INHIBITION OF MITOCHONDRIAL CONTRACTION BY CYTOCHALASIN B

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Received 31 August 1973

1. Introduction

Cytochalasin B (CB), an alkaloid metabolite of the mould *Helminthosporium dematioidium* [1], inhibits a wide variety of cellular processes [2], including cytokinesis [3, 4], cell locomotion [2, 3], cytoplasmic streaming [2], blood clot retraction [5], hormone secretion [6], transport [7–9] and beating of embryonic heart cells [10]. Since many of these cellular events are both energy dependent and contractile in nature, we examined the effects of CB on isolated mitochondria, the main site of energy production in cells. We found that at concentrations around 10^{-4} M, the drug inhibits contraction of these organelles induced by ATP + Mg^{2+}. Mitochondrial ATP synthesis is also inhibited by CB, but the effect appears to be less pronounced.

2. Materials and methods

Intact mitochondria were isolated from Sprague–Dawley albino rats by the procedure of Schnaitman and Greenawalt [11]. The final pellet, after two washes, was suspended in a medium containing 220 mM mannitol, 70 mM sucrose and 2 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonate (HEPES)–NaOH, pH 7.4 at 0°C. The swelling and contraction of mitochondria were monitored optically at 520 nm with a multi-channel Cary 16 recording spectrophotometer [12, 13]. Endogenous substrate supported ATP synthesis activity was determined by ^{32}P incorporation into ATP [14]. Cytochalasin B (CB) was obtained from Imperial Chemical Industries, Ltd. All other reagents were of analytical grade.

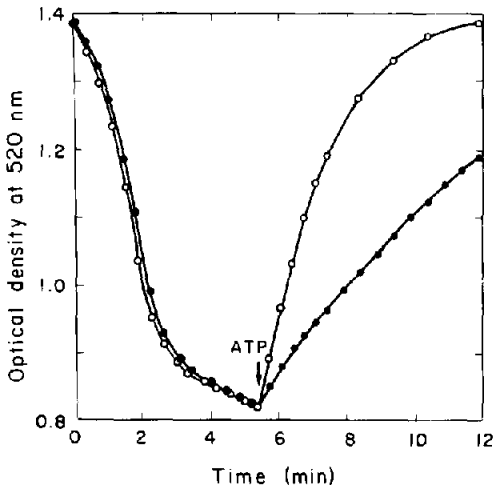

Fig. 1. Inhibition of mitochondrial contraction by cytochalasin B. Mitochondria (0.5 mg protein) were suspended in 1 ml of 0.05 M KCl, 0.03 M Tris–HCl (pH 7.4), 0.85% v/v dimethylsulphoxide, with (•—•—•) or without (○—○—○) 0.17 mM cytochalasin B. After temperature equilibration at 25°C (4 min), 2.5 mM potassium phosphate, pH 7.4 was added to initiate swelling (time zero). When the decrease in absorbance slowed to a constant low rate, 10 mM ATP and $MgCl_2$ were added to induce contraction. In a medium with KCl concentration such as used here, complete reversal of swelling was achieved by addition of ATP + Mg^{2+}. At higher concentrations of KCl, the reversal was not complete, but the degree of inhibition by CB was the same.

3. Results

The time course of the effect of CB on swelling and contraction is shown in fig. 1. Although the phosphate-induced swelling seemed to be unaffected by

0.17 mM CB, the rate of ATP + Mg^{2+} induced contraction was inhibited by 66%. Furthermore, the inhibition of contraction by CB was dependent on the concentration of CB in the medium (fig. 2). Fifty percent inhibition was observed at about 0.1 mM, and at 0.3 mM the inhibition was almost complete.

In order to determine the time required for CB to exert its effect on mitochondrial contraction, CB (0.1 mM) was added at different times during the swelling and contraction cycle. It was found that when CB was added 4 min before the addition of potassium phosphate (fig. 1), or 2 min before the initiation of contraction by ATP + Mg^{2+}, the extent of inhibition was about the same (50%). However, if CB was added 1 min before or simultaneously with ATP + Mg^{2+}, the percent inhibition was less (30 and 20% respectively). Thus, the effect of CB on mitochondrial contraction appears to depend on the time of exposure of mitochondria to the inhibitor and is maximal after 2 min preincubation.

The effect of CB on mitochondrial contraction is reversible. Mitochondria (2.0 to 2.5 mg protein/ml) were first incubated in the presence of 0.1 mM CB for 2 min and then diluted with 5 vol of media with or without 0.1 mM CB. The two suspensions were then induced to swell and contract as described above. The sample which contained 0.1 mM CB showed 50% inhibition of contraction while the sample which had been diluted from 0.1 mM to 0.017 mM CB was inhibited by less than 10%.

Removal of the outer membrane of mitochondria by digitonin treatment [11] had no apparent effect on the swelling–concentration cycle. Moreover, the effect of CB on mitoplasts (mitochondria without the outer membrane) was about the same (40% inhibition of contraction at 0.1 mM CB) as on intact mitochondria, indicating that the effect is not directed at the mitochondrial outer membrane.

The effect of CB on mitochondrial ATP synthesis supported by endogenous substrates is illustrated in fig. 3. The inhibition of ATP synthesis by CB increased with time, and complete inhibition was obtained, in this case, by 5 min. In other experiments, as much as 20 min was needed for maximal inhibition.

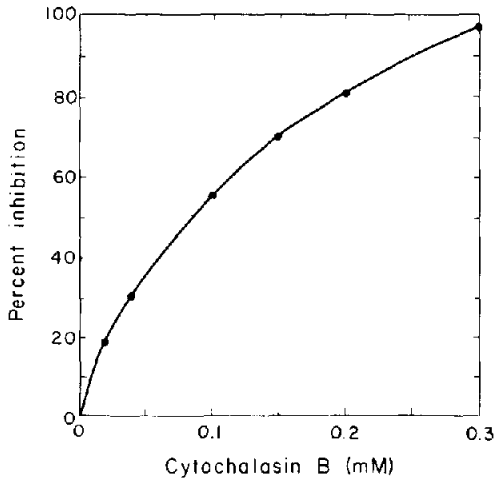

Fig. 2. Dependence of inhibition of mitochondrial contraction on cytochalasin B concentration. The ordinate shows the percent inhibition of the initial rate of contraction (obtained by measuring the slope of the linear portion of the curve; cf. fig. 1). Conditions were the same as in fig. 1 except that the KCl concentration was 0.15 M.

4. Discussion

In summary, CB was shown to have a rapid and re-

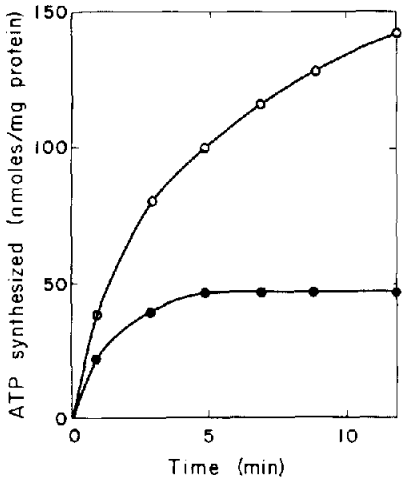

Fig. 3. Inhibition of ATP synthesis by cytochalasin B. The reaction was carried out in small flasks in a shaker-bath at 30° C. The 5 ml of reaction medium contained 0.25 M sucrose, 0.03 M Tris–HCl (pH 7.4), 1 mM ADP and 1 mM potassium phosphate (^{32}P-labeled) and 0.5% v/v dimethylsulphoxide with (●–●–●) or without (○–○–○) 0.1 mM cytochalasin B. The reaction was initiated by adding 2.5 mg of mitochondrial protein.

versible inhibitory effect on mitochondrial contraction induced by ATP + Mg^{2+}. Under similar conditions (no added substrates), ATP synthesis by isolated mitochondria was progressively inactivated in a time–dependent manner. Judging from the length of time needed for CB to be maximally effective, it seems that the effect of CB on the contractile system is probably more direct, whereas the effect on ATP synthesis is only secondary.

The effect of CB on isolated mitochondria were demonstrable at about 10^{-4} M. Since many of the in vivo effects of the drug such as inhibition of glucose transport [7–9] and cytokinesis [3, 4] occur rapidly at 10^{-7} to 10^{-6} M, it seems unlikely that these effects are the result of inhibition of mitochondrial energy production by the drug. In accordance with this conclusion, it was reported [15] that ATP concentrations of fibroblasts were unaffected by CB at 2×10^{-5} M. However, in certain instances, such as the inhibition of cytoplasmic streaming in *Nitella* [2] and in *Elodea* (S. Lin, unpublished) and the inhibition of the beating of embryonic heart cells [10], concentrations of CB approaching 10^{-4} M are used. These cellular processes have been shown to be dependent on high levels of ATP [16, 17] and it is therefore possible that inhibition by CB in these cases can be attributed to the effects of CB on mitochondria of these cells in the manner described in the present report.

Acknowledgements

This work was supported by postdoctoral fellowships from the Bay Area Heart Research Committee (S. L.) and the National Institutes of Health (D. C. L.), American Cancer Society grants VC-121A and BC-47C, Cancer Research Funds of the University of California, and in part by grants of the NIH, HD-01239 and HE 06285. E. K. is a Research Career Awardee of the NIH.

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