SOME ASPECTS OF THE REENFLAGELLATION RESPONSE OF *NAEGLERIA GRUBERI*

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The possible role of increased levels of Ca^{2+} and/or Mg^{2+} in triggering the ameba-to-flagellate transformation (AFT) of *Naegleria gruberi* was investigated using the reenflagellation response (RER). This technique indicates that both of the above divalent cations, separately or in combination, will induce an increase in the flagellate population of cells which have previously undergone the AFT.

INTRODUCTION

The amebo-flagellate *Nagleria gruberi* has been extensively used to study the morphogenesis of an eucaryotic cell. However, the majority of the reported investigations (1-2) use manipulative events that could contribute to the ameba-to-flagellate transformation (AFT), *e.g.*, dilution of the environment; changing the environment from quasi-two dimensions (an agar surface) to three dimensions (an aqueous phase); removing the amebae from their food sources; and the physical trauma associated with these events.

However, some authors (3-4) have suggested that the primary stimulus (i) for the AFT is increasing the influx and/or intracellular concentrations of Ca^{2+} and/or Mg^{2+}. Unfortunately, the elucidation of subcellular events is beyond the state of the art, but if the above factors can be eliminated, a more positive approach to defining the AFT stimulus (i) would be ascertained. The purpose of this investigation was to determine if the above factors could be eliminated and concomitantly establish that the presence of Ca^{2+} and/or Mg^{2+} could promote the AFT.

To accomplish this, we developed a technique to determine the ability of Ca^{2+} and/or Mg^{2+} to promote the AFT in cell populations which had already been stimulated to undergo the AFT, *i.e.*, the reenflagellation response (RER).

MATERIALS AND METHODS

Naegleria gruberi (NB-1) grown on a nutrient agar media (NM) in 100 × 20 mm Petri dishes (2), with *Aerobacter aerogenes* as a food source, was used in this investigation. The experimental protocol was as follows:

Twenty-six hr ($t_{-26\ hr}$) prior to testing the RER, *N. gruberi* plus *A. aerogenes* were spread on NM media and incubated at 30 C. Twenty hr later ($t_{-6\ hr}$), 30 ml of the solution used to initiate the AFT were added to each culture. These suspensions were incubated at 30 C for 2 hr and at $t_{-4\ hr}$ all cell suspensions (now including a high percentage of flagellates) were pooled, redistributed into 250-ml culture flasks at 30 ml/ flask, and agitated at 30 C. After 4 hr (t_0), the respective populations contained approximately 20% flagellates and the test solutions were added using 10 ml of each. (All solutions were made using reagent grade chemicals, including calcium and magnesium chlorides, and all solutions containing HEPES were adjusted to pH 7.5 using NaOH).

Aliquots of each suspension were fixed using Lugol's iodine (2) at t_0, t_{30min} through t_{120min} at 30-min intervals. Differential counts of the fixed cell populations were made using phase contrast microscopy and a minimum cell number of 200 was counted for each suspension at a given time. Each experiment was conducted four times and average values are plotted.

RESULTS

Cells initially transformed in glass distilled water.

Cells, triggered for the initial AFT using glass-distilled water (GDH_2O), were tested at t_0 for the RER using 4m*M* HEPES; 4m*M* HEPES + 4m*M* Ca^{2+}; 4 m*M* HEPES + 4 m*M* Mg^{2+}; and 4 m*M* HEPES, 2m*M* Ca^{2+} and 2 m*M* Mg^{2+}.

This protocol (Figure 1) indicates that the addition of the solution was followed by a decrease in the flagellate population and there was no difference with respect to the solutions tested. However, at $t_{60\ min}$ the flagellate population increased above the initial level at t_0 in all solutions except 4 m*M* HEPES.

With respect to the remainder of the RER, the effect of 4 m*M* HEPES can be eliminated because the flagellate population did not return to the initial level in this solution. However, in the presence of 4 m*M* Ca^{2+}, 4 m*M* Mg^{2+}, or 2 m*M* Ca^{2+} and 2 m*M* Mg^{2+}, the maximum flagellate population increased to 206%, 164%, and 167% of the initial population, respectively.

Cells initially transformed in 4 mM HEPES.

In this experiment the initial AFT was triggered using 4m*M* HEPES and at t_0 the RER was tested by adding GDH_2O, 4 m*M* Ca^{2+}, 4 m*M* Mg^{2+}, or 2 m*M* Ca^{2+} and 2 m*M* Mg^{2+}.

The RER (Figure 2) is essentially the same as given in Figure 1, i.e., the maximum percentage of flagellates formed exceeded the initial level in all solutions containing Ca^{2+} and/or Mg^{2+}, but did not

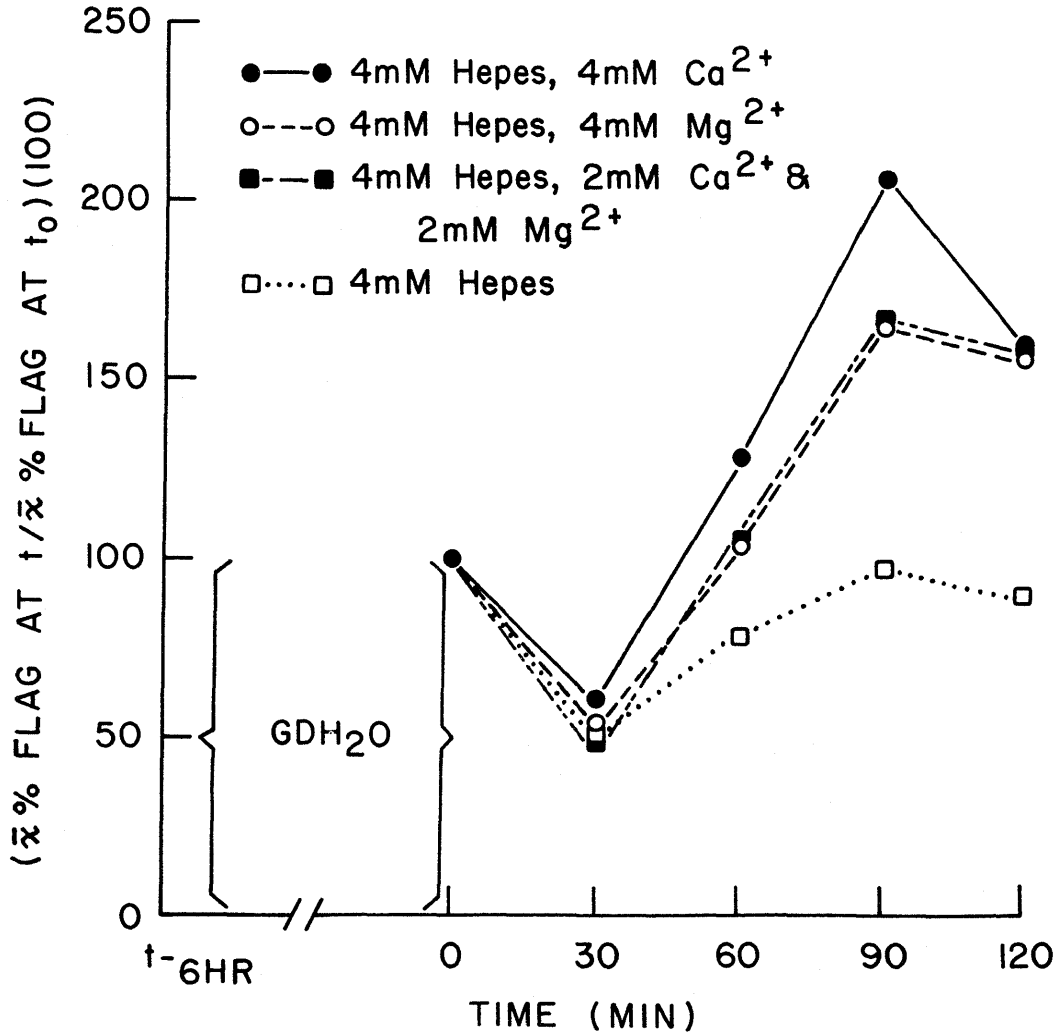

FIGURE 1. The reenflagellation response (RER) of *N. gruberi* initially stimulated for the ameba-to-flagellate transformation using glass-distilled water (GDH_2O). Test solutions added at t_0; concentrations are final.

exceed the initial level in GDH_2O. With respect to the kinetics, the rate of the reenflagellation had reached a maximum by $t_{90\ min}$, but the process continued. At $t_{120\ min}$ the respective values for the flagellate population in 4 m*M* Ca^{2+}, 4 m*M* Mg^{2+}, or 2 m*M* Ca^{2+} and 2 m*M* Mg^{2+} were 195%, 171%, and 202% of the initial population, respectively.

DISCUSSION

Dilution of the environment.

Obviously those solutions which contained Ca^{2+} and/or Mg^{2+} favored the RER (Figures 1 and 2). Thus, the RER was greater in solutions with the greater osmotic concentrations. Therefore, within reasonable limits, dilution of the environment can be omitted as a trigger for the RER.

Changes in the dimensional aspects of the environment.

As stated above, the AFT is associated with a change from a quasi-two- to a three-dimensional environment. This transition includes a change in surface tension. The RER was triggered while the cells were in, and had been exposed for six hr to, a three-dimensional environment. Figures 1 and 2 indicate that the RER occurred in cells

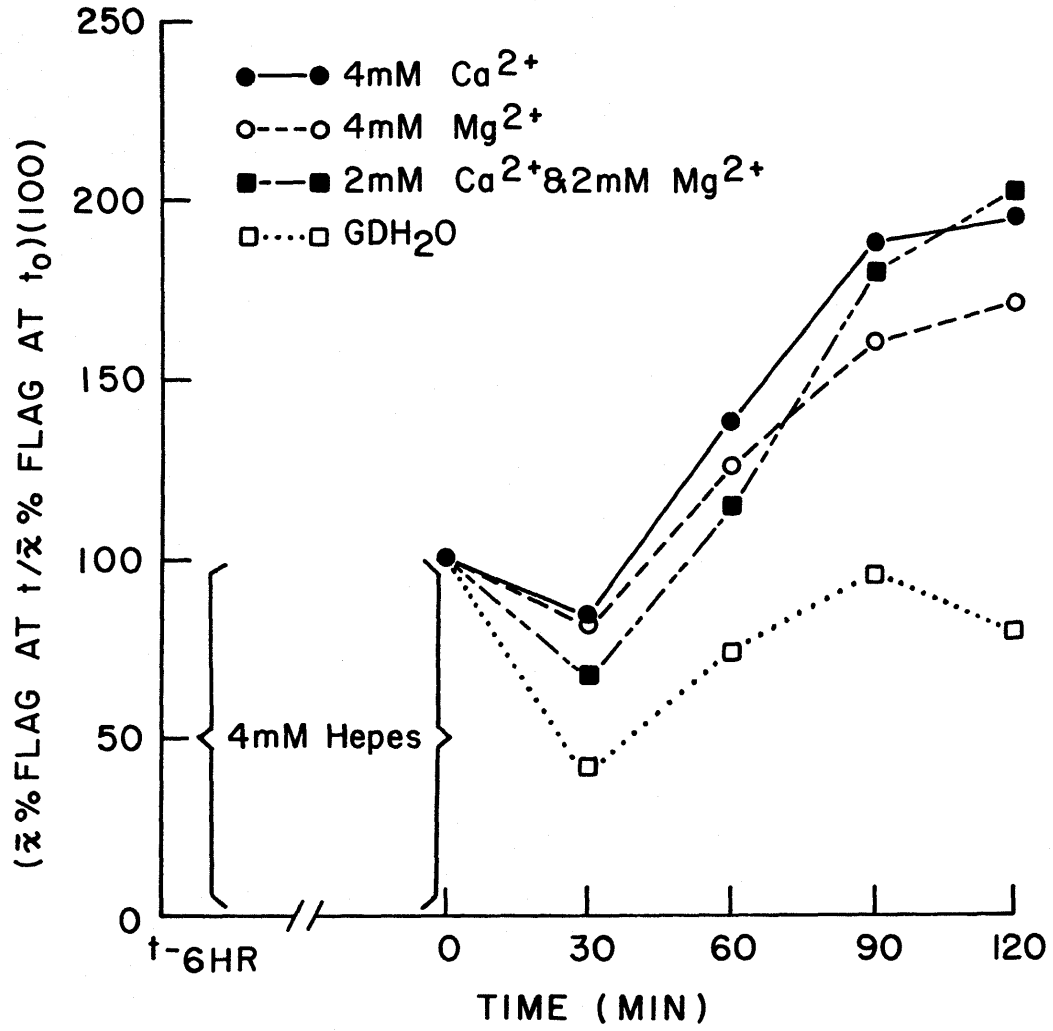

FIGURE 2. The RER of *N. gruberi* which had undergone the initial AFT in 4 m*M* *HEPES*. Test solutions added at t_0; concentrations are final with respect to the cations, *i.e.*, the final *HEPES* concentration. is 3 m*M*.

which remained in a three-dimensional environment. The data also indicate that solutions which have the higher surface tension, *i.e.*, the Ca^{2+} and/or Mg^{2+} solutions, favor the RER. Therefore, the change in surface tension associated with the initial AFT, *i.e.*, a change from high to low surface tension, may be omitted as a primary stimulus for the AFT.

Presence of food source.

During this investigation *N. gruberi* cysts were never observed. Thus, there were enough *A. aerogenes* present to serve as food. Yet the RER took place. Therefore, it is unlikely that the presence of the food is a significant deterrent to the RER.

Physical trauma.

The above investigation minimized and standardized the physical trauma associated with the AFT-RER sequence. Due to the differences in the RER, based on the presence or absence of divalent cations, physical trauma can be eliminated.

Stimulus for the reenflagellation response.

The data indicate that the AFT of *N. gruberi* is correlated (triggered?) with the influx of Ca^{2+} and/or Mg^{2+}. This was proposed by Perkins and Jahn (3, 4) and Perkins (in a manuscript submitted to J. Cell Physiol.) and these data support the influx rather than efflux.

Even though there are apparent quantitative differences between these two divalent cations, both (separately or in combination) favor the AFT. Whether this interaction is due to cation exchange at the subcellular level or to independent pathways for initiating flagella formation remains to be determined.

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