CYCLIC ADENOSINE MONOPHOSPHATE
IN PHAGOCYTIZING GRANULOCYTES
AND ALVEOLAR MACROPHAGES

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The ingestion of particles by phagocytizing cells is accompanied by marked changes in such metabolic
parameters as oxygen consumption, glycolysis, glycogenolysis, \( \text{H}_2\text{O}_2 \) production, and activity of the
hexose-monophosphate shunt, with the extent of these events depending on the particular cell
type studied (for references see 1, 4, 6, 7). In general, these metabolic changes can be observed
within a few minutes or less after the addition of particles and are considered to reflect an increased
energy demand during phagocytosis. It is not clear, however, in which way the contact of par-
ticles with the outer membrane or the initial phase of phagocytosis is signaled to the interior of the cell,
where these metabolic changes take place.

It is now well established that the interaction of many hormones with specific receptor sites at the
outer membrane is signaled to the interior by changes in the intracellular concentration of adenosine
\( 3',5'-\text{monophosphate} \) (\( \text{Ado-3',5'-P} \), cyclic AMP) via stimulation of the membrane-lo-
cated adenylate cyclase (13). This system is not restricted to hormone effects, e.g., lymphocytic
transformation by stimulation of phytohemagglu-
tinins (13). It was therefore tempting to speculate that cyclic AMP may have a messenger function in
the process of phagocytosis. Indeed, results pre-
sented by Park et al. (11) suggest that during
phagocytosis of polystyrene-latex particles by crude leukocyte preparations, the intracellular
concentration of cyclic AMP is increased. Similar results were reported by Manganiello et al. (9). It
was not clear, however, whether the observed in-
crease in cyclic AMP concentration was in fact re-
lated to phagocytosis. On the other hand, Stossel
and colleagues (14) were unable to detect sig-
nificant changes of cyclic AMP concentration in
phagocytizing guinea pig peritoneal leukocytes.

In view of these conflicting observations, it was
of interest to reinvestigate the possible participa-
tion of cyclic AMP in the phagocytosis of poly-
styrene-latex particles by two different cell types, pig peripheral leukocytes and rabbit alveolar
macrophages.

MATERIALS AND METHODS
Alveolar macrophages were obtained from female
New Zealand rabbits sensitized with bacille Calmette-
Guérin (BCG) according to Myrvik et al. (10). Leukocyte suspensions were prepared from pig blood
by the method of Fallon et al. (5) and subjected to
density gradient centrifugation in Ficoll to obtain
granulocyte-rich preparations (15). Differential
counts of this suspension gave 70-92% granulocytes,
6-30% lymphocytes (crude pig leukocyte prepara-
tions contain up to 70% lymphocytes), and 1-3%
monocytes. Contamination was less than one platelet
per granulocyte and one erythrocyte per 100 granu-
locytes. The viability was checked with a dye exclu-
sion test (0.3% trypan blue). Preparations containing
more than 3% trypan blue-positive cells were dis-
carded. The cell isolation procedures were performed
at 0-4°C and cells were suspended in modified
Krebs-Ringer phosphate buffer (pH 7.4) with 8.3
mM glucose (KRP) to contain 100 mg wet cells/ml.

Incubation Conditions
The incubation mixtures contained 500 \( \mu \)l cell
suspension (5 \( \times \) \( 10^7 \)-1 \( \times \) \( 10^8 \) granulocytes or about 5 \( \times \) \( 10^6 \) macrophages). When granulocytes were incubated, 100 \( \mu \)l autologous serum were added. KRP was added to give a final volume of 900 \( \mu \)l. The
tubes were placed in a metabolic shaker (100 oscilla-
tions/min) at 37°C. At the time indicated 100 \( \mu \)l of a suspension of polystyrene-latex particles (0.461 \( \mu \)m diameter, 7.8 \( \times \) \( 10^{10} \) particles in KRP or KRP [controls]) were added. For the estimation of latex
ingestion, the reaction was stopped by transferring
the incubation mixture to 4 ml ice cold KRP without
glucose and subsequently centrifuged at 0°C. For the
estimation of cyclic AMP, the incubation was stopped

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by adding 100 µl ice cold 5 M perchloric acid and placing the tube in a methanol-dry ice bath. 20 µl [3H]cyclic AMP were added for the estimation of the recovery from purification.

Latex uptake was measured by the dioxane procedure of Roberts and Quastel (12). The oxygen consumption was monitored in a standard Warburg respirometer.

Isolation of Cyclic AMP

After sonication for 15 s, 100 mM ZnCl2 and K2CO3, to give a final pH of 7.0 (3), were added to precipitate-interfering nucleotides. After centrifugation the clear supernatant was loaded onto a AG 50 WX 4 column (0.5 X 12 cm). The column was eluted with water and the 6th-9th ml were collected and lyophilized. The recovery was between 57 and 73%.

Cyclic AMP was assayed by measuring the stimulation of a protein kinase with γ-[32P]ATP as phosphate donor and histone as acceptor (modified from Kuo and Greengard (8)). The incubation mixture (total volume 50 µl) contained sodium glycerophosphate 250 µM pH 6.0, 10 mM Mg-acetate, 2 mM theophylline, 0.3 mM EGTA, 40 µg histone, and 10 µl of either cyclic AMP standards or the unknown extract. After incubation for 15 min at 30°C, the reaction was stopped by the addition of bovine serum albumin (640 µg in 100 µl) and 1 ml 20% TCA and cooling the mixture to 0°C. After centrifugation the pellet was washed with 1 ml 5% TCA, hydrolyzed by the addition of 400 µl 1 N NaOH and 100 µl 1 mM KH2PO4 at 100°C, and the total hydrolysate was transferred into counting vials containing 15 ml water. Utilizing the Cerenkov effect, the radioactivity was measured in a Packard 3003 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Internal standards were completely recovered in the final enzymatic assay. Protein kinase was prepared from beef heart according to the method of Kuo and Greengard (8).

Substances Used

Histone type II a (Sigma Chemical Co., St. Louis, Mo.); prostaglandin E1 (kindly donated by Upjohn Co., Kalamazoo, Mich.); [3H]cyclic AMP, sp act 24.1 Ci/mmol (New England Nuclear, Boston, Mass.); γ-[32P]ATP, sp act 12-20 Ci/mmol (Amersham Buchler KG, Braunschweig, Germany); other substances were obtained from standard suppliers.

RESULTS AND DISCUSSION

Both cell types studied rapidly phagocytized polystyrene-latex particles. Uptake of particles was linear from 0 to 10 min and declined thereafter to reach a plateau after 15-30 min. Maximum particle uptake was 35 and 38% of the amount added (2 mg) in granulocytes and alveolar macrophages, respectively. Phagocytosis was accompanied by the expected increase in oxygen consumption from 3.5 to 8.5 µl O2 per h per mg cell protein in granulocytes and from 14 to 15.8 µl O2 per h per mg cell protein in alveolar macrophages.

Cyclic AMP concentration in granulocytes rose sharply at the start of the incubation without addition of particles, reached peak values at 10 min which were almost ten times that of O°C controls, and returned to a stable level after 30 min of incu-
FIGURE 2 Influence of serum and prostaglandin E₁ on cyclic AMP levels in polymorphonuclear leukocytes. Cells were incubated in KRP pH 7.4 at 37°C. Autologous serum (10% of final volume) was added at 20 min. To both serum and serum-free incubates prostaglandin E₁ was added at 30 min (final concentration 10⁻⁵ M). n = four determinations from two cell preparations.
concentration upon particle addition, too, whereas the results shown in detail have been obtained with preparations enriched in polymorphonuclear leukocytes.

In preceding experiments with alveolar macrophages in which phagocytosis was not measured, it was found that the concentration of cyclic AMP increased only by about 10-20% upon transfer from 0° to 37°C. Therefore the preincubation period was reduced to 10 min (Fig. 3).

In contrast to granulocytes, in alveolar macrophages phagocytosis is accompanied by a significant increase in cyclic AMP concentration (Fig. 3). This difference may reflect either the known difference in energy metabolism during phagocytosis or differences in the biochemical mechanisms of phagocytosis in these two cell types (7).

It seems justified to conclude that changes in cyclic AMP concentration are not a prerequisite for or a consequence of phagocytosis in all types of phagocytizing cells. This, however, does not exclude the possibility that in alveolar macrophages the rise in cyclic AMP concentration is the necessary link between contact of particles with the membrane and initiation of phagocytosis and/or metabolic changes. This view is supported by the short latency period between particle addition and onset of cyclic AMP rise.

SUMMARY

The uptake of polystyrene-latex beads, oxygen consumption, and concentration of cyclic AMP were measured during phagocytosis in pig peripheral blood leukocyte preparations rich in polymorphonuclear cells and in rabbit alveolar macrophages. Both cell types took up particles very rapidly and exhibited the expected increase in the oxygen consumption. The cyclic AMP content of the polymorphonuclear fraction did not immediately change after incubation with polystyrene-latex beads. Alveolar macrophages exhibited a small but significant increase in the level of cyclic AMP as early as 30 s after addition of particles.

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