vivo and in vitro) this percentage is reached after 3 h; however, the rate of LH release in vitro does not decline.

Discussion. During continuous stimulation with LH–RH the LH secretion rate in vivo displays the characteristic multiphasic pattern as described before[13]. Similar patterns have been recognized in rats and other species including man[15]. However, these patterns were generally considered to be biphasic: a first phase of low response for 1–2 h (here phases I and II) and a second phase of increased response followed by desensitization (here phases III and IV). The significance of the very early short phase of relatively increased LH release (representing only a very small amount of LH) observed in vivo (phase I) and also in vitro during more frequent sampling[3–5] is not clear. It is unlikely, however, that it represents the first phase of the biphasic response of the human studies described above.

The present study shows that the overall patterns of LH secretion rates in vivo and in vitro fit reasonably well, although there are some differences in the timing of events: the occurrence of phases III and IV is retarded. These differences are probably not the result of poor penetration of LH–RH into the inner area of the glands, since the heights of the phase II responses in vivo and in vitro are similar, indicating an equally efficient induction of LH release. Moreover, pretreatment of the pituitary glands with LH–RH in vivo failed to cause a clear desensitization during subsequent incubation with LH–RH, even if the same percentage of LH had been released as in vivo at the time desensitization had become apparent. Therefore, the delay in occurrence of phases III and IV is rather the result of less efficient processing of LH release under in vitro conditions. A similar phenomenon has been observed in both static incubation[7,8,16] and perifusion[17] experiments with hemi-pituitary glands from OVX rats. Taking these results together, they may point to environmental differences between in vivo and in vitro conditions influencing the efficacy of processing the increment (phase III) and subsequent decrease (phase IV) in the pituitary LH response to LH–RH. Moreover, comparing previous data from Schuiling et al.[18], who also used hemi-pituitary glands from OB-pretreated rats, with the present data shows that perifusion experiments may procure an even better resemblance to LH secretion patterns obtained in vivo, indicating that agents released by the pituitary gland itself interfere in the efficacy of the action of LH–RH.

In conclusion the present results show that the overall patterns of LH secretion rates in vivo and in vitro (using hemi-pituitary glands) fit reasonably well. Allowing for the timing differences, the results indicate that in vitro studies of biochemical aspects of the LH secretion mechanism using hemi-pituitary glands provide results which are pertinent to the in vivo situation.

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0014-4754/86/010060-03$1.50 + 0.20/0

Increase in cellular cyclic GMP level by potassium stimulation and its relation to ciliary orientation in *Paramecium*

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Summary. Physiological roles of cyclic GMP in the control of ciliary movement in *Paramecium caudatum* were investigated. We found that 1) an increase in cellular cyclic GMP level was observed in association with recovery from the ciliary reversal produced by K stimulation, and 2) the presence of cyclic GMP inhibited the Ca-induced ciliary reversal in triton-extracted models. These results suggest that cyclic GMP plays a key role in the control of the Ca-mediated ciliary reversal mechanism.
Key words. Cyclic GMP; calcium; *Paramecium;* triton-extracted model; ciliary reversal; excitable membrane.

Recent biochemical studies revealed that cilia of ciliate protozoa contain various proteins and enzymes which interact with Ca^{2+} and cyclic nucleotides, such as Ca/calmodulin-regulated guanylate cyclase[2], adenylate cyclase[3], cGMP and cAMP-dependent protein kinases[4]. Many investigators demonstrated that Ca^{2+} is a mediator of ciliary reversal in ciliate protozoa. Triton-extracted models of *Paramecium* exhibit ciliary reversal when the Ca^{2+} concentration of the reactivation medium is raised above a certain threshold level (10^{-6}–10^{-5} M)[5]. In live specimens of *Paramecium* ciliary reversal always occurs in association with an entry of Ca^{2+} into cilia through activated voltage-dependent Ca channels in the ciliary membrane[6,7]. In order to understand the role of cyclic nucleotides in the Ca-mediated control of ciliary movement in *Paramecium*, we measured the amount of cellular cGMP in relation to ciliary reversal produced by K stimulation.

Materials and methods. Wild-type *Paramecium caudatum* (stock G3, syngen 3, mating type V, trichocyst nondischarge) was cultured at 25 °C in bacterized lettuce infusion. Cells were harvested in the early stationary phase of growth by filtration, and incubated for at least 2 h in an equilibration medium containing 0.5 mM KCl, 1.0 mM $CaCl_2$ and 1.0 mM Tris-HCl (pH 7.2). A 24-well tissue culture cluster (Costar, No. 3524) was used for the stimulation experiments. 0.25 ml of cell suspension containing about 600 cells was introduced into each well of the culture

Effect of cGMP and cAMP on the swimming direction of triton-extracted models of *Paramecium*

Tube number	Additions to reactivation medium* $CaCl_2$ (mM)	cGMP (μM)	cAMP (μM)	Swimming direction
1	0	0	0	Forward
2	3.0	0	0	Backward
3	3.0	100	0	Forward
4	3.0	0	100	Forward

*Reactivation medium contained 4.0 mM ATP, 4.0 mM $MgSO_4$, 3.0 mM EGTA, 36 mM KCl and 20 mM MOPS-KOH (pH 7.0).

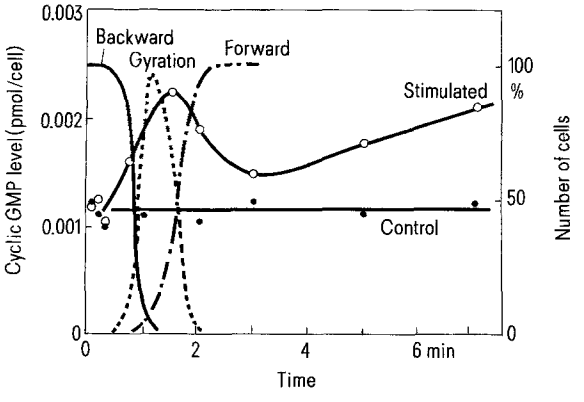

Figure 1. Changes in cellular cGMP level and behavioral responses of *Paramecium* induced by a stepwise increase in [K] from 0.5 to 20 mM. K^+ concentration was raised by adding K^+-rich stimulation medium to the cell suspension (open circles). In control experiments cGMP level was measured after adding the equilibration medium to the cell suspension (closed circles). The number of cells which show a certain type of behavioral response is shown as a percentage of the total number of cells in a suspension. Solid line without symbols; backward swimming. Dotted line; gyration. Half dotted line; forward swimming.

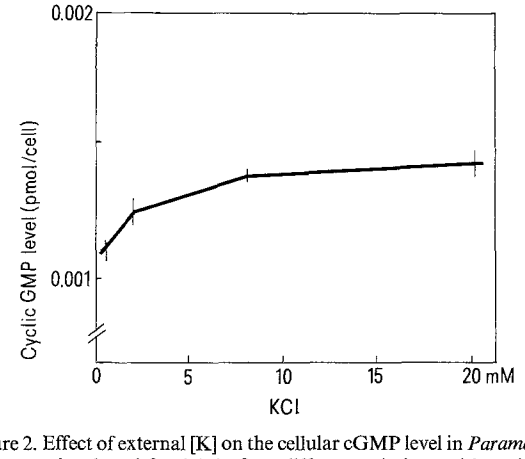

Figure 2. Effect of external [K] on the cellular cGMP level in *Paramecium*. Cells were incubated for 3 h in four different solutions with varied K^+ concentration (0.5, 2, 8 and 20 mM). Ionic compositions of each solution other than K^+ ion are: 1.0 mM $CaCl_2$, 1.0 mM Tris-HCl (pH 7.2).

cluster. After keeping the suspension in the well for 30 min, 0.25 ml of the stimulation medium containing 40 mM KCl, 1.0 mM $CaCl_2$ and 1.0 mM Tris-HCl (pH 7.2) was added to each well to stimulate the cells. After keeping the cells in the stimulation medium for various periods of time, 0.5 ml of 0.2 N HNO_3 was added to each well for extraction of cGMP and cAMP from the cells. The medium in the well was neutralized with 0.5 ml of 0.18 N KOH–0.4 M $NaHCO_3$[8]. 0.1 ml of each neutralized medium

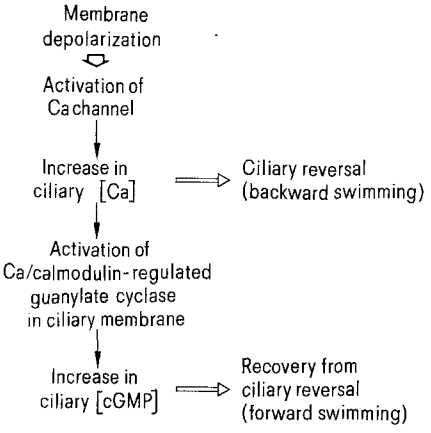

Figure 3. Schematic presentation of physiological roles of Ca^{2+} and cGMP in the control of ciliary orientation.

was taken into an assay tube (each tube contained extract from about 40 cells) and cyclic nucleotide in the sample was acetylated. Amount of cyclic nucleotide was determined by the radioimmunoassay method using New England Nuclear RIA Kit, NEX-132 and NEX-133. (Cross-reactivities of antibodies used with other nucleotides were less than 0.01%. These kits were sensitive to 0.01–0.1 pmoles of acetylated cyclic nucleotides per assay tube.) Behavior of the cells was microscopically observed before and during the stimulation. Triton X-100-extracted models of *Paramecium* were prepared by the method of Naitoh and Kaneko[5] with a slight modification (MOPS was used instead of Tris). All experiments were performed at room temperature, between 20° and 25 °C.

Results and discussion. 1) Change in cellular cGMP level by K stimulation. *Paramecium* shows several types of behavioral responses when it is transferred into a high K solution[9]. First, it exhibits long-lasting backward swimming (continuous ciliary reversal), then gyration, and finally it regains forward swimming. In order to find out the correlation between cellular cGMP level and the behavioral responses, cellular cGMP content was measured in different groups of specimens of *Paramecium* showing different behavioral responses after stimulation of the cells by a high K solution. K^+ concentration was increased from 0.5 to 20 mM for stimulation, while Ca^{2+} concentration was kept constant at 1.0 mM. The membrane of *Paramecium* is known to be markedly depolarized by increases in K^+ concentration[6,7,9]. As shown in figure 1, immediately after the K stimulation, cells exhibited a long-lasting backward swimming. The number of specimens showing backward swimming decreased in the first 30 s after K stimulation. 40–80 s after stimulation, the number of cells showing gyration increased. Some of them began to swim forward again within 60 s after stimulation. By about 90 s after stimulation, half of the cells had begun to swim forward. Cellular cGMP level began to increase after the stimulation with a latent period of about 10 s, and reached its maximum in about 90 s. Then the level began to decrease to its initial level within the next few minutes and increased again gradually. The cellular cGMP level before the K stimulation was 0.001 pmoles/cell as shown in figure 1. The concentration of cGMP in the cilia was estimated to be 2.5 μM. The value was calculated on the basis of the assumption that the cGMP level in the cilia before the stimulation is equal to that in the general cytoplasm. The volume of the cell was estimated to be 4×10^{-10} l, which was calculated by assuming that *Paramecium* is a cylinder of 0.05 mm in diameter and 0.2 mm in length. We also measured the change in cellular cAMP level resulting from K stimulation. We found that the level was about 0.2 fmoles/cell and decreased by less than 10% after K stimulation.

The steady state cellular cGMP level also depended on K^+ concentration in the equilibration medium. In the next series of experiments the cellular cGMP level was determined in four groups of specimens, each of which was incubated for 3 h in four solutions with different K^+ concentrations (0.5, 2, 8 and 20 mM) respectively. As shown in figure 2 the cGMP level increased with increasing K^+ concentration. Schultz et al. observed only a slight increase in cGMP after K^+ stimulation[10]. The relative K^+ concentration ($[K]/\sqrt{[Ca]}$) of the experimental solutions employed by them was rather high (they changed the KCl concentration from 5 to 20 mM in the presence of 0.05 mM $CaCl_2$). Therefore it is conceivable that the cGMP content before stimulation was already too high to detect any change in cellular cGMP level resulting from stimulation.

2) Effect of cGMP on ciliary activities in triton-extracted models. In order to examine the direct effect of cGMP on the ciliary motile mechanism, we observed the locomotion of triton-extracted models of *Paramecium* reactivated by ATP and Mg^{2+} in the presence of cGMP (table). As reported by Naitoh and Kaneko[5], the models swam forwards in the reactivation medium containing 3.0 mM EGTA, in which free Ca^{2+} concentration was less than 10^{-7} M (tube 1). The models began to swim backwards when free Ca^{2+} concentration in the reactivation medium was raised up to 50 μM by adding 3.0 mM $CaCl_2$ to the reactivation medium (tube 2)[11]. The models, however, swam forward even in the presence of 50 μM free Ca^{2+} if 100 μM cGMP was present in the reactivation medium (tube 3). Addition of cAMP also produced forward swimming of the models in the presence of 50 μM of free Ca^{2+} (tube 4). The production of forward swimming was observed even in the presence of 10 μM cGMP or cAMP. Forward swimming velocity was always higher when cyclic nucleotides were present in the reactivation medium. These results are consistent with those obtained by Nakaoka[12].

3) Possible role of cGMP in the control of ciliary activities. We found that both cGMP and cAMP antagonized the action of Ca^{2+} in producing ciliary reversal in triton-extracted models. In the living cells, however, only cGMP level showed a positive correlation with the recovery from ciliary reversal caused by K stimulation. Since the voltage-dependent Ca channels are located only in the ciliary membrane[13,14], the increase in cGMP level is thought to occur only in the cilia due to activation of Ca/calmodulin-regulated guanylate cyclase by Ca^{2+} taken up into the cilia through the activated Ca channels. On the basis of these facts, we propose a possible mechanism by which ciliary orientation is controlled by membrane electric events (fig. 3). Ca^{2+} taken up into the cilia through the Ca channels activated by membrane depolarization may perform a double function; 1) activation of the ciliary reversal mechanism and 2) activation of guanylate cyclase. Since the ciliary reversal takes place promptly after K stimulation, Ca^{2+} seems to interact directly with the reversal mechanism. The activation of guanylate cyclase in the ciliary membrane causes an increase in the intraciliary cGMP concentration. The increase in cGMP causes recovery of cilia from the reversed state probably through activation of a cGMP-dependent protein kinase present in the cilia[4]. Participation of the Ca-activated enzymatic systems in the recovery from ciliary reversal is consistent with the delay observed in the recovery from ciliary reversal induced by K stimulation.

It is well known that membrane hyperpolarization produces acceleration of forward swimming[7]. Schultz et al. reported that an increase in cellular cAMP level relates to the acceleration of forward swimming[10]. We found that forward swimming velocity in the reactivated triton-extracted models was always higher when cAMP was present in the reactivation medium. These facts suggest involvement of cAMP in the mechanism governing beating frequency of cilia in the normal direction.

1 We would like to express our appreciation to Prof. Y. Naitoh, University of Tsukuba, for his valuable discussions during the course of this work and critical reading of this manuscript. This work was supported in part by grants to T.A. from Ministry of Education, Science and Culture of Japan.

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0014-4754/86/010062-03$1.50 + 0.20/0

Superoxide dismutase activity during the plasmodial life cycle of *Physarum polycephalum*

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Summary. Superoxide dismutase activity was slow throughout the cell cycle of surface cultures of *Physarum polycephalum*. This activity increased markedly when the organism was induced to spherulate. Glutathione (GSH) and hydrogen peroxide (H_2O_2) concentrations changed very little during the cell cycle. During spherulation GSH decreased; H_2O_2 and the cyanide-resistant respiration of plasmodial homogenates increased.

Key words. Physarum polycephalum; cell cycle; superoxide dismutase; differentiation.

Recently we reported that the specific activity of superoxide dismutase (SOD; EC 1.15.1.1) in the slime mold, *Physarum polycephalum*, increases dramatically during the starvation-induced spherulation of microplasmodial cultures[1]. The increase in activity was first observed 6 h after transfering the organism from nutrient medium to a salts-only starvation medium[2]; the activity then continued to increase throughout the course of differentiation until a 21-fold enhancement of the original activity was attained. As SOD activity increased the product of its catalysis, hydrogen peroxide (H_2O_2), also increased and the intracellular level of glutathione (GSH) decreased. We have since observed that the activity of the mangano-isozyme of this en-