EFFECTS OF CYTOCHALASIN B ON GLYCOGEN METABOLISM IN PHAGOCYTIZING HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Received 14 November 1978
Revised version received 28 December 1978

1. Introduction

The fungal metabolite cytochalasin B introduces an immediate, but reversible inhibition of locomotion and phagocytic activity of polymorphonuclear leukocytes [1–6]. Bacteria and zymosan particles remain adherent to the cell surface in partial ingestion [3]. The drug also inhibits glucose transport [4].

We have reported [7] that phagocytosis of latex particles by leukocytes is dependent on the extracellular Ca^{2+} concentration and is associated with an immediate, Ca^{2+}-dependent activation of glycogen phosphorylase, resulting in glycogen breakdown. Simultaneously, but independent of extracellular Ca^{2+}, phagocytosis elicits an increase in cyclic adenosine-3′,5′-monophosphate (cAMP) concentration and a conversion of glycogen synthase from a metabolically active R-form [8] into the inactive D-form. As cytochalasin B blocks phagocytosis, it was therefore of interest to investigate whether the mere attachment of latex particles to the plasma membrane is sufficient to initiate the glycogenolytic cascade, which is normally associated to particle ingestion at all levels of extracellular glucose [9]. It is found, that in spite of greatly increased concentration of cAMP elicited by latex particles adhering to cytochalasin B-treated cells, glycogenolysis is blocked as glycogen phosphorylase remains inactive, presumably due to rapid depletion of cellular Ca^{2+} by cytochalasin B.

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2. Materials and methods

Cytochalasin B was purchased from Sigma, dissolved in dimethyl sulfoxide at 4 mg/ml and stored at −20°C until use. Aliquots of the stock solution were diluted with 0.9% NaCl and added to the incubation medium to 5 μg/ml final conc. The resulting concentration of dimethyl sulfoxide was ⩾ 0.165%, which has been shown not to interfere with cell metabolism [3,4]. Latex particles were primed for phagocytosis by dialysis against Krebs-Ringer bicarbonate buffer [7].

2.1. *Incubation of cells*

Human polymorphonuclear leukocytes were isolated, suspended in a (glucose-free) Krebs-Ringer bicarbonate buffer (pH 7.4) with 1.3 mM $CaCl_2$ at 1.2×10^7 cells/ml, and incubated at 37°C under an atmosphere of 94.4% O_2–5.6% CO_2, as in [7]. After incubation for 105 min, 5 μg/ml cytochalasin B was added and 15 min later 500 μl latex particles/10 ml cell suspension injected. At intervals, 600 μl samples were withdrawn, added to 100 μl ice-cold 50 mM Tris–HCl (pH 7.6)–50 mM EDTA buffer, and immediately homogenized by sonic disintegration. The glycogen concentration and glycogen synthase and phosphorylase activities were assayed in the homogenate. The uptake rate of latex particles incubated with or without cytochalasin B was measured as in [7].

2.2. *Analytical procedures*

Glycogen synthase was assayed with 0.4 mM UDP-

[U-^{14}C]glucose (3–4 × 10^6 cpm/μmol), as in [7]. Synthase I activity was determined without glucose-6-P or Na_2SO_4 in the assay mixture, synthase R in the presence of 0.67 mM glucose-6-P, and total activity with 67 mM glucose-6-P [8]. Phosphorylase *a* was determined in the direction of glycogen synthesis [10] with 10 mM [U-^{14}C]glycose-1-*P* (3–4 × 10^5 cpm/μmol). Glycogen was precipitated on filter paper and measured employing amyloglucosidase-hexokinase [11]. Determination of cAMP and the rate of $^{45}Ca^{2+}$-efflux from $^{45}Ca^{2+}$-prelabelled cells were performed as in [7].

3. Results

3.1. *Effect of cytochalasin B on glycogen metabolism*

Although 5 μg/ml cytochalasin B inhibits various cell functions, addition to starved leukocytes initiated immediate, but transient metabolic changes. Phosphorylase *a* activity increased 2-fold within 1 min, but reached baseline-values again at 2 min (fig.1). Concurrently, both glycogen synthase R and I were inactivated and returned to initial activity at 6 min (fig.2). cAMP concentration showed an extremely rapid biphasic response, being doubled within 5 s, then almost undetectable at 30 s, and finally returning to initial levels within 2 min (fig.3). Finally, the rate of $^{45}Ca^{2+}$-efflux was greatly accelerated during the first 1 min of exposure to cytochalasin B and then returned to normal, the subsequent higher level reflecting the initial increase of $^{45}Ca^{2+}$ to the extracellular fluid (fig.4). The changes in phosphorylase *a*

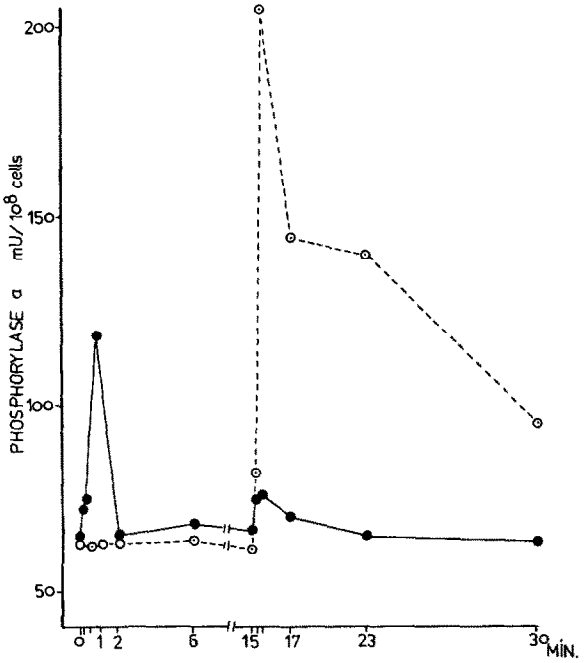

Fig.1. Human polymorphonuclear leukocytes were incubated for 105 min. Then, at zero time, 5 μg/ml cytochalasin B was added (closed symbols) and the incubation continued for 15 min, when latex particles (100 particles/cell) were injected. Open symbols represent incubation without cytochalasin B. The changes in phosphorylase *a* activity (●, ○) are based upon duplicate determination from 4 separate experiments. One mU of phosphorylase *a* activity corresponds to the incorporation of one nmol glucose per min at 30°C.

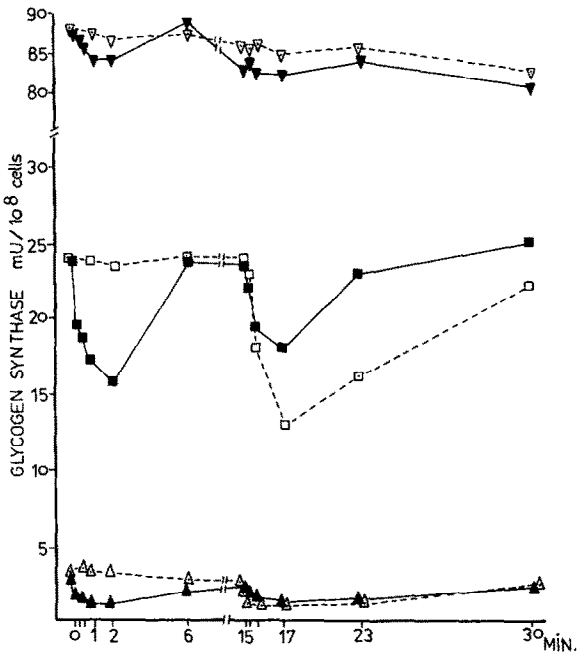

Fig.2. In the same experiments as fig.1, the activity of glycogen synthase I (▲, △), glycogen synthase R (■, □), and the total synthase (▼, ▽) activity was measured in incubations without (open symbols) and with (closed symbols) added cytochalasin B. One mU of synthase activity corresponds to the incorporation of one nmol glucose per min at 30°C.

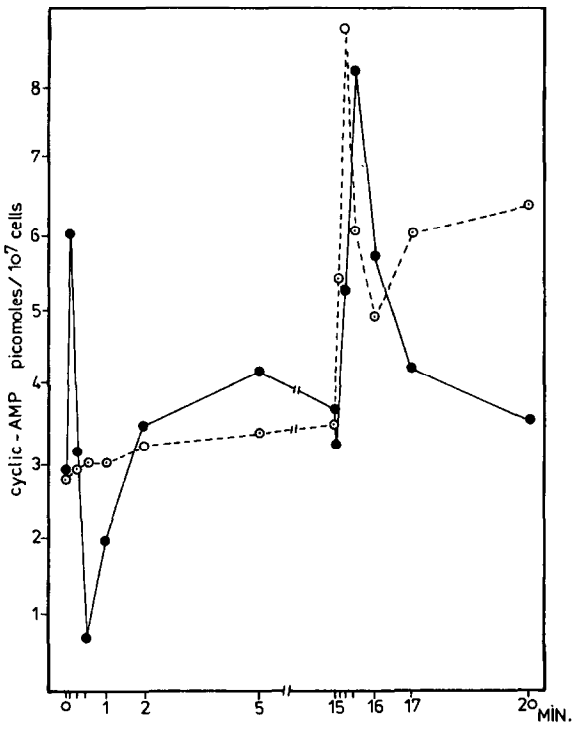

Fig.3. With the same experimental model is in fig.1, cAMP concentration was measured in incubations without (○) and with (●) added cytochalasin B. Duplicate determinations in 2 separate experiments.

and synthase R activity were not sufficient to cause a decrease in glycogen content within the first 6 min (not shown).

3.2. *Effects of latex particles on cytochalasin B treated leukocytes*

After 15 min exposure to cytochalasin B, the activity of phosphorylase *a* and glycogen synthase and the concentration of cAMP were at the same level as before exposure. Addition of latex particles initiated immediate phagocytosis in control incubations, whereas particle uptake was immensely restrained by cytochalasin B (fig.5). The activation of phosphorylase was almost completely blocked, whereas inactivation of synthase was only moderately affected by cytochalasin B. The possibility that the lack of increase in phosphorylase *a* activity was due to phosphorylase phosphatase activity was excluded by control experiments, when 50 mM KF was present in the dilution buffer. The adherence of latex particles to the plasma membrane in cytochalasin B-treated cells was sufficient to cause as great an increase in cAMP concentration as in phagocytosing cells where, however, the cAMP concentration was sustained at a higher level during the following minutes. The rate of $^{45}Ca^{2+}$-

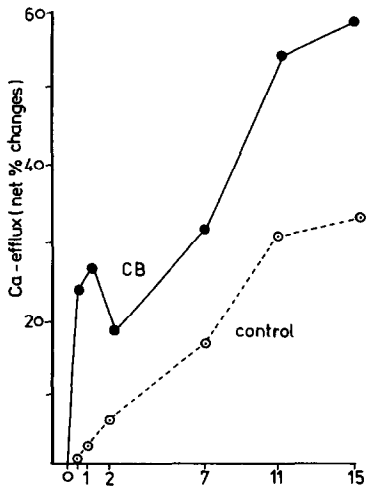

Fig.4. After 105 min incubation of leukocytes (as in fig.1) the % change in $^{45}Ca^{2+}$-efflux from $^{45}Ca^{2+}$-prelabelled cells was measured upon addition of 5 μg/ml cytochalasin B (●) and in control cells (○). The filtrate activity at zero time was 3420 cpm/ml (n = 4).

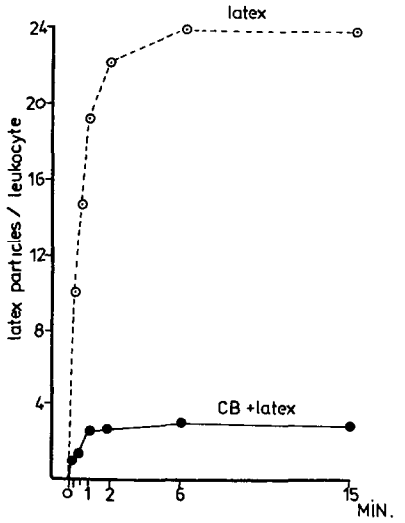

Fig.5. Uptake of latex particles in cytochalasin B (●) and control experiments (○) was determined in experiments depicted in fig.1 and 2 from examination of 50 leukocytes in each experiment (n = 4).

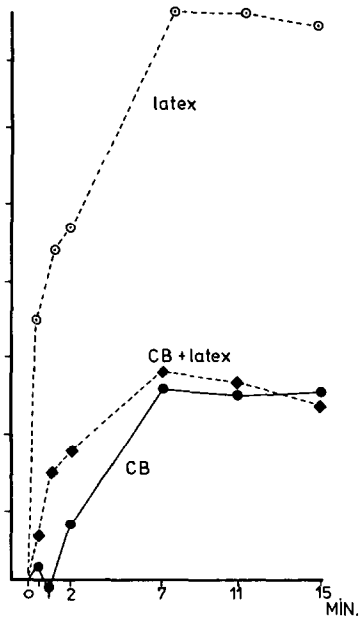

Fig.6. The experiments were conducted as in fig.4, but cytochalasin B (CB) was added 1 min before zero time (♦, •). At zero time, when the effect of cytochalasin B on the rate of $^{45}Ca^{2+}$-efflux had subsided (cf. fig.4), latex particles were added to one of these incubations (♦) and to a control without cytochalasin B (○). Filtrate activity at zero time was 3723 cpm/ml (○), 4072 cpm/ml (♦) and 3830 cpm/ml (•) $n = 4$.

efflux in cytochalasin B-treated cells was slightly further increased upon addition of latex particles, but severely inhibited compared to normal phagocytozing cells (fig.6). The exposure to cytochalasin B before addition of latex particles was in this experiment shortened to 1 min as changes in $^{45}Ca^{2+}$-efflux after 15 min exposure were not safely measured against the high background of $^{45}Ca^{2+}$ present extracellularly at this time (cf. fig.4). The glycogen concentration was not affected by addition of latex particles to cytochalasin B-treated cells, whereas a 10% decrease was seen in normal leukocytes 6 min after initiation of phagocytosis (not shown), as in [7].

4. Discussion

Cytochalasin B increased glycogen phosphorylase *a* concentration briefly, which might have been caused either by the burst of cAMP, activating the cAMP-dependent protein kinase and subsequently phosphorylase kinase, or by an activation of phosphorylase kinase *b* by the increased concentration of Ca^{2+} in the cytosol, as evidenced by the increased rate of $^{45}Ca^{2+}$-efflux.

The biphasic response in cAMP concentration upon addition of cytochalasin B tentatively suggests a stimulation of adenylate cyclase activity followed by inactivation by the simultaneously increased cytosolic Ca^{2+}concentration. Such an explanation implies the immediate binding of formed cAMP by cAMP-dependent protein kinase, and thus phosphorylation of phosphorylase kinase.

In the cytochalasin B-treated cells, glycogen phosphorylase was not activated by the addition of latex particles in spite of a large increase in cAMP concentration. At the same time the response in $^{45}Ca^{2+}$-efflux to latex particles was severely reduced, when evaluated 1 min after addition of cytochalasin B. This suggests that an intracellular Ca^{2+} store of significance for phosphorylase kinase activation may be rapidly depleted by cytochalasin B and enhances the significance of Ca^{2+} metabolism for the regulation of glycogen breakdown.

It is not known, why the greatly increased cAMP concentration did not result in phosphorylase activation. Recent experiments [13] have shown that the activity of leukocyte phosphorylase kinase *a* is ~50% reduced at (calculated) $Ca^{2+} < 10^{-10}$ M, whereas half-maximal activation of phosphorylase kinase *b* occurred at 10^{-6} M Ca^{2+}. The state of these enzymes, as well as that of the cAMP-dependent protein kinases during the influence of cytochalasin B would have to be determined in order to further understand why the cAMP signal is not implemented.

Conversion of synthase R into D has been found independent of Ca^{2+}, but to be elicited by various stimuli such as phagocytosis, glucagon, epinephrine and isoproterenol and in each case in association with a simultaneous increase in cAMP concentration. This was also the case in the present experiments, but the extent of conversion was not as marked upon addition of latex particles to cytochalasin B-treated cells as to normal cells, despite a similar increase in cAMP concentration. It was found [12] that phenylephrine caused an R into D conversion of glycogen synthase in the absence of changes in cAMP concentration.

The above finding would tend to support the suggestion that the phosphorylation reaction involved in the R into D conversion of synthase is not solely explanable in terms of a cAMP-dependent protein kinase-catalyzed phosphorylation reaction.

Acknowledgements

This work was supported by a grant (no. 512-10031/78) from the Danish Medical Research Council to V.E.

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