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INVOLVEMENT OF THE SECOND MESSENGER CAMP IN GRAVITY-SIGNAL TRANSDUCTION IN *PHYSARUM*

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ABSTRACT

The aim of the investigation was to clarify, whether cellular signal processing following graviperception involves second messenger pathways. The test object was a most gravisensitive free-living ameboid cell, the myxomycete (acellular slime mold) *Physarum polycephalum*. It was demonstrated that the motor response is related to acceleration-dependent changes in the levels of the cellular second messenger cyclic adenosine monophosphate (cAMP). Rotating *Physarum* plasmodia in the gravity field of the Earth about a horizontal axis increased their cAMP concentration. Depriving the cells for a few days of the acceleration stimulus (near weightlessness in a space experiment on STS-69) slightly lowered plasmodial cAMP levels. Thus, the results provide first indications that the acceleration-stimulus signal transduction chain of *Physarum* uses an ubiquitous second messenger pathway.

INTRODUCTION

The interaction of cells with their environment can be investigated using various external stimuli. Gravity effects can be conveniently studied in the gravisensitive free-living ameboid cell, the *Myxomycete* (acellular slime mold) *Physarum polycephalum*. The multi-nucleated giant cell (plasmodium) is typically several square centimeters in area and 1-2 mm thick. It is differentiated into a rear network (vein) area and a leading front zone. During its actomyosin-based locomotion (Wohlfarth-Bottermann, 1979) it uses the gravity vector for spatial orientation (gravitaxis; Wolke *et al.*, 1987). Plasmodia show, in addition, distinct gravisensitivities of the underlying cellular motion phenomena (rhythmic contraction activity, protoplasmic streaming) and morphology (asymmetry) (Block *et al.*, 1994a,b, 1996).

One approach to investigating the influence of gravity on cellular functions uses single 180° -horizontal turns. Another approach is signal deprivation, that is, actual weightlessness. Both methods were used to test whether the initial steps of the signal transduction chain, leading from the acceleration-stimulus perception to the motor response, involve the cellular second messenger cyclic adenosine monophosphate (cAMP).

MATERIAL AND METHODS

Physarum polycephalum (ATCC No. 44912) was cultured according to Daniel and Rusch (1961). Microplasmodia were allowed to fuse and grow for 2-3 days on agar to a macroplasmodium either under

normal gravity conditions ("1 g") or in actual weightlessness ("0 g", Space Shuttle Mission STS-69). Plasmodia grown at 1 g were turned "upside down" with a slow (3 s) 180°-horizontal rotation, as illustrated in Figure 1. Cells were fixed after different times in cold trichloroacetic acid (TCA)/Ethanol. The 0-g samples were grown in middeck-locker accomodated canisters (BRIC, i.e., Biological Research in Canisters) and frozen in a -196°C Gaseous Nitrogen Freezer on the second and third days of the mission. Because baseline cAMP levels in the cultures can vary, control and test samples were taken from identical cultures. Plasmodia were either processed as a whole, or front area and plasmodial network area were processed separately. cAMP concentration was determined in 3 to 5 samples 4 times using a fluorescence immunoassay (FIA). Protein concentration was determined according to Lowry *et al.* (1951). Results are expressed in pmol cAMP/mg protein as means +/-SD from the number of experiments indicated. Means were compared by using an f-test. P values of <0.05 were considered to indicate significant differences.

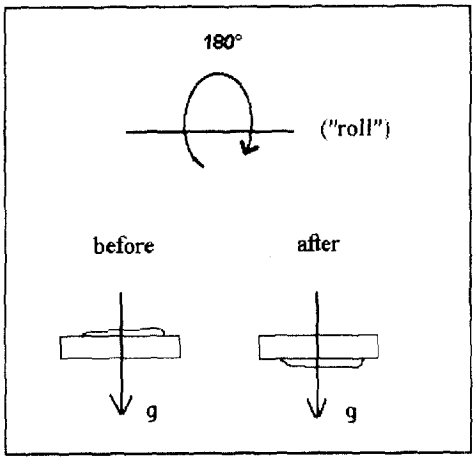

Fig. 1. Direction in which plasmodia were rotated once 180°.

RESULTS

A short-term gravistimulation of plasmodia by rotating them 180° in the gravity field of the Earth induced a slow increase in cellular cAMP levels (Figure 2): 0.5 min after rotating the cAMP levels of the stimulated cells were not markedly different from those in the controls. However, 20 and 30 min after the stimulation the cAMP levels had significantly increased. 1 hour after the horizontal turn the cAMP levels had returned to baseline values.

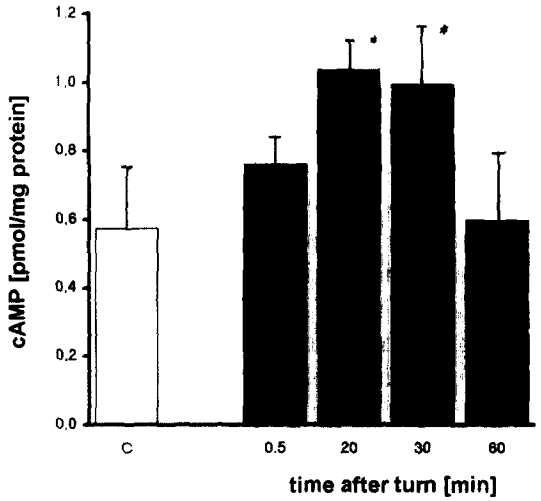

Fig. 2. Total cellular cAMP levels of *Physarum* plasmodia following a 180°-horizontal turn. cAMP concentration (pmol/mg protein) is plotted against time (min) showing +/-SD. C: control, n=5. Note the significant (*) increase in cAMP ($P < 0.02$) 20 and 30 min after the stimulation (n=5).

Whether or not the plasmodial contraction activity influences cellular cAMP levels was investigated. For this purpose non-stimulated whole plasmodia were fixed during maximum contraction as well as during maximum dilatation of the plasmodial veins. Two experiment series revealed no significant differences between the overall cAMP levels during contraction and dilatation phases (n=7; $P > 0.4$).

During prolonged weightless conditions, the overall plasmodial cAMP levels were markedly lower than in the ground controls (3 days of 0 g; n=6, $P < 0.01$). The separate processing of the plasmodial front and rear areas demonstrated for the controls the normal cAMP distribution (higher levels in the rear than in the front, Ueda *et al.*, 1986), but revealed a significant reduction in cAMP in the rear areas after 3 days of actual weightlessness (Figure 3).

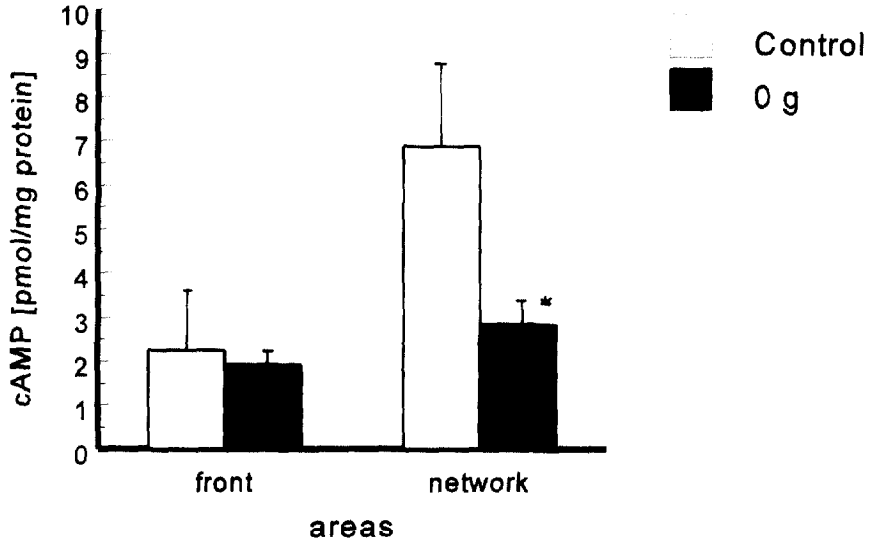

Fig. 3. Cyclic AMP levels during prolonged actual weightlessness (3 days) in the front and network (rear) areas of plasmodia. Note the significant reduction in cAMP only in the network areas (n=3; +/-SD; for network $P < 0.04$).

DISCUSSION

The presented results suggest that a short-term gravity stimulation (180°-horizontal turn) transiently increases the cellular levels of the second messenger cAMP. A comparison with the work of other authors (Garrison and Barnes, 1980; Ueda *et al.*, 1988; Akitaya *et al.*, 1984) shows that the applications of light and chemical stimuli to *Physarum* raised plasmodial cAMP levels within 15 min by the same factor as gravity stimulation did within 20 min.

As the plasmodial contraction activity does not influence total cellular cAMP levels, the observed variations in total cAMP concentrations following gravity stimulation can be attributed to stimulus-processing mechanisms. The weightlessness studies indicate a decrease in cAMP levels during prolonged acceleration-stimulus free conditions restricted to the plasmodial network - the motive-force generating part of the cell.

The results indicate that in *Physarum polycephalum* acceleration-stimulus signal transduction might use or

even partly shares the ubiquitous cAMP second-messenger pathway comparable to other stimulus signal transduction chains.

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