Acta Protozool. (2001) 40: 153 - 157

ACTA
PROTOZOOLOGICA

Short Communication

Alterations of Photophobic Motile Response in *Stentor* by Cyclic Guanosine 3', 5'- Monophosphate-elevating Agents

Mirosława WALERCZYK and Stanisław FABCZAK

Department of Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa, Poland

Summary. The effects of exogenous cyclic nucleotides and modulators of the cyclic nucleotide signaling pathway on the step-up photophobic response have been studied in the ciliate *Stentor coeruleus*. To attain this, the latency time for the ciliary stop response and the ciliary reversal duration has been estimated using microscope videorecording and dark-field macro-photography. Membrane-permeable, slowly hydrolyzable analogs of cyclic GMP, 8-Br-cGMP and db-cGMP, significantly increase the latency time of ciliary stop response and decrease the period of ciliary reversal. By contrast, cyclic AMP analogs, 8-Br-cAMP or db-cAMP, had no measurable effect on the photophobic response parameters. The effects of the exogenous cGMP are mimicked by the application of the non-specific PDE inhibitor IBMX or zaprinast, a specific cGMP-PDE inhibitor. These behavioral observations suggest that *in vivo*, the internal level of presumed cGMP, but not cAMP, is possibly a limiting factor for the time course of the motile avoiding response of *Stentor*, and they further support the hypothesis that cGMP metabolism may play a role in photosignal transduction in these cells.

Key words: cGMP phosphodiesterase, ciliary stop and reversal response, cyclic nucleotide analogs, cyclic nucleotide phosphodiesterase inhibitors, photophobic response, phototransduction, *Stentor coeruleus*.

Abbreviations: cAMP = adenosine 3', 5'- cyclic monophosphate; 8-Br-cAMP = 8-bromo-cAMP; db-cAMP = N^6, 2'-o-dibutyryl-cAMP; cGMP = guanosine 3', 5'- cyclic monophosphate; 8-Br-cGMP = 8-bromo-cGMP; db-cGMP = N^6, 2'-o-dibutyryl-cGMP; DMSO = dimethyl sulfoxide; PDE = cyclic nucleotide phosphodiesterase; IBMX = 3-isobutyl-l-methylxanthine; zaprinast = 1,4-dihydro-5- [2-propoxyphenyl]-7H-1,2,3-triazolo [4,5-d]pyrimidine-7-one.

INTRODUCTION

The avoidance of an illuminated area by ciliate *Stentor coeruleus* results from a step-up photophobic response (Tartar 1961). This motile response may be elicited by a spatial or temporal increase in light intensity and it consists of a delayed stop of forward swimming, a period of ciliary reversal during which cell moves backwards and turns to one side, and subsequent stop of movement followed by resumption of forward swimming in a new direction (Wood 1976, Song *et al.* 1980, Fabczak *et al.* 1993b). The ciliary reversal in *Stentor* is caused, as in other ciliates, by an action potential, triggered, in turn, by a delayed depolarizing photoreceptor potential (Wood 1976, Fabczak *et al.* 1993b). The generation of receptor potential is a consequence of light absorption by numerous blue-green subpellicular pigment granules, arranged in longitudinal strips between

Address for correspondence: Stanisław Fabczak, Department of Cell Biology, Nencki Institute of Experimental Biology, ul. Pasteura 3, PL-02-093 Warszawa, Poland; Fax: (4822) 822 53 42; E-mail: sfabczak@nencki.gov.pl

each pair of ciliary rows (Tartar 1961, Tao *et al.* 1994). These granules contain stentorin, a hypericin derivative, as a chromophore (Møller 1962, Tao *et al.* 1994, Song 1995). At present, little is known about functional link between photoreceptors and the bioelectrical events preceding the observed photomotile behavior of *Stentor*. In preliminary studies, we suggested an existence a signaling chain in *Stentor*, which may involve cGMP as a second messenger (Fabczak *et al.* 1993a). In this paper, we have used a microscope videorecording system and pharmacological approach to further investigate the role of cGMP metabolism in the light-dependent motile responses in the ciliate *Stentor coeruleus*.

MATERIALS AND METHODS

Stentor coeruleus ciliates were grown in 0.5 l Petri dishes in a manner previously described (Koprowski *et al.* 1997). For cell harvesting, a chosen dish was placed on a dark or moderate illuminated background and then it was lighted from one side. In a short time, most ciliates collect at the side opposite to the light source (negative photodispersal effect). Thus, accumulated cells were transferred with a pipette to fresh culture medium devoid of nutritional components, referred to as a control medium. The selected cell sample was then placed in an appropriate solution filling a test chamber, located on a microscope stage. Each recording session of cell movements was performed usually after 60 min of cell adaptation to darkness and applied test solution. A constant temperature of 22°C of the solution filling recording chamber was maintained automatically throughout the experiment by an electronic device, based on a semiconductor Peltier element (Fabczak 1990). Test solutions containing derivatives of cGMP or cAMP were prepared simply by its addition to control medium, prepared daily from stock solution. Cyclic nucleotide PDE inhibitors, IBMX and zaprinast, were first dissolved in ethanol and DMSO, respectively, and then added to the control solution. The maximal final concentrations of both solvents in test solutions were 0.1% and the same amount of the solvents was included in the control solution for each series of experiments. All cyclic nucleotide analogs, 8-Br-cGMP, db-cGMP, 8-Br-cAMP and db-cAMP and IBMX were purchased from Sigma, whereas zaprinast was obtained from Alexis. All chemicals were of analytical reagent grade.

The photostimulation and video recordings of cell swimming pattern were carried out with methods described elsewhere (Fabczak *et al.* 1994, Fabczak 2000a). The effects of the tested substances on cell photobehavior were achieved by measurements of photophobic response parameters, such as latency of stop response (T_{CRL}, time between cell entry into light spot from a dark background and stop of cell forward swimming) and duration of backward swimming (T_{CRD}, time of ciliary reversal) expressed as percentage change in relation to the control values (taken arbitrarily as 100%).

A dark-field and low-magnification photography was used for an illustration of cell motility pattern under different experimental conditions (Ferguson 1957, Dryl 1958).

RESULTS AND DISCUSSION

To test the effects of cyclic nucleotides on cell photophobic movement, both the T_{CRL} and T_{CRD} parameters of photophobic responses were evaluated in cells exposed to dibutyryl- or 8-bromo-substituted cyclic nucleotide analogs, 8-Br-cAMP and 8-Br-cGMP or db-cAMP and db-cGMP. These compounds, being lipophilic analogs of cAMP and cGMP, possess considerable membrane permeability and are relatively poorly hydrolyzed by cyclic nucleotide PDEs (Braumann and Jastorff 1985, Zimmerman *et al.* 1985, Krass *et al.* 1997). When 8-Br-cGMP or db-cGMP analogs in final concentrations between 0.25 and 2.00 mM were applied to *Stentor via* bath addition, the time course of photophobic response underwent significant alterations in a dose-dependent manner (Fig. 1). In cells treated by 2 mM 8-Br-cGMP for 5 min, the T_{CRL} value was increased two-fold and T_{CRD} reduced by about 73% compared with controls (Fig. 1A). As illustrated in Fig. 3A-D, the inhibitory effect of cGMP was larger with prolongation of incubation time. The influence of db-cGMP analog on the cell response latency or ciliary reversal duration was also very large (Fig. 1B) but somewhat lower than that of 8-Br-cGMP, probably due to differences in membrane permeability or metabolic turnover of a particular cyclic nucleotide analog by PDEs. In contrast, both 8-Br-cAMP and db-cAMP analogs were ineffective at the same applied concentrations and incubation.

These results demonstrate that application of membrane-permeable cGMP analogs, which are expected to increase intracellular cGMP levels, significantly modulates the light avoiding motile behavior of *Stentor* cells. The negative results obtained using membrane-permeable cAMP analogs serve, as a control, to evidence the specificity of the action of cGMP on cell photobehavior. These might indicate that, *in vivo,* the level of presumed cGMP, but not cAMP, in *Stentor* is perhaps a limiting factor for the cGMP signaling pathway and increased cGMP levels might suppress the signaling chain efficiency. This might demonstrate a direct or indirect causal connection between the kinetics of the signaling process and time course of the cell motile response to light. A slower processing of light signals resulting from cGMP accumulation could lengthen the T_{CRL} and decrease T_{CRD} periods. On the other hand, a higher efficiency of signal pathway could lead to a shortening and prolongation of the T_{CRL} and T_{CRD} response parameters,

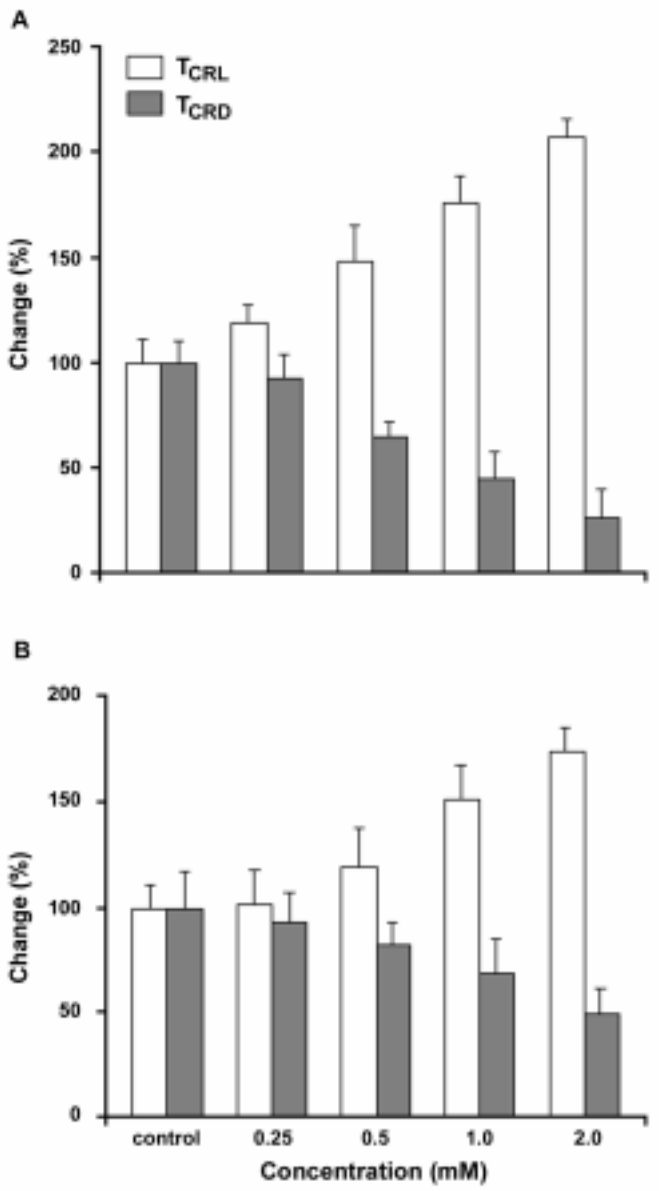

Fig. 1. Effect of 8-Br-cGMP (A) and db-cGMP (B) analogs on photophobic response elicited with standard white light stimuli (5 x 10^{-4} Wcm^{-2}). The ciliary stop response latency (T_{CRL}) and duration of backward swimming (T_{CRD}) were determined in dark-adapted cells in control medium and in this medium supplemented with cGMP analogs at the indicated concentrations. All data (in percent) are given as mean ± S.E of measurements on 60-80 cells

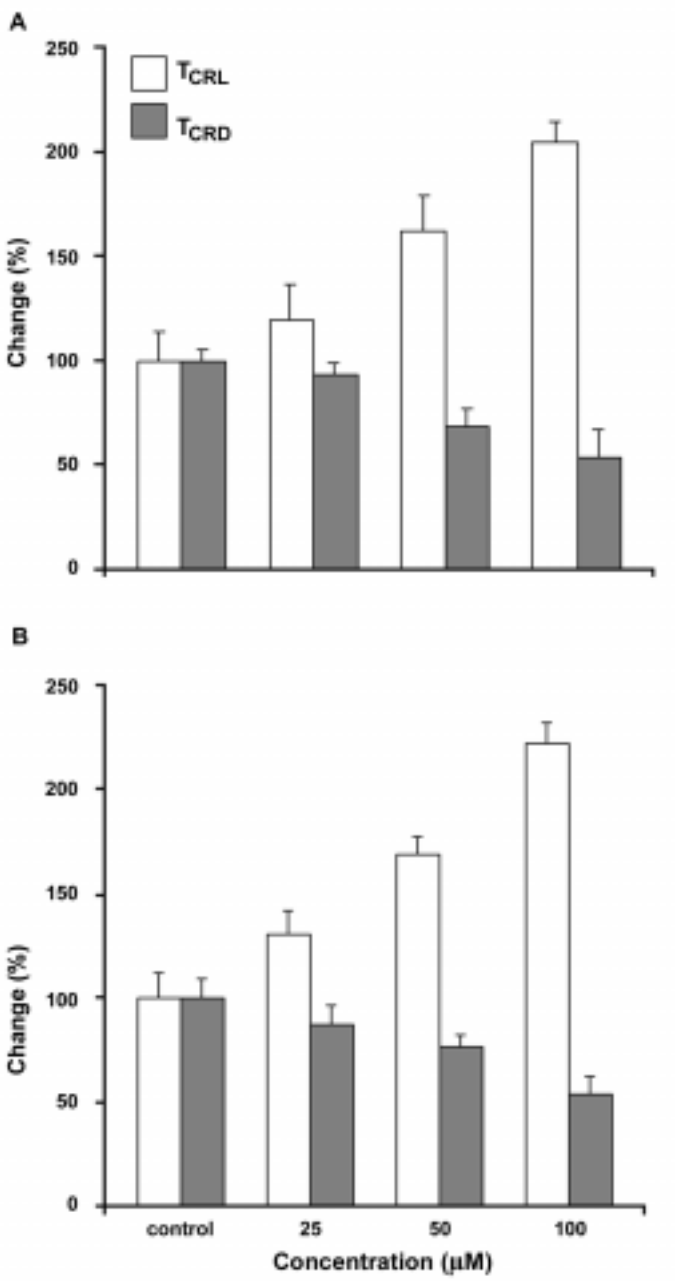

Fig. 2. Effect of IBMX (A) and zaprinast (B) on photophobic response. The T_{CRL} and T_{CRD} values were estimated in control medium including appropriate solvent concentrations (ethanol or DMSO) and in medium supplemented with IBMX and zaprinast at the indicated concentrations. Other details as for Fig. 1

respectively. This closely resembles earlier behavioral observations on the reciprocal relationship between the response latency and duration of ciliary reversal in *Stentor*, when photophobic responses were elicited with light stimuli of variable intensity (Fabczak *et al.* 1993a). Thus, the photophobic response of prolonged T_{CRL} and decreased T_{CRD} can be obtained under both experimental circumstances - when signal turnover is disturbed by an application of cGMP-elevating agents, as found in the present experiments, or during cell stimulation with light stimuli of low fluence rates, as observed earlier (Fabczak *et al.* 1993a).

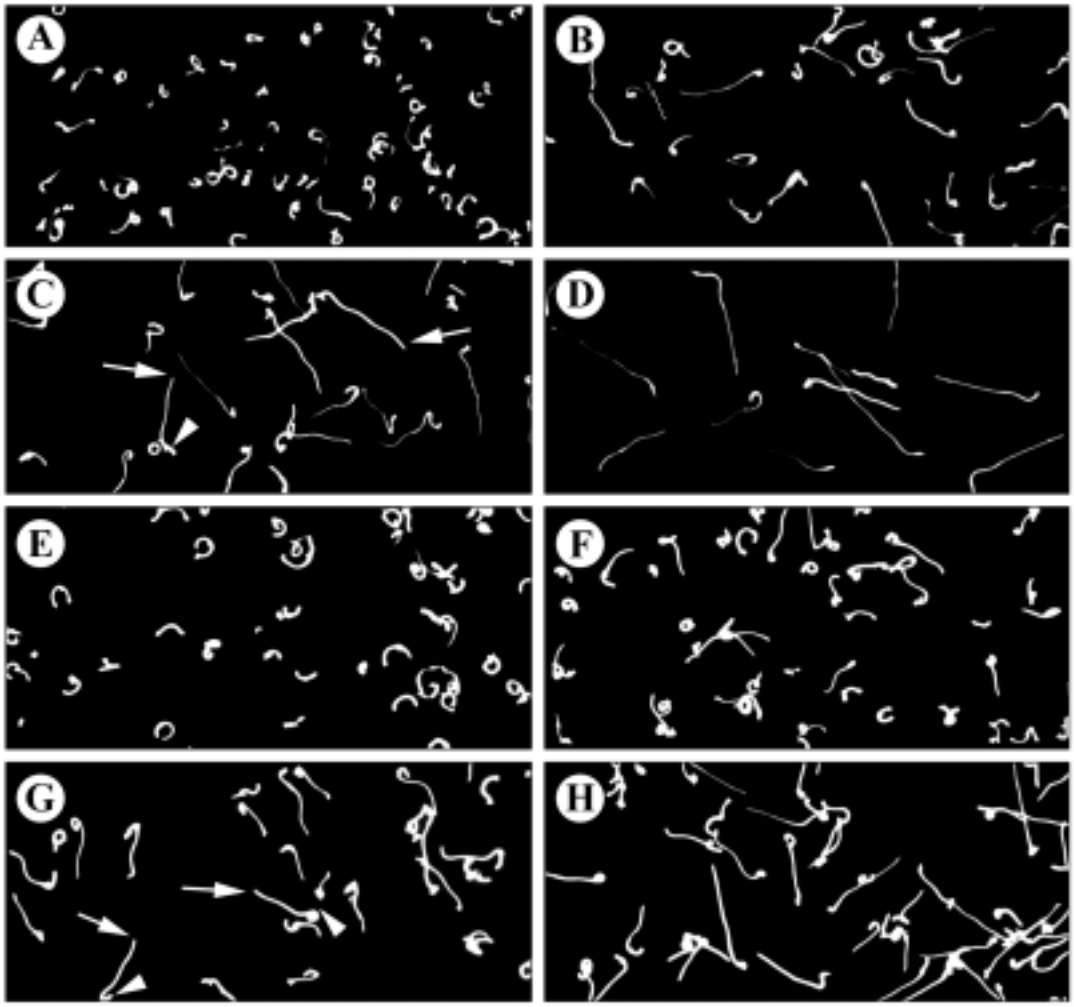

Fig. 3. Dark-field and low-magnification photographs of *Stentor* photobehavior (motility tracks) under different experimental conditions. The photophobic response of dark-adapted cells to standard light stimulus in control medium (A) and in the presence of 3 mM 8-Br-cGMP analog (B-D) applied for 1 min (B), 10 min (C) and 30 min (D) or in control medium supplemented with 0.1% ethanol (E) and in the presence of 25 μM IBMX (F-H) applied for 1 min (F), 10 min (G) and 30 min (H). Arrows and arrowheads in (C) or (G) indicate the onset of light stimulus and ciliary reversal, respectively. The photographs were taken with an exposure time of 6 s

To verify whether *Stentor* cells contain an endogenous enzymatic pathway for cGMP metabolism, we externally applied a non-specific PDE inhibitor, IBMX and a specific cGMP PDE inhibitor, zaprinast (Amer and Kriegbaum 1975, Goldberg and Haddox 1977, Turko *et al.* 1999). IBMX and zaprinast are known to effectively raise the intracellular concentration of cGMP, as a consequence of inhibition of cGMP degradation by PDE, when cGMP synthesis catalyzed by a guanylate cyclase remains unaffected (Goldberg and Haddox 1977, Burns *et al*. 1992). Both of these agents applied at micromolar concentrations (25-100 μM) modulated T_{CRL} similarly to the effects of cGMP analogs (Fig. 2). An application of IBMX (Fig. 2A) or zaprinast (Fig. 2B) at a concentration of 100 μM extended the T_{CRL} two-fold and more than 2,2-fold, respectively, whereas T_{CRD} was decreased in both cases by about 46% compared with those photoresponse parameters in appropriate control solutions (Figs. 2A,B). As in the case of cGMP analogs, an increase in the incubation time caused more pronounced inhibitory effects of IBMX (Fig. 3E-H). No toxic effects for either drug were observed, as judged by the continuing regular cell swimming at the inhibitor concentrations used. These observations indicate that a decrease in PDE activity in *Stentor* may cause changes in cytoplasmic cGMP levels and, as a consequence, the photophobic response coupled to the cGMP messenger might be suppressed.

Since the inhibitory effects of the used compounds, cGMP analogs and antagonists to cyclic nucleotide PDE are presumably due to the accumulation of internal cGMP in *Stentor*, the decrease in cellular cGMP concentration is considered to be implicated in the cell phobic response to light stimuli. This supposition is consistent with the recently reported preliminary results of *in vivo* estimation of endogenous cyclic nucleotide levels in *Stentor* cells using highly specific cyclic nucleotide radioimmunoassay (Walerczyk *et al.* 2000, Walerczyk and Fabczak 2001). These experiments evidently show that, in dark-adapted cells following illumination, a decrease in intracellular cGMP level occurs. The measured alterations in cGMP levels were observed to be suppressed strongly by cyclic nucleotide PDE inhibitors, such as IBMX or theophylline (Darcy and Fisher 1990). Moreover, a presence of cGMP-activated channels and 63 kD protein that specifically bind cGMP were confirmed in ciliate cortex fraction with an electrophysiological patch-clamp and immunochemical methods, respectively (Fabczak 2000b, Walerczyk *et al.* 2000). Also in *Stentor* cells, a transducin-like G-protein was detected using α-transducin antibody (Fabczak *et al.* 1993a). Based on the presented findings, it appears that the photosensitive unicellular organism *Stentor, as* in olfactory- and photo-receptor cells (Fesenko *et al.* 1985, Nakamura and Gold 1987), possess biochemical machinery that involve events from light absorption by photoreceptor pigments (hypericin-like stentorin) *via* G-protein-coupled phosphodiesterase activation to directly modulate membrane cGMP-gated ion channels.

Acknowledgments. This work represents a part of the Ph. D. thesis project (of M. W.) and was supported by Committee for Scientific Research Grant 6-P04C-011-19 (to S. F.).

REFERENCES

Amer M. S., Kriegbaum W. E. (1975) Cyclic nucleotide phosphodiesterase properties: Activators, inhibitors, structure-activity relationship and possible role in drug development. *J. Pharmacol. Sci.* **64:** 1-64

Braumann T., Jastorff B. (1985) Physicochemical characterization of cyclic nucleotides by reversed phase high-performance liquid chromatography. II. Quantitative determination of hydrophobicity. *J. Chromatogr.* **350:** 105-118

Burns F., Rodger I. W., Pyne N. J. (1992) The catalytic subunit of protein kinase A triggers activation of the type V cyclic GMP-specific phosphodiesterase from guinea-pig lung. *Biochem. J.* **283:** 487-491

Darcy P. K., Fisher P. R. (1990) Pharmacological evidence for a role for cyclic AMP signaling in *Dictyostelium discoideum* slug behavior. *J. Cell Sci.* **96:** 661-887

Dryl S. (1958) Photographic registration of movement of protozoa. *Bull. Acad. pol. Sci. Cl. II Sér. Sci. biol.* **6:** 429-430

Fabczak H. (2000a) Contribution of phosphoinositide-dependent signaling to photomotility of *Blepharisma* ciliate. *J. Photochem. Photobiol.* **55:** 120-127

Fabczak H. (2000b) Protozoa as a model system for studies of sensory light transduction: mechanism of photophobic response in the ciliate *Stentor* and *Blepharisma. Acta Protozool.* **39:** 171-181

Fabczak H., Park P. B., Fabczak S., Song P.-S. (1993a) Photosensory transduction in ciliates. II. Possible role of G-protein and cGMP in *Stentor coeruleus. Photochem. Photobiol.* **75:** 702-706

Fabczak S. (1990) Free potassium and membrane potential in cells of *Blepharisma japonicum. Acta Protozool.* **29:** 179-185

Fabczak S., Fabczak H., Tao N., Song P.-S. (1993b) Photosensory transduction in ciliates. I. An analysis of light-induced electrical and motile responses in *Stentor coeruleus. Photochem. Photobiol.* **57:** 696-701

Fabczak S., Fabczak H., Song P.-S. (1994) Ca^{2+} ions mediate the photophobic response in *Blepharisma* and *Stentor. Acta Protozool.* **33:** 93-100

Ferguson M. L. (1957) Photographic technique for quantitative physiological studies of *Paramecium* and other motile cells. *Physiol. Zool.* **30:** 208-215

Fesenko E. E., Kolesnikov S. S., Lyubarski A. L. (1985) Induction of cyclic-GMP of cation conductance in plasma membrane of retinal rod outer segment. *Nature* **313:** 310-313

Goldberg N. D., Haddox M. K. (1977) Cyclic GMP metabolism and involvement in biological regulation. *Annu. Rev. Biochem.* **46:** 823-896

Koprowski P., Walerczyk M., Groszyńska B., Fabczak H., Kubalski A. (1997) Modified patch-clamp method for studying ion channels in *Stentor coeruleus*. *Acta Protozool.* **36:** 121-124

Krass J. D., Jastorff B., Genieser H.-G. (1997) Determination of lipophilicity by gradient elution high-performance liquid chromatography. *Anal. Chem.* **69**: 2575-2581

Møller K. M. (1962) On the nature of stentorin. *Comp. Rend. Trav. Lab. Carlsberg* **32:** 471-497

Nakamura T., Gold G. H. (1987) A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* **325:** 442-444

Song P.-S., Hader D.-P., Poff K. L. (1980) Step-up photophobic response in the ciliate *Stentor coeruleus. Arch. Microbiol*. **126:** 181-186

Song P.-S. (1995) The photomechanical responses in the unicellular ciliates. *J. Photosci.* **2:** 31-35

Tao T., Deforce L., Romanowski M., Meza-Keuthen M. S., Song P.-S., Furuya M. (1994) *Stentor* and *Blepharisma* photoreceptors: structure and function. *Acta Protozool*. **33:** 199-211

Tartar V. (1961) The Biology of *Stentor.* In: International Series of Monographs on Pure and Applied Biology, (Ed. G. A. Kerkut), Pergamon Press, Oxford, London, New York, Paris, **5:** 11-25

Turko I. V., Ballard S. A., Francis S. H., Corbin J. D. (1999) Inhibition of cyclic GMP-binding cyclic GMP-specific phosphodiesterase (Type 5) by sildenafil and related compounds. *Mol. Pharmacol.* **56:** 124-130

Walerczyk M., Fabczak H., Fabczak S. (2000) Cyclic cGMP-gated channels are present in ciliate *Stentor coeruleus*. *Post. Hig. Med. Doświad*. **54:** 329-339

Walerczyk M., Fabczak S. (2001) Pharmacological evidence for a role for cyclic GMP signalling in *Stentor coeruleus* motile behavior. *Photochem. Photobiol.* (in press)

Wood D. C. (1976) Action spectrum and electrophysiological responses correlated with the photophobic response of *Stentor coeruleus. Photochem. Photobiol*. **24:** 261-266

Zimmerman A. L., Yamanaka G., Eckstein F., Baylor D. D., Stryer L. (1985) Interaction of hydrolysis-resistant analogs of cyclic GMP with the phosphodiesterase and light sensitive channel of retinal rod outer segment. *Proc. Natl. Acad. Sci. USA* **82:** 8813-8817

Received on 14th March, 2001; accepted March 23rd, 2001