INHIBITION OF MUSCLE CELL FUSION *IN VITRO* BY Mg^{2+} AND K^+ IONS

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1. Introduction

Fusion of myoblasts in tissue culture has been shown to be triggered by Ca^{2+} ions [1]. The calcium concentration has to be changed only by a factor of five to produce a rise from 10 to 90% fusion [2]. This effect of Ca^{2+} is pH-dependent, i.e. lowering the pH decreases the fusion index and higher concentrations of Ca^{2+} are needed to restore the original fusion capacity. These findings raise the question whether Ca^{2+} is the only ion which is able to trigger fusion or whether it can be substituted by other ions. The experiments presented provide further evidence for the specific role of Ca^{2+}. Other cations except Sr^{2+} act inhibitory. In the case of Mg^{2+} and K^+ this inhibitory action can be reversed by increasing Ca^{2+} concentrations.

2. Materials and methods

Chick embryo breast muscle cells were explanted and cultured as described previously [2]. After seeding, the cells were grown for 52 hr at low (28 μM) $[Ca^{2+}]$ in Dulbecco's modification of Eagle's medium supplemented by 10% Ca^{2+}-free dialysed calf serum, 5% Ca^{2+}-free dialysed embryo extract and 40 mM bicarbonate. Only 5–7% of the cells fused under these conditions. After 52 hr the medium was changed and the fusion experiment was started by addition of 2 ml of fresh medium. The ion concentrations of the fusion experiment are given in the diagrams. At least 800 nuclei per dish were counted and fusion index equals

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

3. Results

3.1. *Fusion induction experiments*

If Mg^{2+} up to concentrations of 40 mM in the presence of 28 μM Ca^{2+} is added to cultures after 52 hr growth, Mg^{2+} is not able to initiate fusion. Cell viability is not impaired by these concentrations as is confirmed by fig. 1. Experiments with Zn^{2+}, Mn^{2+}, Ba^{2+}, Cu^{2+}, Cd^{2+}, La^{3+}, Li^+ show that none of these cations can substitute for Ca^{2+} as fusion triggering agent. A slight fusion promoting effect could be observed only in the presence of 2.4 mM Sr^{2+}. These ions were selected with regard to their charge, size and hydrate water exchange rate – properties in which they are related to Ca^{2+} or differ from it in a known degree [3].

3.2. *Fusion inhibition experiments*

Fusion is inhibited by increasing $[Mg^{2+}]$ at fixed $[Ca^{2+}]$. Raising the $[Ca^{2+}]$ shifts the Mg^{2+}-concentration necessary for half maximal inhibition to higher values, as shown in fig. 2. Reducing $[Mg^{2+}]$ gradually from 0.8 to 0.04 mM does not change the position of the curves within experimental error. In addition to the parallel shift, we observe the curves flattening with increasing Mg^{2+}-concentrations. As shown in fig. 3. K^+ also inhibits fusion. Obviously, increasing $[K^+]$ beyond its normal concentration reduces fusion, and this inhibition can be reversed by increasing $[Ca^{2+}]$. All the metal cations tested except Sr^{2+} were inhibitory at different concentrations as given in table 1.

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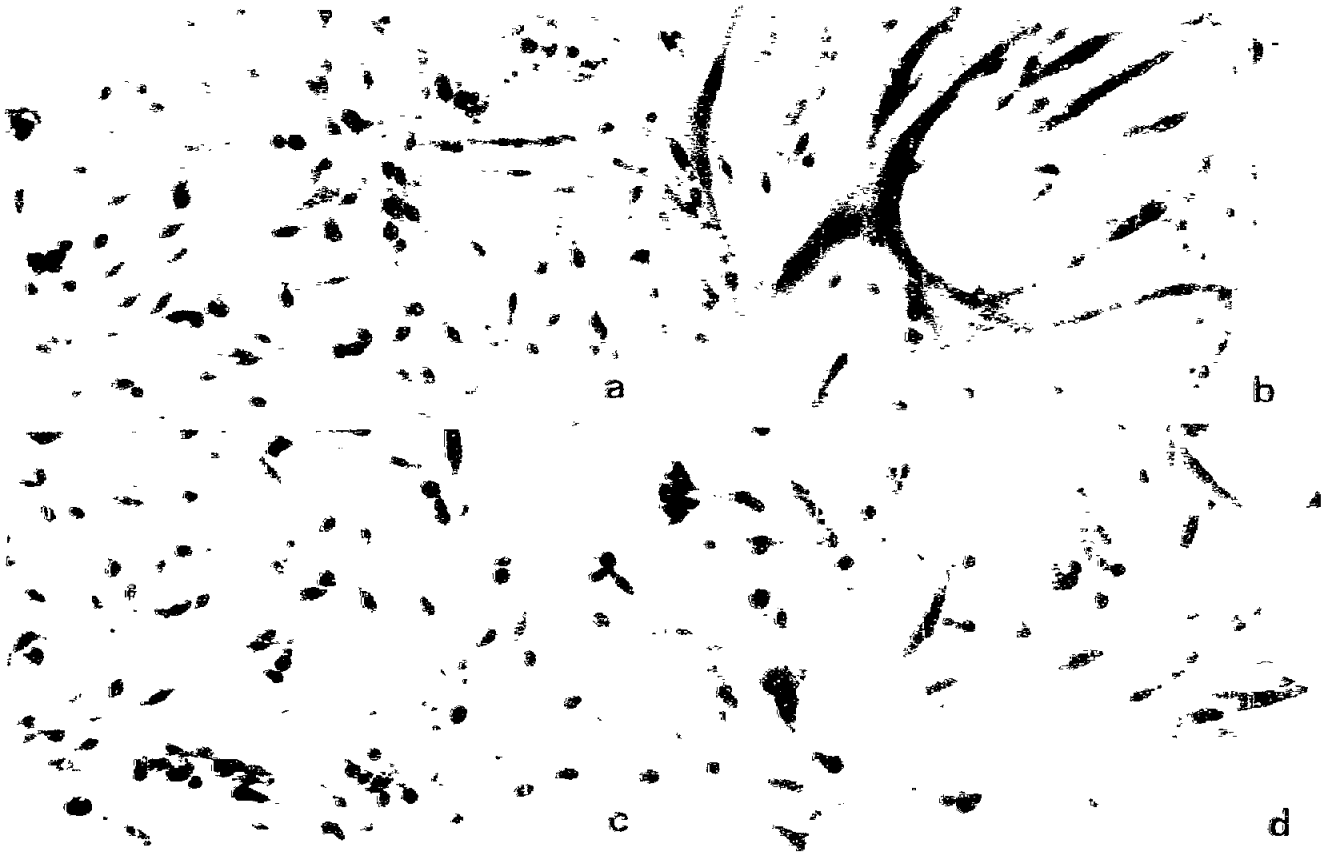

Fig. 1. Muscle cells 12 hr after fusion has been started under different conditions: a) 0.8 mM Mg^{2+}; 44 μM Ca^{2+}. b) 0.8 mM Mg^{2+}; 1400 μM Ca^{2+}. c) 40 mM Mg^{2+}; 44 μM Ca^{2+}. d) 40 mM Mg^{2+}; 5800 μM Ca^{2+}.

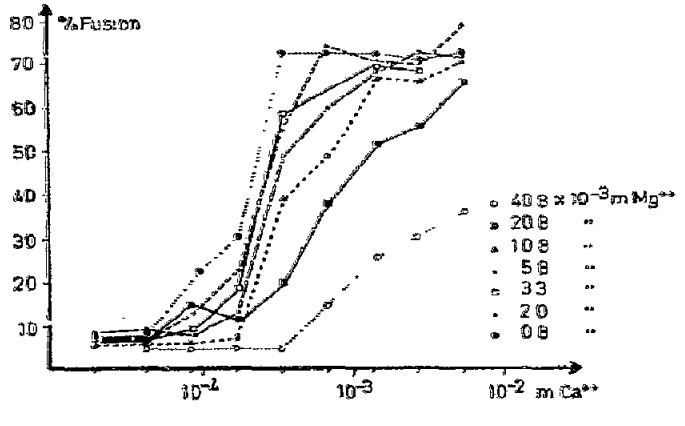

Fig. 2. Ca^{2+}-dependence of fusion at different Mg^{2+}-concentrations: a) Plot of fusion percentages versus Ca^{2+}.

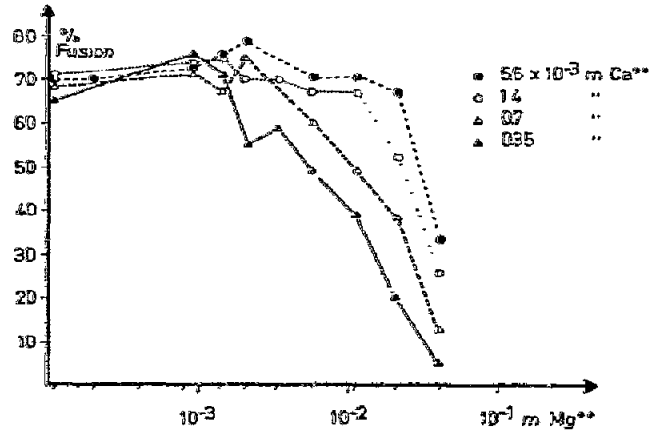

Fig. 2b) Plot of fusion percentages versus Mg^{2+}.

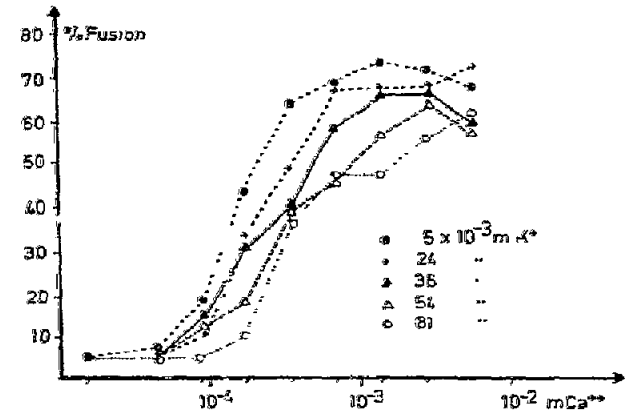

Fig. 3. Ca^{2+}-dependence of fusion at different K^+ concentrations.

4. Discussion

The specificity of interaction of Ca^{2+} with the cell components mediating fusion suggests a specific binding of Ca^{2+} to these cell structures. Thus, the curves of the fusion index versus Ca^{2+} may be, in essence, binding isotherms for Ca^{2+} ions to these specific sites.

The steep rise of the fusion versus Ca^{2+} curves may be interpreted in terms of cooperative binding of Ca^{2+}. Assuming that Mg^{2+} has to be bound, too, in order to exert its inhibiting effect, the steep decrease of the fusion index in fig. 2b suggests that this binding should also be a cooperative process. One possible explanation for this effect is that Ca^{2+} and other cations are competing for one single kind of binding site which promotes fusion only if it is occupied by Ca^{2+}. On the other hand if two different sites are responsible for the Ca^{2+}–Mg^{2+}-antagonism these two should be coupled energetically. Binding of Mg^{2+} to the one site may thus reduce Ca^{2+} binding to the fusion promoting site.

Table 1

Cation concentrations [mM] necessary for 50% inhibition of fusion in the presence of 1.4 mM Ca^{2+}.

Li^+	K^+	Mg^{2+}	Mn^{2+}	La^{3+}
50	80	20	0.15	2
Ba^{2+}	Zn^{2+}	Cd^{2+}	Cu^{2+}	
0.2	0.2	0.2	1.5	

Results obtained in the presence of high extracellular $[K^+]$ can be interpreted in the same way as for Mg^{2+}. In any case, the K^+-binding site should discriminate sharply between K^+ and Na^+, since Na^+, which is always present at 140 mM concentration, does not interfere with fusion.

On the other hand, K^+ might also interfere by modifying cell membrane potential. Such an effect has recently been reported on the growth of BHK-cells [4]. The K^+-induced depolarization preceded all further events in these cells.

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