INFLUENCE OF THE IONOPHORE A 23 187 ON MYOGENIC CELL FUSION

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1. Introduction

Spontaneous myogenic cell fusion can be prevented by growing the cells in a medium with Ca^{2+}-concentrations below 50 μM [1]. Fusion of myoblasts can then be triggered by raising the Ca^{2+}-concentration of the medium [2]. In order to study the action of calcium in the mechanism of cell fusion as well as in related cytosis processes it is necessary to know whether Ca^{2+}-ions act on sites at the outer or inner face of the myoblast membrane or inside of the cytoplasm. We tried to approach this problem by taking advantage of the ionophore A 23 187 which is supposed to increase selectively the passive transport of divalent cations across membranes [3]. It is suggested that the ionophore increases the intracellular free concentration of calcium whereas the Ca^{2+}-concentration in the medium remains constant. Processes which are regulated by intracellular Ca^{2+}-concentration should thus be accelerated in the presence of the ionophore [4–8].

2. Materials and methods

Cultures of chick embryo breast muscle cells were prepared as by v. d. Bosch et al. [2]. In order to prevent fusion, the cells were kept at a low Ca^{2+}-concentration of 28 μM. For measurements of the fusion rates, the cells were triggered by addition of fresh medium adjusted to the indicated Ca^{2+}-concentration. After 2, 4, 6 and 10 hr cultures were fixed, stained and at least 1500 nuclei per dish were counted in order to estimate the fusion percentage. Fusion percentage is given as

$$\frac{\text{number of nuclei in myotubes}}{\text{total number of nuclei}} \times 100$$

For Ca^{2+}-uptake experiments cells were grown in 6-cm dishes. After 50 hr the medium was exchanged against another medium with 44 μM Ca^{2+}, buffered with 20 mM Hepes to maintain a pH of 7.3 at 37°C. The Ca^{2+}-concentration of 44 μM has been chosen in order to avoid any permeability changes eventually related to the fusion process, which would be triggered by higher Ca^{2+}-concentrations. After 2 hr this medium was replaced by another medium containing the same Ca^{2+}-concentration, 1 μCi ^{45}Ca/ml and in addition the indicated concentration of the ionophore A 23 187. Since equilibrium is reached within 60 min each dish was washed after 90 min with icecold Earle's solution. The washing procedure was repeated four times. The cells were solubilized in 1 ml 0.1% SDS and counted in 10 ml of Triton/Xylol/PPO scintillation fluid. For each experimental condition 5 dishes were measured in separate determinations. Protein was determined in 5 separate dishes according to Lowry [9]. Ca^{2+}-efflux measurements were performed in 14.5-cm dishes. Cells were labelled during 20 hr in Hepes buffered medium (pH 7.3 at 37°C) with 44 μM Ca^{2+} in the presence of 1 μCi ^{45}Ca/ml. The dishes were washed carefully 5 times with 30 ml icecold buffer. Finally 20 ml of a buffer solution were added which contained the indicated concentration of the ionophore. The dishes were shaken gently at 37°C and 1 ml samples were taken at different times. These samples were immediately centrifuged in order to sediment eventually detached cells. Radioactivity was determined in the supernatants.

^{45}Ca was purchased from Buchler/Amersham.

A 23 187 was a kind gift of Dr. Hamill from Eli Lilly Co.

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3. Results and discussion

Fig.1 shows fusion rates in the presence and absence of the ionophore at increasing Ca^{2+}-concentrations from 44 to 5600 μM Ca^{2+}. The ionophore concentration was increased from 0.125×10^{-6} to 8×10^{-6} M. Identical curves as shown in fig.1 were obtained at all concentrations up to 0.5 μM. Above 0.5 μM A 23 187 all fusion percentages and fusion rates showed a slight depression regardless of the Ca^{2+}-concentration. This is probably due to a toxic effect of the drug at higher concentrations. In these experiments it could be shown that no change in fusion rate occurs at any Ca^{2+}-concentration in the presence of non-toxic concentrations of the ionophore. Since small increases of the Ca^{2+}-concentration of the medium caused a steep increase in the fusion rate (fig.1), an effect of the ionophore should be detected especially at low Ca^{2+}-concentrations if the binding site as well as the site of action of the Ca^{2+}-ion is located on the inner face of the plasma membrane or within the cytoplasm.

Because of the difficulties to measure directly the increase of the intracellular free Ca^{2+}-concentration, we tried to show indirectly that A 23 187 is influencing the various Ca^{2+}-pools of the cell. Fig.2 shows the equilibrium data for the amount of total intracellular Ca^{2+} after 90 min incubation with various concentrations of A 23 187. With increasing amounts of ionophore the cells are more and more depleted from calcium. These data indicate that A 23 187 is entering the cells and that it is distributed in the membranes

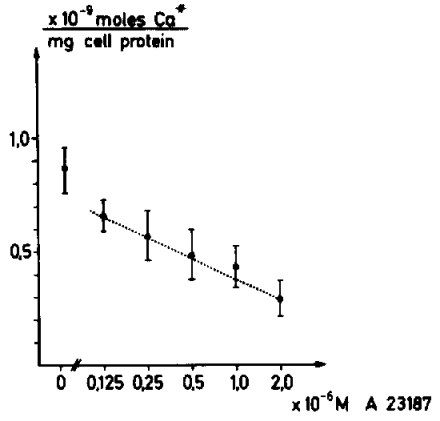

Fig.2. Ca^{2+}-content of myoblasts in the presence of A 23 187. Ca^{2+}-concentration of the medium is 44 μM Ca^{2+}.

which are sequestering calcium inside the cells. Since A 23 187 most probably is accelerating the passive fluxes from the extracellular compartment as well as from the mitochondria to the cytoplasm [10] it is conceivable to expect an increased cytoplasmic concentration of free Ca^{2+}.

In parallel experiments we tried to obtain further indirect evidence for increased Ca^{2+}-concentrations in the cytoplasm by measuring the efflux of labelled calcium from the cells in the presence of A 23 187. These data are given in fig.3. Due to the fact that cells represent a multi-compartment system for Ca^{2+}-ions [11] it is not possible to measure definite kinetic

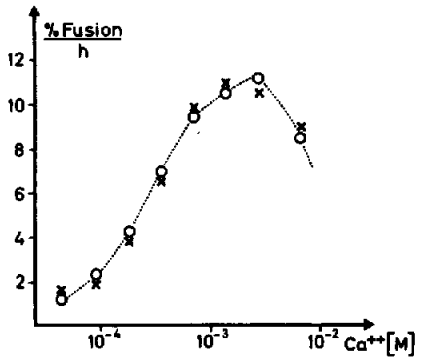

Fig.1. Ca^{2+}-dependence of fusion rate. (○) control; (×) A 23 187 0.25 μM.

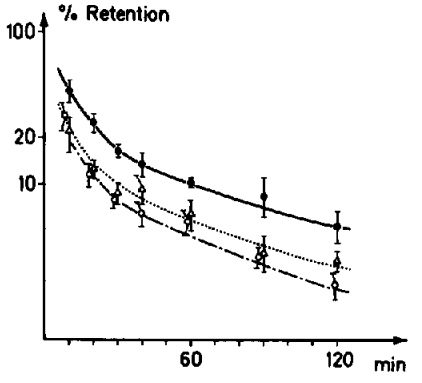

Fig.3. Ca^{2+}-efflux in the presence of A 23 187 after 20 hr labelling, 1 μCi ^{45}Ca/ml, 44 μM Ca^{2+}. (●) control; (△) 0.25 μM; (○) 1.0 μM.

constants in this type of experiment. Although Ca^{2+}-transport from cytoplasm to the surrounding medium is supposed to be an active process, we realize that this efflux is stimulated by the ionophore. Analogous effects of A 23 187 on barnacle muscle fibre have been described recently [12]. Considering the high concentration gradient from the medium to the cytoplasm, the most plausible explanation for this phenomenon is the increased cytoplasmic Ca^{2+}-concentration in the presence of the ionophore which causes a depletion of the intracellular Ca^{2+}-pools. As a consequence of the increased Ca^{2+}-concentration it appears probable that the Ca^{2+}-pump is transporting calcium through the plasma membrane at higher rates.

The following conclusions may be drawn:

1. The ionophore A 23 187 is suggested to enter the myoblasts and to increase passive permeability for Ca^{2+}-ions of intracellular Ca^{2+}-sequestering membranes and of the plasma membrane.

2. The efflux of ^{45}Ca from prelabelled cells is stimulated by A 23 187 which is also a strong indication for increased free Ca^{2+}-concentration produced by this drug.

3. The Ca^{2+}-dependence of myoblast fusion is not affected by the ionphore at any Ca^{2+}-concentration – except inhibitions at toxic concentrations of A 23 187

These results suggest that the Ca^{2+}-concentration dependence of the fusion process reflects the concentration dependence of Ca^{2+}-binding sites that are exposed at the outer face of the plasma membrane.

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