SOME ASPECTS OF THE ENFLAGELLATION INDEX OF *NAEGLERIA GRUBERI*

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The interaction of Ca^{2+}, Mg^{2+} and osmotic concentration in altering the ameba-to-flagellate transformation (AFT) of *Naegleria gruberi* was investigated using the enflagellation index (EI). The EI, which includes a consideration of the magnitude and duration of the enflagellation response, is decreased by increasing osmotic concentration, increasing Ca^{2+} concentration, and increasing Ca^{2+} /Mg^{2+} ratio (CMR). In addition, an antagonistic role of Ca^{2+} and Mg^{2+} was found.

INTRODUCTION

Naegleria gruberi, an ameboflagellate, is one of the most common protozoa found in soil and fresh water (1). Undoubtedly, the ubiquitous occurrence of this particular organism is due to its ability to undergo an ameba-to-flagellate transformation (AFT). Also, the dispersal of the flagellated phase of *N. gruberi* is based on both the percentage and duration of the enflagellation response.

In the natural environment the ameboid phase of *N. gruberi* is exposed to many stimuli which may alter the AFT (2), but the two stimuli of primary concern are changes in the osmotic and divalent cation concentrations of the environment. As outlined by Perkins and Jahn (3), the principal stimulus for the AFT could be due to an increase in the divalent cations, e.g., Ca^{2+} and/or Mg^{2+}, associated with the cell, which could be induced by decreasing the osmotic concentration of the environment as well as cationic increases.

Several investigators (4, 5) have noted that the effects of increasing electrolyte concentrations, and the concomitant decrease in the AFT, are qualitatively similar, but quantitatively different, with respect to similar concentration changes of non-electrolytes. In addition, it has been shown that the AFT can be inhibited by several compounds which do not significantly contribute to the osmotic concentration. (6). Also, by using the reenflagellation response, Perkins (7) has shown that increasing the divalent cation concentration favors the AFT even though the osmotic concentration is increased. However, it has not been established whether the initial AFT is primarily correlated with osmotic or divalent cation parameters.

The following investigation is a report of the initial AFT in solutions which, through the use of EDTA (8), have the Ca^{2+} and Mg^{2+} concentrations stabilized at variable osmotic concentrations. Through a statistical analysis of the data by the least squares technique (9) it was determined whether the principal stimulus for the AFT was based on osmotic or divalent cation alterations of the environment.

MATERIALS AND METHODS

The experimental model used in this investigation was *Naegleria gruberi* (NB-1) grown on a nutritient agar medium (NM) in 100 × 20 mm Petri dishes using *Enterobacter aerogenes* as a food source (10).

Approximately twenty hr prior to testing for the AFT, *N. gruberi* amebae were contiguous (t_o); an aliquot of the NM culture was suspended in 50 m*l* of the chosen solution and centrifuged until the amebae were pelleted (2,000 RPM for 3 min), the resulting supernatant was discarded; and the pellet was resuspended in 50 m*l* of the same solution. The above washing, via centrifugation, was repeated two more times; after the third washing, the amebae were resuspended in 5 m*l* of the solution and transferred to 25 m*l* of the respective solution in 250-m*l* culture flasks at 28 C with mild agitation.

Aliquots of each cell suspension were fixed using Lugol's iodine (10) at t_{60}, t_{75}, t_{90}, t_{105}, t_{180}, and t_{210} min. Differential counts of the fixed cell populations were made using phase-contrast microscopy and a minimum of 200 cells were counted for each suspension at a given time. The percent flagellated cells at a given time were

plotted to give an enflagellation profile for each solution and the respective analyses were made using these profiles.

To determine the influence of environmental parameters on the enflagellation response the concept of an enflagellation index (EI) was used. The EI was determined by measuring the relative areas of the enflagellation profiles and used as the dependent variable in the least squares technique. Thus, both the percentage of flagellated cells formed and the duration of the enflagellation response have been considered.

All solutions were made using reagent grade chloride salts and all solutions were adjusted to a final pH of 7.1 using KOH and/or NaOH. The respective solutions were formulated using the technique of Zucker (8) over Ca^{2+} and Mg^{2+} concentration ranges of 10^{-8} to 10^{-2} M; a Ca^{2+}/ Mg^{2+} or calcium-to-magnesium ratio (CMR) of 10^{-6} to 10^{6}; and, by varying the concentration of EDTA, an osmotic range of approximately 20 to 100 mOsm was used as measured using an Osmette.

RESULTS

Effect of osmotic concentration.

As given in Fig. 1, there is a negative correlation between an increase in osmotic concentration and the EI. The corresponding R value for these data is –0.459 (N = 39), which indicates that there is a highly significant negative correlation over the osmotic concentration range tested.

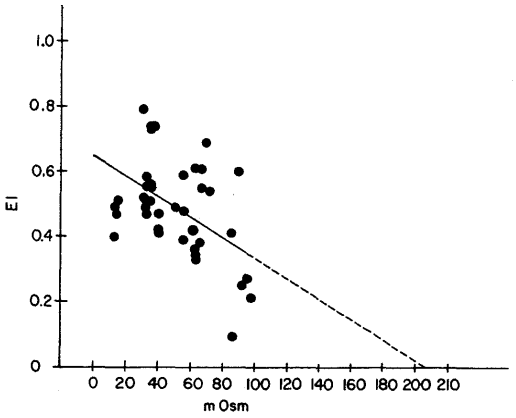

FIGURE 1. The enflagellation index (EI) of *N. gruberi* as a function of osmotic concentration. (Broken line is extrapolation.)

Effect of Ca^{2+} and Mg^{2+} concentrations.

If the EI is evaluated in terms of increasing Ca^{2+} concentration (Fig. 2) the results also indicate a highly significant negative correlation (R = –0.56, N = 39). However, the same relationship does not exist for Mg^{2+} (Fig. 3), i.e., the EI is positively correlated, but not significantly, with increasing Mg^{2+} concentration (R = +0.11, N = 39). An additional analysis indicated that neither the Ca^{2+} or Mg^{2+}, as log functions, were significantly correlated with osmotic concentration (R = +0.202 and R = +0.189, respectively).

Effect of Ca^{2+} /Mg^{2+}.

The above data indicate that Ca^{2+} and Mg^{2+} tend to be antagonistic to each other with respect to effect on EI. Thus, it would be expected that the Ca^{2+} -to-Mg^{2+} ratio

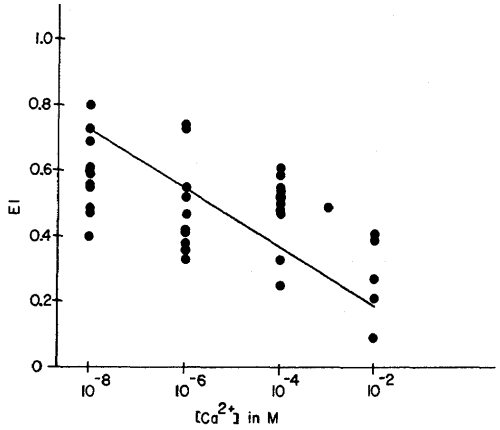

FIGURE 2. The EI of *N. gruberi* as a function of the Ca^{2+} concentration.

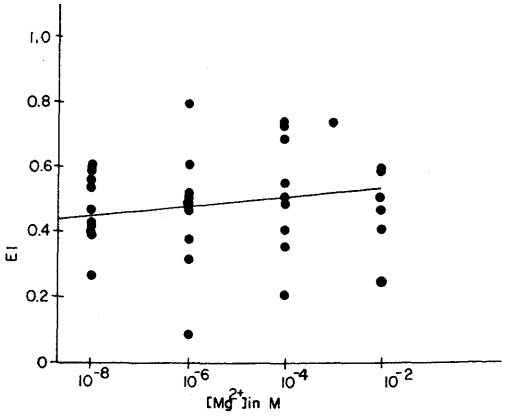

FIGURE 3. The EI of *N. gruberi* as a function of the Mg^{2+} concentration.

(CMR) would be negatively correlated with the EI and *vice versa.* Also, a consideration of both ions should decrease the significance of the Ca^{2+} on the EI. Fig. 4 indicates that there is indeed a negative correlation between the CMR and the EI. Although this correlation is not significant (R = –0.229, *N* = 39) it does tend to establish that in this situation Mg^{2+} is antagonistic to Ca^{2+} . As before, an additional analysis indicated no significant correlation between the CMR and osmotic concentration (R = +0.153, *N* = 39).

DISCUSSION

The above data indicate that the osmotic concentration is negatively correlated with the EI (Fig. 1) and will prevent enflagellation at approximately 200 mOsm. This correlation, as shown by additional analysis, also exists for the rate of the AFT (R = –0.61) and the duration of the enflagellation response (R = 0.58). Thus, dilution of the natural environment is a significant component of the enflagellation response. Whether the decrease in osmotic concentration brings about an increase in the intracellular level of associated or bound divalent cations (3) or a decrease of bound divalent cations remains to be determined.

With respect to the divalent cations being considered, i.e., Ca^{2+} and Mg^{2+}, only Ca^{2+} is negatively correlated with the EI (Fig. 2) and the same relationship exists for the rate and duration of the enflagellation response (R = –0.53 and R = –0.29, respectively). Conversely, Mg^{2+}, which is positively correlated with the EI (Fig. 3), is also positively correlated with the duration (R = +0.13), but negatively correlated with the rate of the AFT (R = –0.08). Even though the level of significance for the positive correlations of Mg^{2+} does not establish that Mg^{2+} is facilitory, there is a difference in the EI with respect to these ions, i.e., Ca^{2+} is more inhibitory than Mg^{2+}. This relationship is also apparent in the data of Jeffery and Hawkins (5), who report that 50 mM Ca^{2+} contains only 5.8 percent of the average flagellate population of a 50 mM solution of Mg^{2+}. It is rather surprising that we did not find a significant correlation between the divalent cations and osmotic concentration. Thus it appears that both osmotic and ionic events influence the enflagellation response.

If the above relationship is true, the CMR should indicate an antagonistic relationship between Ca^{2+} and Mg^{2+}. As shown in Fig. 4, this relationship, even though not significant, exists. Since the EI includes all components of the enflagelation response we looked at the rate and duration of enflagellation as correlated with the CMR. There is a highly significant negative correlation between the CMR and the duration (R = –0.43) and a non-significant correlation (R = –0.18) with the rate of the AFT. Thus, the antagonism of Ca^{2+} and Mg^{2+} is masked when considering the EI, but such antagonism does exist.

The above data indicate that a decrease in osmotic concentration and/or a decrease in the CMR favor the flagellated form of *Naegleria gruberi* in situations where both osmotic and divalent cation concentrations are being altered, *i.e.,* the natural environment.

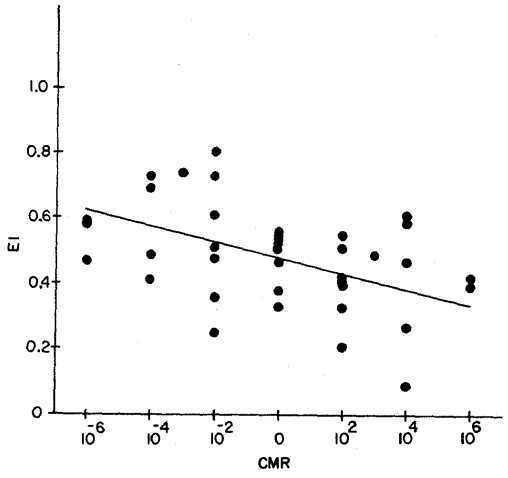

FIGURE 4. The EI of *N. gruberi* as a function of the Ca^{2+} /Mg^{2+} (CMR).

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