Immunological Phagocytosis: Effect of Drugs on Phosphodiesterase Activity

Adenosine 3′, 5′-monophosphate (cAMP) has been shown to modulate several intracellular processes[1]. Intracellular levels of cAMP are under control of at least two enzymes, adenyl cyclase which catalyses the formation of cAMP from ATP, and cAMP phosphodiesterase, which hydrolyses the phosphodiester bond of cAMP converting it to 5′-AMP. Agents that inhibit phosphodiesterase activity have been found to magnify cAMP effects by increasing the persistence of cAMP levels. One of the most important questions pertaining to the effects of cAMP in different tissues is the remarkable selectivity of its action, possibly depending on the enzymatic composition of the cell. In many cases, e.g. thyroid hormone[2], amylase in the parotid gland[3], ACTH[4], growth hormone[5], serotonin[6], the release process appears to be mediated by an increase in cAMP levels. In contrast, agents which raise cAMP levels inhibit the antigen-induced release of histamine and SRS from the leukocytes and lung of sensitized individuals[7–10] and the release of histamine from peritoneal mast cells by compound 48–80[11,12].

The binding and engulfment of antibody-coated particles by macrophages (immunological phagocytosis) has been shown to be a specific phenomenon mediated by the presence of receptor sites on macrophage membrane for the Fc part of the antibody molecule[13]. The interaction between the combining sites of the immunoglobulin molecule with the antigen, results in configurational changes in the Fc region with subsequent activation of the intracellular actomyosin-like microfilaments[14] and phosphorylation of microtubular proteins[15]. Although the possible role of cAMP in phagocytic cell function has already been suggested[15], its real meaning has not yet been evaluated. In this communication we present the results of experiments carried out with drugs active on phosphodiesterase and their influence on endocytosis by mouse peritoneal macrophages.

Materials and methods. Random-bred SW adult female mice were used. Sheep red blood cells (SRBC) antiserum was obtained after repeated i.v. injection of 0.1 ml of 1×10^8/ml SRBC suspension into mice. γ-globulins were precipitated with ammonium sulfate at 33% saturation.

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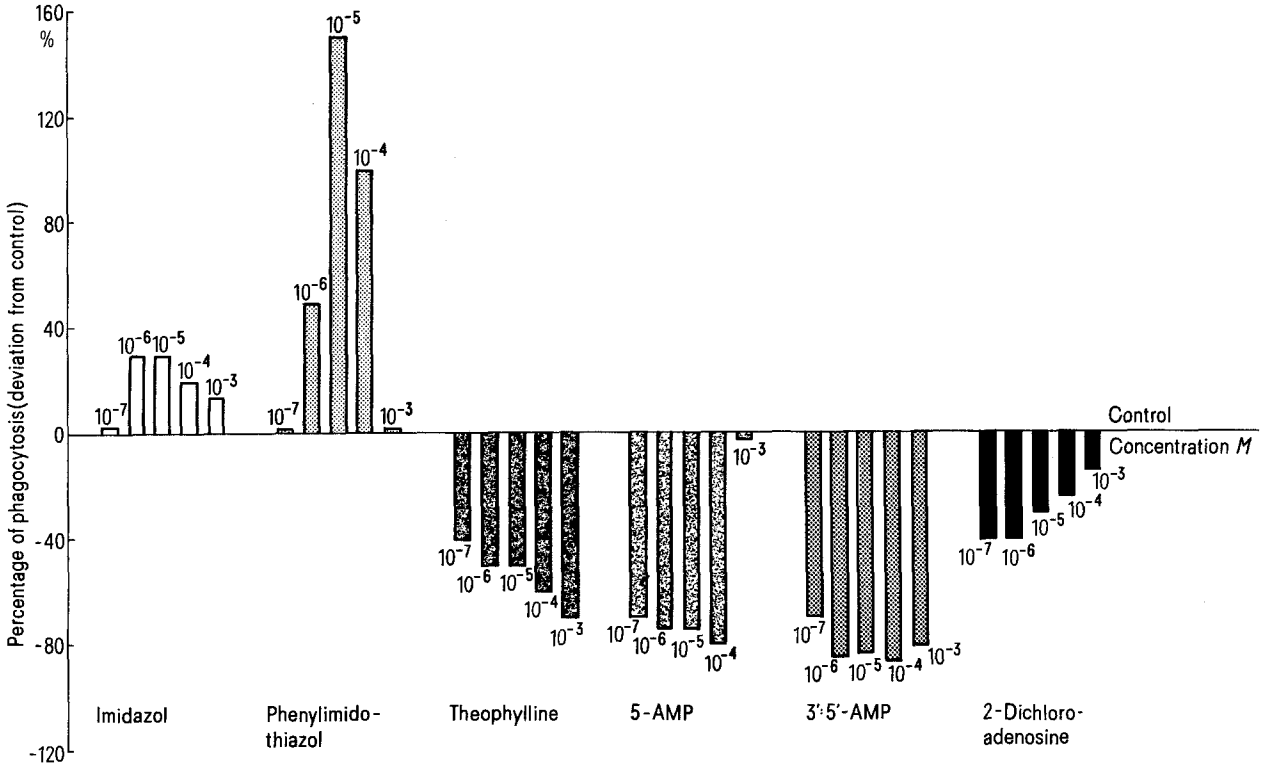

Immunologic phagocytosis of SRBC by mouse peritoneal macrophages in the presence of drugs which inhibit or enhance phosphodiesterase activity.

The 7S immunglobulin was separated by chromatography in Sephadex G-200 and DEAE-cellulose column. Peritoneal macrophages were harvested by injecting 3 ml of heparinized Hanks' solution into the peritoneal cavity of exsanguinated mice. $^2/_{10}$ of a suspension containing 1×10^6 cells/ml were added to wells of acrylic rings attached to microscopic slides and incubated at 22–24 °C for 60 min. The adherent macrophages were washed and 0.2 ml of sensitized SRBC with 7S antibody (EA-7S) containing the drugs were then added to each well. After incubation for 30 min at 22–24 °C the slides (test and control) were kept for 45 sec in Hank's solution diluted 1/5 with water to affect lysis of bound but non-ingested SRBC. The macrophages were fixed with glutaraldehyde, treated with benzidine-H202 mixture for erythrocytes staining followed by Wright staining. The number of macrophages with SRBC phagocytized in randomly microscopic fields was estimated by scoring 200 macrophages in duplicated slides. Drug dilution inducing more that 10% of cells taking up Trypan blue were discarded. Solutions of the drugs were prepared each day before use in Hanks' solution, with the final pH adjusted to 7.4 .The following drugs were used: theophylline, 3′,5′-AMP, 5′-AMP, 2-chloradenosine, imidazol and phenylimidothiazol.

Results and discussion. The results are summarized in the Figure. The drugs recognized as capable of raising the levels of intracellular cAMP through a blockade of the phosphodiesterase activity showed a potent inhibition of phagocytosis. In contrast the drugs capable of reducing the levels of intracellular cAMP by activation of phosphodiesterase, such as imidazol and phenylimidothiazol, were found to be potent stimulators of phagocytosis.

The alterations in cellular cAMP levels during phagocytosis, and the influence of adenyl cyclase, specially in PMN leukocytes, have been the subject of recent controversy. Concentrations of cAMP above 10^{-4} *M* induced by theophylline, or prostaglandine and theophylline, have the capacity to retard the phagocytosis of ^{125}I-labelled heataggregated BSA by mouse peritoneal macrophages, whereas very low concentrations of cAMP (10^{-10} *M*) stimulated the taking up of these particles[15]. These data are compatible with the hypothesis that cAMP may exert a byphasic effect on endocytosis. It was also found that increased levels of intracellular cAMP inhibited lysosomal enzyme release of PMN leukocytes exposed either to particles of zymosan or to immune precipitates. This finding raised a possible analogy between the inhibitory effects of cAMP on lysosomal enzyme release and on histamine liberation[15]. It was demonstrated that high intracellular levels of cAMP impair granulocytes's ability to kill *C. albicans* and that this effect could be consistently obtained with theopylline[16]. It has been suggested that at least one consequence of raising the level of cAMP within cells is to impede the traffic of lysosomes to the phagocytic vacuoles, retarding significantly the extrusion of acid hydrolases[15]. It is generally assumed that peritoneal macrophages usually utilize energy from glycolysis for phagocytosis and that oxidative pathways may also be used[17]. Since the only events common to both glycolysis and respiration are synthesis of ATP from ADP, and oxidation and reduction of NAD, it has been widely inferred that membrane invagination and particle interiorization during endocytosis are processed through ATP. Thus, the energy required to induce membrane excitation, for activation of the intracellular actomyosin-like microfilaments[14] and phosphorylation of tubulin[15], are provided by ATP. The results described herein showing that engulfment of antibody-coated erythrocytes proceed optimally in conditions in which the macrophages cAMP content is lower, indicate that the modulation of this process may follow the same mechanism as histamine release from leukocytes[7,8,11,12]. Accordingly, a decrease in the cAMP level of macrophages may follow the interaction of the receptor located on cell surface with the Fc portion of immunoglolulins after antigen-antibody combination. A direct determination of cAMP content in macrophages undergoing immunological phagocytosis has, however, to be made before a final conclusion can be drawn on its role in this process. It has recently been proposed[18] that in a number of cell types, cyclic 3′,5′-guanosine monophosphate (cGMP) promotes cellular events that are antogonistic to those believed to be mediated by cAMP. The influence of cGMP on macrophage phagocytosis is now being studied in our laboratory[19].

Zusammenfassung. Nachweis, dass Drogen, die einen Anstieg des cAMP-Gehalts der Makrophagen durch Verhinderung der Phosphodiesterase hervorrufen, die immunologische Phagozytose verhindern. Andererseits aber wird die Phagozytose durch Drogen gefördert, welche den cAMP-Gehalt der Makrophagen herabsetzen.

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Stimulation of Human Lymphocytes in vitro by Bacterial Hydrolysates[1]

Various antigenic components of microorganisms, such as streptolysins, staphylococcal filtrate and tuberculine amongst others have been reported to induce lymphocyte blastogenesis in vitro[2,3]. The mitogenic activity of non-immunogenic subfractions of such substances, however, has not yet been systematically analyzed. This paper reports the property of a bacterial hydrolysate with a molecular weight below 10,000 to stimulate human lymphocytes in vitro.

Material and methods. Peripheral venous blood lymphocytes were obtained from 16 healthy male volunteers at weekly intervals for 8 weeks. Lymphocyte separation