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EDITORIAL REVIEW

The role of cAMP regulation in controlling inflammation

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In 1958, Sutherland and Rall identified adenosine 3′, 5′-monophosphate (cAMP) as an intracellular second messenger of hepatic glycogenolysis [1]. Subsequently, cAMP was shown to act as second messenger for a variety of hormones, inflammatory mediators and cytokines, and has been shown to modulate models of immune and non-immune inflammation *in vivo* and a variety of cellular processes *in vitro*. Indeed, the current paper by Ottonello *et al.* is typical of research in this area. The authors show that in a population of adherent neutrophils, the oxidative burst induced by exposure to granulocyte-monocyte colony-stimulating factor is reduced by agents that elevate cAMP [2]. They speculate that therapeutic elevation of cAMP will result in reduced oxidative damage to tissues in neutrophil-dominated inflammatory reactions.

Production of cAMP in leucocytes is stimulated by β-adrenergic catecholamines, histamine and the E series prostaglandins by a receptor-coupled activation of adenylate cyclase, an enzyme which catalyses the conversion of adenosine triphosphate to cAMP [3]. Rises in intracellular cAMP are usually transient, cAMP being rapidly broken down by phosphodiesterases (PDEs) to 5′AMP. A role for cAMP in a particular cell function can be inferred from the use of agents that activate adenylate cyclase (receptor-coupled activation or direct activation with agents such as cholera toxin [4] or forskolin [5]), duplication of the cell response with a hydrophobic (i.e. membrane-permeable) analogue of cAMP (e.g. dibutyryl cAMP), inhibition of PDEs with methylxanthines (e.g. theophylline [6]) or isoenzyme-specific agents (see below) and by assessing the effects of these various treatments on intracellular cAMP levels.

At an inflammatory site, mast cells are stimulated to degranulate, causing release of vasoactive and other inflammatory mediators. Circulating leucocytes adhere to vascular endothelium and accumulate at the inflamed site under the direction of chemotactic factors. Phagocytic stimuli cause release of lysosomal enzymes and reactive oxygen species (ROS) from neutrophils, eosinophils and macrophages. Antigen recognition causes proliferation and differentiation of lymphocyte subsets. *In vitro* work has suggested that following cell stimulation, agents that elevate cAMP reduce: immunological release of histamine and leukotrienes from mast cells [7], monocyte [8] and neutrophil [9,10] locomotion, release of lysosomal enzymes [11], ROS [12], platelet-activating factor [13] and leukotriene B_4 [14] from neutrophils, release of ROS from eosinophils [15], release of cytokines [16,17] and nitric oxide [18] from macrophages, proliferation of lymphocytes [19] and effector functions of cytotoxic T lymphocytes [20]. However, it is important to realize that the ability of cAMP elevating agents to suppress cell functions is not uniform but depends on the initial stimulus. In *in vivo* models of inflammation it has been shown that in different types of experimental pleurisy (carrageenan [21], pyrophosphate [22], Arthus [23] and delayed hypersensitivity [24]) cAMP levels vary during the reactions, low levels being observed as the reactions proceed and normal or higher levels being observed as the reactions subside [25]. The experimental data therefore suggest that cAMP is part of an endogenous mechanism for down-regulating the inflammatory response and preventing the beneficial effects of acute inflammation from progressing to chronic inflammation and its associated tissue destruction. This view is supported by the clinical finding that leucocytes from atopic individuals appear to have higher than normal PDE activity [26].

The targeting of a single mediator or group of mediators for treatment of inflammation has the drawback that other mediators could partially compensate for the loss, thereby limiting the efficacy of the treatment. Therapeutic elevation of cAMP to treat inflammatory disorders is attractive because a whole host of inflammatory cell functions can in theory be inhibited. In addition, *in vitro* work suggests that a synergy exists between activators of adenylate cyclase and PDE inhibitors in elevating cAMP. If this is true also *in vivo* then the production of agents such as prostaglandin E_2 (PGE_2) at a site of inflammation should ensure that the inflamed tissue is more responsive to PDE inhibitors than non-inflamed tissues.

Interestingly, and somewhat paradoxically, many non-steroidal anti-inflammatory drugs (NSAIDs) appear to elevate cAMP [27] despite blocking the synthesis of PGE_2 which stimulates adenylate cyclase. The reasons for this are unclear, but blockade of cyclooxygenase by these drugs could lead to an accumulation of its substrate, arachidonic acid, which has been shown to have second messenger properties [28]. It is clear that signal transduction pathways do not work in isolation, instead they interact to modulate cell responses [29]. Arachidonic acid appears to be able to elevate cAMP [28], which may explain the effects of NSAIDs on cAMP levels.

Theophylline has been used in the treatment of asthma for many years, and appears to be effective due to a combination of anti-inflammatory and bronchodilatory activities. However,

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theophylline is associated with side-effects in the gut, cardiovascular system and the central nervous system, and these side-effects seem to be mainly due to inappropriate inhibition of PDEs in these tissues and additional actions such as antagonism of adenosine receptors and stimulation of catecholamine release [30]. The future of PDE inhibitors as therapeutics therefore looked bleak until the realization that hydrolysis of cAMP (and cGMP) is not dependent on a single enzyme but on a range of isoenzymes which differ in their tissue distributions.

Seven families of PDEs (types I–VII) are currently recognized based on protein sequence and cDNA analysis. These enzymes differ in substrate selectivity, sensitivity to calcium/calmodulin, allosteric regulation by cGMP, sensitivity to phosphorylation and distribution both in tissues and subcellular compartments [30–33]. Each family can contain subfamilies, and further diversification may arise from genes that can give rise to two or more alternatively spliced RNAs. Tissues may express more than one family of PDEs, but in inflammatory cells (with the exception of lymphocytes) it seems to be members of the PDE IV family that are dominant. Lymphocytes appear to have both PDE III and PDE IV enzymes; whether particular isoenzymes are confined to particular subsets of lymphocytes is not known. PDE IV enzymes are cAMP-specific, are calcium/calmodulin-independent, and are not regulated by cGMP. In addition to inflammatory cells, PDE IV enzymes are found in smooth muscle, brain, liver, heart and kidney. PDE IV inhibitors should lack activities other than PDE inhibition and be more tissue selective than theophylline. However, the distribution of PDE IV enzymes suggests that major side-effects could still be a problem. Indeed, PDE IV inhibitors are being developed as anti-depressants; what effect these drugs would have on unaffected individuals is not known. As subfamilies of PDE IV are investigated, isoenzymes that are truly specific to inflammatory cells may become apparent which will prove more effective targets.

Interest in PDE inhibitors has increased enormously since the discovery of isoenzymes with differing tissue distributions. The potential therapeutic advantages of PDE IV inhibitors in the treatment of inflammatory diseases are clear. However, it is only as data become available from clinical trials that we will see whether these compounds live up to their potential.

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