Switching phenomena in thin amorphous films and chalcogenide glasses have been extensively studied (*4*, *5*). Since more information is becoming available on these materials, we wished to determine to what extent the results for melanins parallel those reported for some inorganic amorphous semiconductors. Switching behavior in these materials is usually reported for samples less than 10 μm thick and with potential gradients greater than 10^5 volt/cm (*4*). Melanins, however, switch at 3.5×10^2 volt/cm and through at least 1 cm of material. It should be noted that this potential gradient exists in some biological systems.

Switching at low gradients and through bulk samples poses interesting theoretical questions. However, the consistent appearance of melanin in living organisms at locations where energy conversion or charge transfer occurs (the skin, retina, midbrain, and inner ear) is of particular interest in view of the evidence for a role for melanin in such human disorders as parkinsonism (*6–8*), schizophrenia (*7*), and deafness (*8*). The role of melanin in these disorders may be in some way related to its ability to function as an electronic device. This possibility is supported by the observation that the electronic properties of the melanin persist in intact melanosomes.

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9. We thank M. Romsdahl and S. Moss for their assistance in this work. Supported in part by PHS training grant CA-05099 and by AEC contract AT-(40-1)-2832.

14 December 1973

Hormonal Control of Neutrophil Lysosomal Enzyme Release: Effect of Epinephrine on Adenosine 3′,5′-Monophosphate

Abstract. *Human neutrophilic leukocytes release neutral protease and β-glucuronidase during cell contact with, and phagocytosis of, zymosan particles treated with rheumatoid arthritic serum. Release of lysosomal enzymes is inhibited by epinephrine and adenosine 3′,5′-monophosphate (cyclic AMP), but not by phenylephrine or adenosine 5′-monophosphate. Inhibition of enzyme release by epinephrine may be mediated by cyclic AMP because the cyclic AMP in the neutrophils is increased by epinephrine treatment at the time when enzyme release is reduced.*

Extracellular release of lysosome granule enzymes from polymorphonuclear leukocytes is provoked by cell contact with various immunologic reactants (*1*, *2*). Local tissue injury is probably a direct consequence of the release of granule constituents (*3*), as lysomal proteins are capable of mediating inflammation (*4*) and cartilage degradation (*5*). Therefore, inhibition of lysosomal enzyme release from leukocytes in contact with immune reactants might be of value in attenuating the severity of inflammation and tissue destruction.

Catecholamines, and adrenergic mechanisms in general, are thought to be involved in the regulation of the inflammatory process. In fact, certain catecholamines can elicit anti-inflammatory effects in various models of acute and chronic inflammation, including polyarthritis (*6*). Moreover, specific actions of catecholamines at the cellular level include inhibition of allergic release of histamine from sensitized human leukocytes (*7*) and lung tissue (*8*). At the subcellular level, catecholamines inhibit the release of enymes from isolated lysosome granules (*9*), probably via a mechanism involving adenosine 3′,5′-monophosphate (cyclic AMP).

The findings that catecholamines and cyclic AMP inhibit the phagocytic release of lysosomal enzymes from human mixed leukocytes (*10*) prompted an analysis of the effects of these agents on purified human neutrophils. We report here that the adrenergic neurohormone epinephrine inhibits the extracellular release of β-glucuronidase and neutral protease from human neutrophils, in contact with zymosan particles treated with rheumatoid arthritic (RA) serum, and that inhibition of enzyme release is accompanied by a concomitant elevation of intracellular cyclic AMP. Thus, the influence of epinephrine on neutrophil function may be mediated by changes in the endogenous concentration of cyclic AMP.

Human neutrophils were isolated from fresh heparinized venous blood of healthy volunteers (*11*). Final leukocyte suspensions were prepared in Hanks balanced salt solution containing 1 percent glucose (weight to volume), and final cell concentrations were adjusted to 5×10^6 neutrophils per milliliter. Neutrophils constituted 95 to 98 percent of all cells present. Mononuclear cells were present to the extent of 0 to 3 percent, and erythrocytes and platelets were absent. Viability of the neutrophils was greater than 99 percent, as determined by eosin Y or trypan blue exclusion.

Zymosan particles (0.5 to 3.0 μm in diameter) were boiled in Hanks balanced salt solution (10 mg/ml), resuspended in RA serum (1 : 20,480 titer rheumatoid factors as determined by latex agglutination) at a concentration of 25 mg/ml, and incubated at 37°C for 30 minutes (with slight agitation). The treated zymosan particles were washed with cold saline and suspended at 10 mg/ml in the Hanks-glucose solution (4×10^8 particles per milliliter).

Neutrophils (5×10^6 in 1.0 ml of Hanks-glucose solution) and test agent (or agents) were incubated at 37°C for 5 minutes prior to the addition of 0.1 ml of zymosan suspension, and were then incubated at 37°C in a Dubnoff metabolic shaker (120 excursions per minute). After incubation, samples were centrifuged at 200*g* for 10 minutes at 4°C, and the supernatants were assayed for neutral protease, β-glucuronidase, and lactate dehydrogenase (LDH) activities (*12*). Determinations of total neutrophil enzyme activities were made after cell lysis, by eight freeze-thaw cycles, from a Dry Ice–acetone mixture to cool tap water, and centrifugation as described above. Epinephrine solutions contained 0.01 percent (weight to volume) sodium metabisulfite to prevent spontaneous oxidation of the catecholamine, and were used immediately.

Cyclic AMP in neutrophils was determined by a protein-binding method (*13*), after rapid freezing of individual incubation mixtures containing cells,

acidification, and ether extraction of samples (*14*).

Epinephrine inhibits the phagocytic release of β-glucuronidase from human neutrophils in contact with particulate suspensions of zymosan treated with RA serum (Table 1). Isoproterenol also inhibits enzyme release. Initial data indicate that epinephrine does not reduce particle uptake (neutrophils that took up two or more particles within 15 minutes were considered phagocytic). Phenylephrine, a sympathomimetic amine that lacks a catechol moiety and therefore does not interact directly with β-adrenergic receptors, does not affect β-glucuronidase release. These data suggest that interaction with β-adrenergic receptors accounts for the inhibitory action of epinephrine on phagocytic enzyme release. This suggestion is supported by the finding that propranolol, a β-adrenergic receptor antagonist, but not phentolamine, an α-adrenergic receptor antagonist, blocks the inhibitory action of epinephrine on enzyme release.

Table 1. Inhibition of phagocytic release of β-glucuronidase from human neutrophils by epinephrine and adenosine 3′,5′-monophosphate. Data represent the mean ± standard error of the mean from four separate experiments. Human neutrophils (5×10^6) were incubated at 37°C for 5 minutes in 1.0 ml of Hanks balanced salt solution containing the test agent or agents, and then further incubated at 37°C for 15 minutes in the presence of zymosan treated with RA serum particles. Control incubations (no agents added) yielded values of 51.6 ± 3.1 μg of phenolphthalein per 18 hours per 5×10^5 neutrophils (18.6 percent of total cell activity) for release of β-glucuronidase. Neither propranolol ($10^{-6}M$) nor phentolamine ($10^{-6}M$), when tested alone, affected enzyme release. Theophylline, at the concentrations indicated, did not affect enzyme release, but 18 ± 1.0 percent inhibition of β-glucuronidase release was observed with $10^{-5}M$ theophylline.

Agent*	Percent inhibition of release of β-glucuronidase at concentrations of the agent:		
	$10^{-6}M$	$10^{-7}M$	$10^{-8}M$
Epinephrine (E)	51 ± 3.8	30 ± 1.9	18 ± 1.2
Isoproterenol	48 ± 2.9	31 ± 2.6	15 ± 1.0
Phenylephrine	0	0	0
E + propranolol ($10^{-6}M$)	9 ± 0.6	0	0
E + phentolamine ($10^{-6}M$)	55 ± 4.3	34 ± 2.5	21 ± 1.4
Theophylline	0	0	0
E + theophylline ($10^{-6}M$)	78 ± 6.2	52 ± 5.1	37 ± 3.3
Cyclic AMP	27 ± 1.1	11 ± 0.6	0
Cyclic AMP + theophylline ($10^{-6}M$)	43 ± 2.6	29 ± 2.2	15 ± 1.0
N^6,O^2-Dibutyryl cyclic AMP	45 ± 2.2	31 ± 1.8	19 ± 1.4
AMP	0	0	0

* Abbreviations and forms of agents tested: *l*-epinephrine bitartrate; *l*-isoproterenol hydrochloride; *l*-phenylephrine hydrochloride; *dl*-propranolol hydrochloride; phentolamine mesylate; cyclic AMP, adenosine 3′,5′-monophosphate sodium; AMP, adenosine 5′-monophosphate sodium.

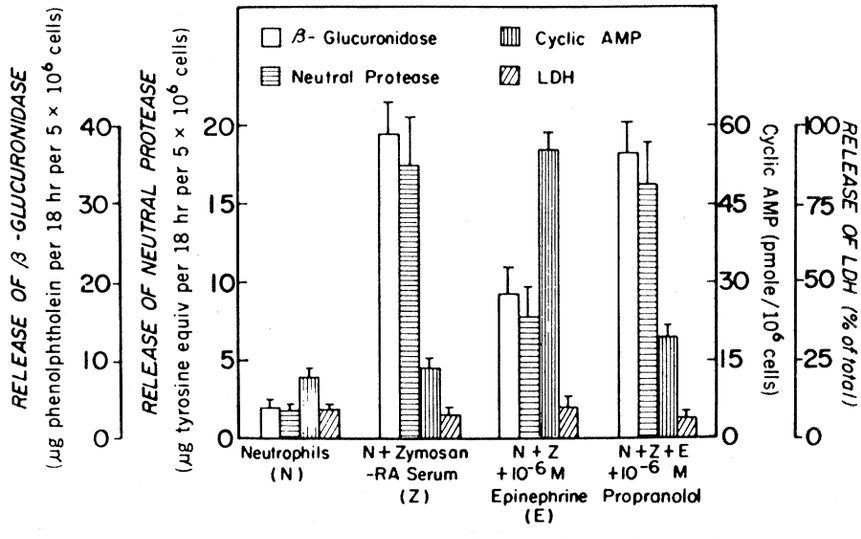

Fig. 1. Effects of epinephrine on release of β-glucuronidase and neutral protease from and on concentrations of cyclic AMP in human neutrophils exposed to zymosan particles treated with RA serum. Data represent the mean ± S.E.M. from three to five separate experiments. Human neutrophils (5×10^6) were incubated at 37°C for 5 minutes in 1.0 ml of Hanks balanced salt solution, in the presence or absence of epinephrine or propranolol (or both), and then further incubated at 37°C for 15 minutes in the presence or absence of zymosan particles treated with RA serum. Propranolol, when tested alone, did not modify enzyme release or cellular levels of adenosine 3′,5′-monophosphate. Epinephrine and propranolol, respectively, were used as the *l* isomer of the bitartrate salt and the *dl* isomer of the hydrochloride salt.

The observation that theophylline, an inhibitor of the hydrolytic degradation of cyclic AMP catalyzed by phosphodiesterase, potentiates the action of epinephrine (Table 1) signifies that endogenous neutrophilic cyclic AMP may be involved in expressing the action of epinephrine. Indeed, cyclic AMP inhibits enzyme release, an effect potentiated by theophylline. The dibutyryl analog of cyclic AMP, which is resistant to degradation by phosphodiesterase, elicits an effect comparable to that of a combination of cyclic AMP and theophylline. Inhibition of phagocytic release of β-glucuronidase from neutrophils is specific for cyclic AMP, as adenosine 5′-monophosphate is without effect on enzyme release. Cyclic AMP does not appear to inhibit phagocytic uptake of particles. Similar data to those indicated above for β-glucuronidase release were obtained when release of lysosome-associated neutral protease was measured.

In order to implicate endogenous cyclic AMP as "second messenger" mediating the action of epinephrine on neutrophil function, it is imperative to demonstrate an increase in cyclic AMP in neutrophils during cell contact with epinephrine. The data in Fig. 1 illustrate that the inclusion of epinephrine in the test system, consisting of neutrophils and zymosan particles, results in elevation of cyclic AMP. This elevation occurs at the time that lysosomal enzyme release is inhibited by epinephrine. Thus, epinephrine-induced inhibition of lysosomal enzyme release is associated with a concomitant increase in neutrophil cyclic AMP. Initial data indicate that isoproterenol also elevates neutrophil cyclic AMP. The association between these two cellular events is substantiated by the finding that propranolol blocks the action of epinephrine, not only on enzyme release, but also on accumulation of cyclic AMP. Release of lysosome granule enzymes is not accompanied by release of cytoplasmic constituents (that is, LDH), and cell viability is maintained throughout the incubations.

Neutrophil concentrations of cyclic AMP do not increase during phagocy-

tosis of, or cell contact with, zymosan particles. These data are in accordance with those of Manganiello *et al.* (*15*) which illustrate that cyclic AMP concentrations in phagocytic cells do not increase on phagocytosis of particles. Similarly, Seyberth *et al.* (*16*) reported that the concentrations of cyclic AMP in phagocytic granulocytes do not increase during particle ingestion.

In view of these data, it is most tenable to consider that epinephrine inhibits the phagocytic release of lysosomal enzymes from human neutrophils via a mechanism involving an increase in intracellular cyclic AMP. The intracellular events that might link cyclic AMP to inhibition of enzyme release are not understood at the present time. Intracellular, rather than extracellular, actions of cyclic AMP, and therefore of epinephrine, appear to predominate since neither of these agents affect phagocytic uptake of particles when enzyme release is reduced. This failure of cyclic AMP to inhibit phagocytosis has been reported (*1*). One might suppose that cyclic AMP, as it does in other cells and tissues, activates a specific protein kinase, thus catalyzing the phosphorylation of important intracellular components. The consequent alteration of physical or functional properties (or both) of such intracellular components might produce changes in the degree to which granule constituents are released. Whether such intracellular mechanisms in the neutrophil exist and, if so, whether such events can explain adequately the inhibitory action of cyclic AMP on phagocytic enzyme release remain to be determined. Lysosome granules appear to be likely candidates for the intracellular effects of cyclic AMP, as cyclic AMP is capable of inhibiting leakage or release of contents from isolated lysosomes (*9*). In fact, epinephrine and other catecholamines also inhibit enzyme release from isolated lysosomes (*9*). Therefore, alterations in certain properties of the lysosome granule membrane by elevated intracellular cyclic AMP might explain the inhibitory action on lysosomal enzyme release from neutrophils by epinephrine. Such intracellular changes could interfere, conceivably, with certain normal functions of lysosome granules, such as peripheral migration and subsequent fusion of granules with heterophagic vacuoles or the plasma membrane, that lead to extrusion of granule contents.

Regardless of exact mechanism, inhibition of the phagocytic release of granule contents from human neutrophils by epinephrine and cyclic AMP, together with the concomitant elevation of neutrophil concentrations of cyclic AMP by epinephrine, suggests that the autonomic nervous system can regulate the inflammatory process by modulating intracellular neutrophil levels of cyclic nucleotides.

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26 September 1973

Penetration of Somatic Mammalian Cells by Sperm

Abstract. *Penetration of somatic mammalian cells by spermatozoa occurred after simple admixture in culture. With sperm labeled in vivo, autoradiography revealed incorporation of DNA into nuclei of recipient cells, indicating release of DNA after entrance by sperm. This system provides a new approach to study the molecular biology of information transfer and of haploid gene expression.*

Mammalian cells can acquire genetic components by simple exposure to nucleic acids, viruses, microorganisms, other cells, and cellular organelles (*1*). A great variety of cultured mammalian cells can ingest not only these entities but also nonbiological substances such as asbestos and polystyrene spheres (*2*). Active participation by the target cells in processes analogous to phagocytosis or pinocytosis is required, since many of the ingested materials are either inert themselves or play only a passive role in the uptake process. Sperm, on the other hand, are specifically endowed with motility and enzymic mechanisms to enhance delivery of their genome to the egg. We have studied their interaction with somatic cells to see whether genetic information can be acquired from the sperm, as occurs in fertilization (*3*).

In orienting experiments, living sperm collected aseptically from Swiss white mouse epididymus and vas deferens were added to monolayer cultures of various malignant or diploid normal mammalian lines maintained in our laboratory. These included mouse, hamster, rat, and human lines. Periodic examination of living or fixed and stained specimens by phase contrast microscopy revealed that apparent penetration of cells in all the lines had occurred.

Mouse sperm and normal, diploid Chinese hamster (CH) fibroblast cells were used. In this pair of species heritable expression of mouse genes results after exposure of CH cells to either mouse tumor cells or the DNA isolated therefrom (*4*). Monolayer cultures of CH cells (*4*) were brought into suspension by routine trypsin treatment (0.125 percent trypsin and 0.01 percent ethylenediaminetetraacetate; 5 to 10 minutes; 37°C), and the rounded, washed cells were mixed with one to five times as many freshly collected sperm, which had been previously washed in Tyrode solution. The mixtures were suspended (final concentration, 2 to 4 × 10^5 CH cells per milliliter)