Calcium Ions, Airway Function, and Asthma

Ca^{2+} hypothesis of asthma

Altered control of cellular Ca^{2+} ion fluxes may represent the final biochemical pathway which could account for the pathological changes in the lung and the pathogenetic mechanisms which lead to the syndrome of asthma. While this idea, which can be considered the Ca^{2+} hypothesis of asthma[1] may seem overly reductionistic, there is much evidence that directly or indirectly supports its basic tenent, namely, that all of the features of the disease are ultimately dependent on the translocation of Ca^{2+} ions in the relevant cells which are involved in the disease.

All the principle features of the pathogenesis of asthma are Ca^{2+} -dependent phenomena.[1] This includes Ca^{2+} -dependent stimulus-secretion coupling in mast cells (and basophils) and mucous gland secretion, Ca^{2+} -dependent excitation-contraction coupling in agonist -induced smooth muscle contraction, Ca^{2+} -dependent vagus nerve impulse initiation and conduction, and the Ca^{2+} -dependent movement of cells to the site of immunological injury to produce the characteristic inflammatory infiltrate in the airways. Therefore, any proximal stimulus to the development of asthma, such as an allergic reaction, a viral respiratory infection, exercise, cold air, or an emotional upset, must ultimately lead to the increased availability of Ca^{2+} ions to the relevant cell machinery in the above mentioned cell types that will enhance or permit secretion, contraction, nerve related, or inflammatory responses to occur. It follows, therefore, that any effective antiasthmatic therapy must reduce the availability of Ca^{2+} ions to the cellular machinery that leads to secretion of chemical mediators from mast cells (and basophils), stimulation of mucous gland secretion, contraction of smooth muscle,[2] nerve impulse initiation and conduction in the vagus, and the development of the characteristic inflammatory response. Thus, hypothetically, all features of asthma may ultimately be the result of abnormal regulation of Ca^{2+} movements in the involved cells. The ultimate cause of this abnormality and how it affects the Ca^{2+} -regulating systems remain to be elucidated. By analogy, in clinical and experimental hypertension there are changes in vascular smooth muscle reactivity which could be explained by altered control of Ca^{2+} movements.[2a]

Relationship of airway calibre to cell Ca^{2+}

Under normal conditions airway smooth muscle tone is maintained by low grade cholinergically driven smooth muscle contraction which can be reversed by inhaling the muscarinic anticholinergic agent atropine. In pathological states such as asthma, airway calibre can be compromised by increased smooth muscle contraction stimulated by the vagal bronchoconstrictor reflex and by chemical mediators such as leukotriene C and D (slow reacting substance of anaphylaxis, SRS-A) and histamine released from mast cells, and also by the hypersecretion of mucous glands and the ingress of inflammatory cells, especially eosinophils, into the bronchial wall.[3-6] The specific functions of these cells are ultimately dependent upon the availability of Ca^{2+} ions, as noted above, that is, exitation-contraction coupling in agonist-induced smooth muscle contraction,[7-11] stimulus secretion coupling in mast cell (and basophil) mediator secretion[12,13] and mucous gland secretion,[14] nerve impulse initiation and conduction[10,15] and the motility of inflammatory cells. Of course, it is well recognized that Ca^{2+} ions are extremely important informational cations in the function of all cells[13,16] and so it is plausible that altered control of Ca^{2+} mobilization and translocation in various pulmonary cells could profoundly affect the function of the lung (and indeed the nasal airways as well).

Mechanisms controlling intracellular Ca^{2+} concentration

All cells in the body maintain a steep inside - to - outside unbound Ca^{2+} concentration gradient. The concentration of free Ca^{2+} ions in the cytoplasm is approximately

10^{-7} M as compared to an extracellular concentration of approximately 10^{-3} M, a 10,000-fold higher concentration than inside the cells. Clearly mechanisms must exist for maintaining this extraordinary concentration gradient and, in addition, other mechanisms that selectively permit the entry of Ca^{2+} ions into the cytoplasm of cells that have been stimulated to perform a particular Ca^{2+} -dependent function such as smooth muscle contraction or chemical mediator secretion from mast cells, for example.

Low cytosolic Ca^{2+} concentrations are normally maintained in resting nonstimulated cells by pumping Ca^{2+} to the outside through the action of the Ca^{2+} --ATPase extrusion pump or via Na $-Ca^{2+}$ exchange mechanisms, amongst others.[9,10,17-19]

In cells stimulated to perform a particular Ca^{2+} -dependent function, Ca^{2+} can be mobilized from subcellular stores such as sarcoplasmic or endoplasmic reticulum, plasma membrane-bound Ca^{2+}, or from extracellular sources via so-called transmembrane Ca^{2+} channels.[7-10,20-23] An activated cell may utilize several of these Ca^{2+} sources to elevate intracellular cytosolic Ca^{2+} concentrations in order to accomplish particular contractile,[20] secretory, motile, or other functions before intrinsic regulatory mechanisms restore the low cytosolic Ca^{2+} concentration.

Transmembrane Ca^{2+} channels are of at least two types, the potential dependent channel (PDC) and receptor operated channel (ROC).[10] The chemical nature of these putative Ca^{2+} channels and their topographical relationship to receptors on the cell surface for various ligands is not understood in detail. PDC are activated by various stimuli with associated decrease in membrane potential which is accompanied by action potential generation. ROC are specifically activated by neurotransmitters and agonists such as acetylcholine, histamine and the leukotrienes C and D (SRS-A)[21] to produce Ca^{2+} -dependent contraction with little or variable degrees of accompanying membrane depolarization.

Ca^{2+} antagonists and airway function

In recent years compounds called Ca^{2+} channel antagonists or Ca^{2+} entry blockers such as verapamil, D-600 (methoxyverapamil), nifedipine, and diltiazem, amongst many others, have been developed. These compounds have found a place in the treatment of cardiac arrhythmias, angina, and hypertension by blocking the entry of Ca^{2+} ions into myocardium and vascular smooth muscle[8,18,24-26] or to alter intracellular Ca^{2+} mobilization.[18-20] They appear to act in part by competitively inhibiting the entry of Ca^{2+} into cells and have affinity and selectivity for PDC.[8] The studies performed on the effect of vascular smooth muscle Ca^{2+} antagonists on airway smooth muscle contraction suggest that they may be somewhat less active than in cardiovascular smooth muscle.[27,28] These observations suggest, however, that it might be possible to synthesize Ca^{2+} channel antagonists which would have specificity for airway smooth muscle or, for that matter, other cell systems as well, e.g. mast cells and mucous glands which require Ca^{2+} for their specific functions. It is of interest in this connection that some Ca^{2+} antagonists appear to act intracellularly to antagonize, for example, cholinergically induced catecholamine secretion from the adrenal medulla (cf.[1])

With some knowledge of PDC and ROC in smooth muscle it became of interest to study what kind of Ca^{2+} channel is activated, or "opened", in other cell systems such as mast cells and basophils stimulated by an immunologic reaction between antigen and membrane-bound IgE. That differences clearly exist between the Ca^{2+} channels of agonist-stimulated smooth muscle and antigen-activated basophils has recently been shown in experiments demonstrating that the Ca^{2+} antagonists verapamil, D-600 (methoxyverapamil), nifedipine, dantrolene, and TMB-8 (a trimethoxybenzoate compound) failed to inhibit antigen-induced human basophil histamine release.[29] However, Norn et al[30] Jensen et al[31] found that relatively high concentrations of nifedipine and verapamil inhibited immunologic and ionophore-induced basophil histamine release both *in vitro* and from cells obtained from subjects who had been given an oral dose of nifedipine. Similar results with passively sensitized, antigen-challenged human pulmonary mast cells have been reported.[32] Whether these observations represent Ca^{2+} channel blockade or a nonspecific action of the Ca^{2+} antagonists must be determined since the concentration of Ca^{2+} channel blockers employed in the *in vitro* experiments was quite high. Weiss and coworkers[28,33] also concluded that nifedipine and verapamil could inhibit experimental anaphylaxis. Thus, Ca^{2+} "channels" are complicated structures and it is likely that the nature of Ca^{2+} channels differs within cells and from one cell type to another.[10,23] This notion is supported by the remarkable structural diversity of the known calcium antagonists and the uncertainty of predicting effectiveness in different tissues. An exciting advance is the development of a radioligand assay to measure Ca^{2+} channels directly.[34] This technique may make possible the quantitation of PDC- Ca^{2+} channels in lung smooth muscle and other tissues of asthmatics.

Very few studies have been conducted on the effect of the "classical" Ca^{2+} channel antagonists (i.e. ones effective in vascular smooth muscle and myocardium, such as verapamil and nifedipine) on responses of airway smooth muscle to stimulation by various agonists.[28,33] Several reports indicate, however, that the currently avail-

able Ca^{2+} channel antagonists such as verapamil and nifedipine do have some inhibitory activity on airway smooth muscle of dog[35-41] and guinea pig[42-45] and may reduce resting tone.[37] In general, it appears that the concentration of Ca^{2+} antagonist required to inhibit agonist-induced airway smooth muscle contraction may be about one-two orders of magnitude higher than that necessary to antagonize similar contractile responses in vascular smooth muscle and that isoproterenol is still the most effective relaxing agent on a molar basis. Indeed, certain Ca^{2+} channel antagonists may enhance the relaxing effect of the classical bronchodilators, i.e. the beta agonists and theophylline,[45] suggesting yet another mechanism for their potential as antiasthmatic drugs.

Clinical experiments in humans are beginning to be reported. The experiments of Cerrina[46] and Patel[47] suggest that exercise-induced asthma can be inhibited by prior administration of nifedipine but this Ca^{2+} channel antagonist failed to alter the impaired baseline pulmonary functions of patients with asthma. The explanation for this discrepancy is not evident. However, another Ca^{2+} antagonist, cinnarazine, has been reported to exert beneficial effect in patients with chronic asthma.[48] Jaiprakash et al[49] also showed that patients with coexistent angina and fixed or labile airway obstruction treated with nifedipine not only had reduced angina (and reduced blood pressure in the hypertensives) but also had improved peak expiratory flow rates, although changes in FEV_1 were not detected. A lack of effect of verapamil in obstructive airway disease had been reported earlier.[50]

Williams et al[51] were unable to show any significant bronchodilating effect of orally administered nifedipine in asthmatics but did demonstrate that the drug provided significant protection against histamine-induced bronchoconstriction. On the other hand, Patel[52] and Patel and Kerr[27] found that aerosol verapamil had no effect on the airway responsiveness of asthmatics or nonasthmatics given histamine or methacholine by inhalation. Furthermore, So et al[53] found that neither inhaled verapamil or sublingual nifedipine caused bronchodilation nor did they prevent allergen-induced bronchoconstrition. Thus it appears that the classical Ca^{2+} channel antagonists have variable effects on human asthmatic airway responsiveness but it is highly predictable that some future compounds may prove to have important effects on the peculiar hyperresponsiveness of asthmatic airways and be useful in asthma therapy. While most of the studies of Ca^{2+} antagonists briefly reviewed herein deal with their presumed action on airway smooth muscle, it is possible that they may also have effects on other cells pertinent to the pathogenesis of asthma such as mast cells, mucous glands, vagus nerves, and inflammatory cells.

It should be noted that the concentration of the classical smooth muscle Ca^{2+} channel antagonists required to affect various secretory processes is considerably higher (ca. 10-100 fold) than the concentrations required to inhibit agonist-induced smooth muscle contraction.[10] Whether different mechanisms of action account for the concentration-effect differences in secretory and contractile tissues remains to be established. The effect of Ca^{2+} channel blockers on mucous gland secretion has not been reported but as this secretory process in all likelihood shares Ca^{2+} -dependence as with other exocrine glands[14] it would not be surprising to find that some of these compounds inhibit the stimulated secretion of mucous glands. Perhaps Ca^{2+} channel antagonsists more-or-less specific for mucous gland secretion will be synthesized. Ca^{2+} channel blockers have also been shown to have weak effects on nerve function[15] and so it is conceivable that some Ca^{2+} channel antagonists might exert an effect on the bronchoconstrictor reflex by altering vagus nerve impulse initiation and conduction. Finally, since the motility of various inflammatory cells is also Ca^{2+} -dependent it is conceivable that Ca^{2+} channel antagonists active in these cells might affect their ingress into the site of immunologic injury. Specific experiments to verify this possibility remain to be performed.

In summary, the "biochemical lesion" which accounts for the unique hyperresponsiveness of asthmatic airway smooth muscle may reside in cell membranes and be manifested as abnormal control of Ca^{2+} translocations. It seems desirable to investigate new Ca^{2+} antagonists with specificity for airway smooth muscle (and possibly other cells) which might prove to be of value in the treatment of asthma.[54,55]

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