Adv. Space Res. Vol. 9, No. 11, pp. (11)75–(11)78, 1989
Printed in Great Britain.

0273–1177/89 $0.00 + .50

POTENTIAL SITES FOR THE PERCEPTION OF GRAVITY IN THE ACELLULAR SLIME MOLD *PHYSARUM POLYCEPHALUM*

I. Block and W. Briegleb

DFVLR – Institute for Aerospace Medicine, Linder Höhe, D–5000 Cologne 90, F.R.G.

ABSTRACT

Recently a gravisensitivity of the acellular slime mold Physarum polycephalum, which possesses no specialized gravireceptor, could be established by conducting experiments under simulated and under real near weightlessness. In these experiments macroplasmodia showed a modulation of their contraction rhythm followed by regulation phenomena. Until now the perception mechanism for the gravistimulus is unknown, but several findings indicate the involvement of mitochondria: A) During the impediment of respiration the 0g-reaction is inhibited and the regulation is reduced. B) The response to a light stimulus and the following regulation phenomena strongly resemble the behavior during exposure to 0g, the only difference is that the two reactions are directed into opposite directions. In the blue-light reaction a flavin of the mitochondrial matrix seems to be involved in the light perception. C) The contraction rhythm as well as its modulations are coupled to rhythmic changes in the levels of ATP and calcium ions, involving the mitochondria as sites of energy production and of Ca^{++}-storage. - So the mitochondria could be the site of the regulation and they possibly are the receptor sites for the light and gravity stimuli. - Also the observation of a morphologic polarity of the slime mold's plasmodial strands has to be considered: Cross-sections reveal that the ectoplasmic wall surrounding the streaming endoplasm is much thinner on the physically lower side than on the upper side of the strand - this applies to strands lying on or hanging on a horizontal surface. So, in addition to the mitochondria, also the morphologic polarity may be involved in the perception mechanism of the observed gravisensitivity and of the recently established geotaxis. - The potential role of the nuclei and of the contractile elements in the perception of gravity is also discussed.

INTRODUCTION

Like most of the unicellular organisms the acellular slime mold Physarum polycephalum does not possess a specialized gravireceptor. Nevertheless this organism turned out to be gravisensitive showing at least 4 responses to a changing or a lacking gravistimulus:

1.) In the experiments macroplasmodia were used. They are the multinucleated motile stage composed of a network of strands performing rhythmic contractions. These macroplasmodia showed under conditions of simulated weightlessness (fast-rotating clinostat) and under real near weightlessness (Spacelab experiment) as well as after a 180° horizontal turn under continuous 1g-conditions a transient acceleration of their contraction rhythms (shortening of the periods) followed by regulation phenomena /1,2/.

2.) Directly correlated with the contractile activity is the locomotion of a macroplasmodium, since the oscillating contractions of the strands' ectoplasmic walls are the motive force for the endoplasmic shuttle streaming which is a hydrostatic-pressure flow system. A preferred transport of the endoplasm in one direction determines the direction of the locomotion. This direction can be changed by external stimuli, for example gravity: it was only recently that a positive geotaxis could be demonstrated for the acellular slime mold /3/.

3.) The morphologic polarity of plasmodial strands, revealed in cross-sections of macroplasmodia, shows a striking dependence upon the g-vector: in strands lying on a horizontal surface the ectoplasmic wall, enclosing the streaming endoplasm, is much thinner on the physically lower side than on the upper side of the strand (Figure 1a) /4/. The same aspect could be demonstrated in strands hanging on an inversed horizontal surface /5/: the ectoplasm still

is thinner on the now physically lower side, the endoplasmic channel seems to have moved downwards in direction of the gravity vector (Figure 1b).

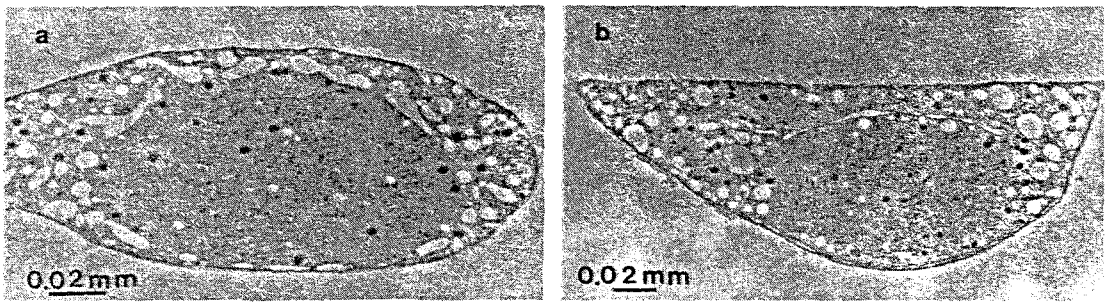

Fig. 1. Cross-sections of plasmodial strands a) lying on agar; b) hanging on an agar substratum. Note in both cases the very thin ectoplasm and the small and flattened plasmalemma invaginations on the physically lower sides of the strands.

4.) In experiments using microplasmodia, tiny plasmodia grown in liquid culture, the time course of the synchronous mitoses of the nuclei was observed during 0g-simulation. Interestingly enough, here also an acceleration could be registered; especially the metaphase was significantly shortened (by about 50 %) compared to 1g-control plasmodia /6/.

RESULTS AND DISCUSSION

What could be the perception mechanism initiating all these responses - is there only one receptor for the 4 above-mentioned reactions? The primary perception may take place via slight pressure and tension effects or dislocations in the vicinity of cell compartments of differing density (for calculations of forces see /7,8,9/). In search for such cell compartments playing a role in g sensing, experiments conducted with Physarum indicated at least 4 potential sites of gravity perception:

Mitochondria

Prime candidates for the graviperception are the mitochondria. Their mass and volume are only at a physical threshold concerning the mitochondria's ability for sedimentation /7,8,9/, but several findings indicate their involvement not only in the normal regulation of the contractile activity, but also in g-dependent reactions:

1.) The contractile activity of macroplasmodia depends on two energy (ATP) - producing processes: respiration and glycolysis taking place in the mitochondria and the cytosol, respectively. During the impediment of respiration with KCN the rhythmic contractions continue, but the response to simulated 0g is inhibited and the regulation is reduced, at least indicating that mitochondria are involved in the 0g-reaction /1/. Experiments involving a blockage of glycolysis indicate that this metabolic pathway has neither an influence on the 0g-reaction nor on its regulation.

TABLE 1 An Increase in Frequency Leads to a Positive Taxis, a Decrease in Frequency to a Negative Taxis. This Seems to Be a General Mode of Reaction /14/, Because it Could Be Demonstrated for Geotaxis, Phototaxis, and Chemotaxis of Plasmodia of Physarum Polycephalum.

Stimulation / Reaction	Gravity (1g+0g-simulation)	Light (< 570 nm)	Glucose
frequency modulation (inhibited by impediment of respiration)	increase	decrease	increase
↓	↓	↓	↓
taxis	positive geotaxis (impediment of respiration not tested)	negative phototaxis	positive chemotaxis (inhibited by impediment of respiration)

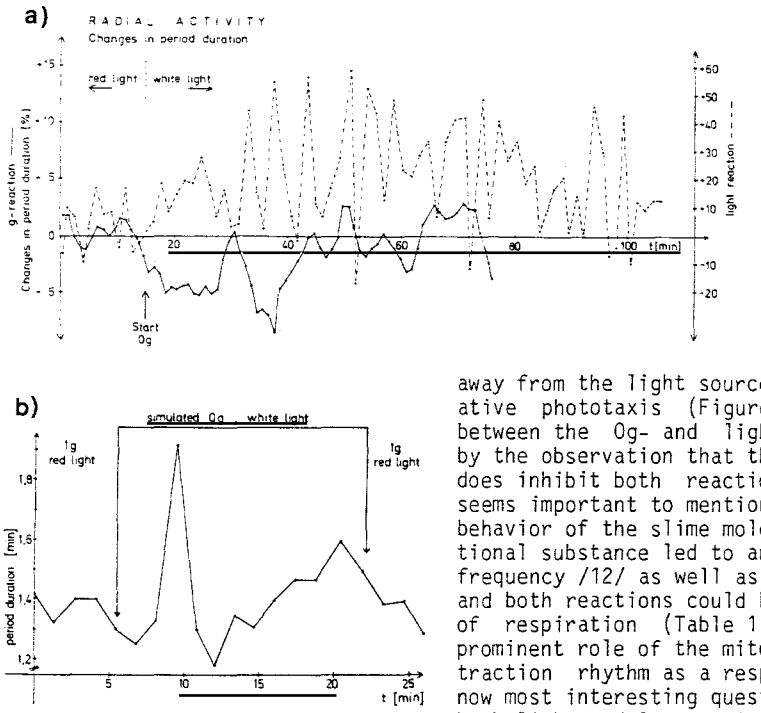

Fig. 2. Frequency evaluation curves of the contractile activities recorded during a) 1-h simulation of 0g (solid curve, mean values of 15 experiments) and during stimulation (1,5 h) with white light (dashed curve, one experiment); note that the two reactions are directed into opposite directions; b) simultaneous application of 0g and white light for 20 min.; note the delayed light reaction starting after 7 min. of illumination.

2.) Young macroplasmodia show a distinct response to illumination with light containing the shorter wavelengths of the visible spectrum, that is, the blue-green portion. The time pattern of this response and the following regulation phenomena strongly resemble the 0g-reaction /10/, the only difference is that the two reactions are directed into opposite directions: In the light response the frequency of the contractions is decreased and - in moving away from the light source - the plasmodia show a negative phototaxis (Figure 2a) . The strong similarity between the 0g- and light reactions is expressed also by the observation that the impediment of respiration does inhibit both reactions /11/ . In this context it seems important to mention the effect of glucose on the behavior of the slime mold. The addition of this nutritional substance led to an increase in the contraction frequency /12/ as well as to a positive chemotaxis /13/, and both reactions could be inhibited by the impediment of respiration (Table 1). These findings stress the prominent role of the mitochondria in changing the contraction rhythm as a response to external stimuli. The now most interesting question is: What will happen, if both light and 0g-reactions are induced simultaneously? The type of superposition of the two opposite reactions of the contraction rhythm should give further indications concerning the role of the mitochondria not only as sites of the regulation of the contractile activity, but also as the receptor sites for the light and gravity stimuli. Already in 1983 /11/ a flavin in the α -ketoglutarate and pyruvate dehydrogenase complexes, located in the mitochondrial matrix, was suggested to be involved in the primary blue-light reaction. As mentioned above, the experiment including the impediment of respiration pointed to an involvement of the mitochondria also in the 0g-reaction. In this scope we began to perform light experiments during simultaneous 0g-simulation on the fast-rotating clinostat. The most interesting aspect of the prelimenary results revealed in these experiments is that the 0g-reaction can not be observed and that the light reaction is not only reduced but also delayed by about 10 minutes (Figure 2b). This mutual influence of the light and gravity reactions on one another represents one further indication pointing to a role of the mitochondria not only in the regulation of these two reactions, but also in the perception of the respective stimuli.

3.) The contraction rhythm itself as well as its slight frequency modulations which occur under stimulus-free conditions /15,16/, i.e., when all environmental factors such as light, temperature and humidity are kept constant, are coupled to rhythmic changes in the intracellular levels of ATP and calcium ions. Responsible for such changes again are the mitochondria being main sites of energy production and of Ca^{++}-storage. So, the mitochondria are, in fact, regulating the contraction frequency, thereby also responding to external stimuli.

Nuclei

With regard to their size and density the nuclei, nucleoli, and especially the condensed chromosomes are perfectly suited as gravireceptors. The influence of simulated 0g on the time course of mitosis may be an indication for this function. However, the shortened metaphase does not necessarily indicate a statolith role for the chromosomes. It remains open, if the g-sensitivity of nuclei expressed in mitosis is used by the cell, and if the g-stimulus is transduced via unknown messengers to the mitochondria which, as part of the reaction chain, act as modulators of the contractile behavior.

Morphologic Polarity

The morphologic polarity may also be involved in the perception mechanism or may even act as a primary receptor. In this case one may speculate that the endoplasm - due to a lower con-

tent of vacuoles and the lack of invaginations - may have a higher density than the ectoplasm enabling the endoplasm to move in direction of the g-vector. This sedimentation may then be the signal which will eventually cause the mitochondria to modulate their contraction frequency. A second mode of perception is discussed in /5/.

Contractile Elements

Another possibility for a graviperception are the contractile elements themselves, since the weight of the whole cell or parts of it may induce tensions in the actomyosin system /5/. A detailed evaluation of the contraction curves revealed that the Og-reaction takes place only in the contraction phase of the contraction-relaxation cycle /17/, which may indicate such an influence of gravity on the force-generating actomyosin system itself and on the regulation of this system, respectively. However, by providing most of the energy (ATP) and the calcium ions and thus enabling the contractile activity again the mitochondria are involved.

We have discussed 4 possibilities a cell has to perceive gravity : 4 potential gravireceptors were presented, but up to now it is nearly impossible neither to decide which way was chosen by the cell to register the gravistimulus nor to exclude other organelles totally from considerations concerning a key role in graviperception.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. K.E. Wohlfarth-Bottermann for helpful discussions and support in preparing and conducting the experiments, and Mrs. B. Bromeis for technical assistance.

REFERENCES

1. I. Block, W. Briegleb, and K. E. Wohlfarth-Bottermann, Gravisensitivity of the acellular slime mold Physarum polycephalum demonstrated on the fast-rotating clinostat, Eur. J. Cell Biol. 41, 44 (1986)
2. I. Block, W. Briegleb, V. Sobick, and K. E. Wohlfarth-Bottermann, Contraction behaviour and protoplasmic streaming in the slime mould Physarum polycephalum (Physarum kinetics), in: Biorack on Spacelab D-1, ESA SP-1091, 55 (1988)
3. A. Wolke, F. Niemeyer, and F. Achenbach, Geotactic behavior of the acellular myxomycete Physarum polycephalum, Cell Biol. Int. Repts. 11, #7, 525 (1987)
4. F. Achenbach, W. Naib-Majani, and K.E. Wohlfarth-Bottermann, Plasmalemma invaginations of Physarum dependent on the nutritional content of the plasmodial environment, J. Cell Sci. 36, 355 (1979)
5. W. Briegleb, I.Block, and A. Wolke, How does Physarum polycephalum perceive and use gravity? in: Proc. 3rd Eur. Symp. Life Sci. Res. Space, ESA SP-271, Graz, Austria 1987, p.129
6. V. Sobick and W. Briegleb, Influence of zero gravity simulation on time course of mitosis in microplasmodia of Physarum polycephalum, Adv. Space Res. 3, #9, 259 (1983)
7. E. C. Pollard, Theoretical studies on living systems in the absence of mechanical stress, J. theor. Biol. 8, 113 (1965)
8. A. Schatz and W. Briegleb, Physikalische Grundlagen und Anwendung der Og-Simulation nach H. J. Muller, DLR-FB 72-15, 169 (1973)
9. I. L. Silver, The dynamics of a discrete geotropic sensor subject to rotation-induced gravity compensation, J. theor. Biol. 61, 353 (1976)
10. K. E. Wohlfarth-Bottermann and I. Block, Function of cytoplasmic flow in photosensory transduction and phase regulation of contractile activities in Physarum, Cold Spring Harbor Symp. Quant. Biol. 46, 563 (1982)
11. W. Korohoda, Z. Shraideh, Z. Baranowski, and K. E. Wohlfarth-Bottermann, The blue-light reaction in plasmodia of Physarum polycephalum is coupled to respiration, Planta 158, 54 (1983)
12. Z. Baranowski, Z. Shraideh, W. Korohoda, and K. E. Wohlfarth-Bottermann, Consequences of impeding in mitochondrial function in Physarum polycephalum. II. Influence on "de novo" generation of contractile activities and responses to glucose and blue light, Eur. J. Cell Biol. 31, 187 (1983)
13. Y. Mito, K. Kurihara, and Y. Kobatake, Selective suppression of positive chemotaxis in Physarum polycephalum by treatment with rotenone or under anaerobic conditions, Eur. J. Cell Biol. 21, 43 (1980)
14. U. Achenbach and K. E. Wohlfarth-Bottermann, Synchronization and signal transmission in protoplasmic strands of Physarum. The endoplasmic streaming as a pacemaker and the importance of phase deviations for the control of streaming reversal, Planta 151, 584 (1981)
15. U. Achenbach and K. E. Wohlfarth-Bottermann, Synchronization and signal transmission in protoplasmic strands of Physarum. Reaction to varying temperature gradients, Planta 150, 180 (1980)
16. Z. Baranowski, Consequences of impeding in mitochondrial functions in Physarum polycephalum. III. Reversible cessation of the contraction-relaxation cycle and the temperature sensitivity of the alternate respiratory pathway, Eur. J. Cell Biol. 39, 283 (1985)
17. I. Block, W. Briegleb, and K. E. Wohlfarth-Bottermann, Influence of simulated weightlessness on the motility of the acellular slime mold Physarum polycephalum, in: Proc. 2nd Eur. Symp. Life Sci. Res. in Space, ESA SP-212, Porz-Wahn, Germany 1984, p.27