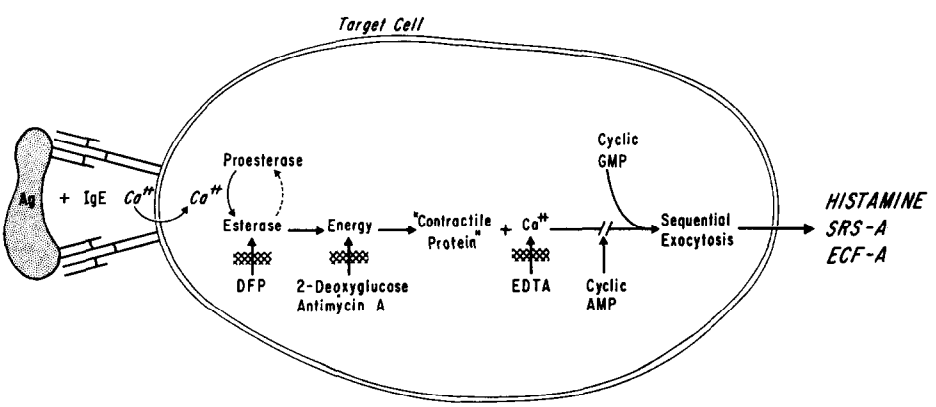

FIG. 2. Schematic representation of the sequence of the antigen-induced, IgE-dependent biochemical events in the secretion of chemical mediators from human lung tissue.

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