Ionic regulation of adenylate cyclase from the cilia of *Paramecium tetraurelia**

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The kinetics of the ionic regulation of an adenylate cyclase associated with the excitable ciliary membrane from *Paramecium tetraurelia* was examined. Glycerol (30%, v/v) stabilized the enzyme, and activated by an increase in $V_{\max}$ (3-fold) and a decrease in the apparent K_m for MgATP (6-fold). Kinetic analysis of Mg^{2+} effects showed a stimulation via a single metal-binding site separate from the substrate site, with a dissociation constant, K_s , of 0.27 mM. Analysis of Ca^{2+} effects showed (i) an uncompetitive inhibition with respect to substrate MgATP, and (ii) dependence of the extent of inhibition on the free Mg^{2+} concentration. K_i values ranged from 4 to 130 μM - Ca^{2+} in the presence of 0.55–2 mM- Mg^{2+} respectively. This indicates competition between Mg^{2+} and Ca^{2+} at the metal-binding site. The Ca^{2+} effect was specific; Sr^{2+} and Ba^{2+} were almost without effect, and 100 μM - Ba^{2+} did not interfere with the Ca^{2+} inhibition. The actions of Ca^{2+} were readily reversible after addition of EGTA. K^+ activated the adenylate cyclase at concentrations around 20 mM. The stimulatory potency of K^+ was dependent on the free Mg^{2+} concentration. At 1 mM free Mg^{2+} , 20 mM- K^+ doubled the adenylate cyclase activity. The inhibitory Ca^{2+} and stimulatory K^+ inputs were independent of each other.

INTRODUCTION

Mainly because of the early electrophysiological studies by Eckert [1–3], the freshwater protozoan *Paramecium* is nowadays used extensively for biochemical studies designed to integrate sensory transduction, membrane excitation and behaviour at a molecular level. An adenylate cyclase of high specific activity is present in the excitable ciliary membrane from *Paramecium tetraurelia* [4]. Hormone-responsive adenylate cyclases in most eukaryotic cells usually consist of several distinct building blocks: (a) a hormone-receptor unit facing the cells' exterior, (b) GTP-binding proteins, designated G_s and G_i for their stimulatory and inhibitory input respectively, and (c) a catalytic subunit at the cytoplasmic side, which generates cyclic AMP [5,6]. Unlike metazoan adenylate cyclases, the protozoan enzyme does not display the characteristic properties which are indicative of a functional cooperativity of several individual regulatory subunits as outlined above [7]. Thus the adenylate cyclase from *Paramecium* is not stimulated by NaF or forskolin, it is unaffected by cholera-toxin pretreatment or by addition of 10 μM -GTP and its stable analogues guanosine 5'-[β -imido]triphosphate or guanosine 5'-[γ -thio]triphosphate. Furthermore, an adenoribosylation of ciliary proteins was not found with cholera toxin or pertussis toxin [7], even under similar experimental conditions where recently a 'hidden' substrate for adenoribosylation by pertussis toxin was identified in spermatozoa [8,9]. Typically, the G_s and G_i subunits of several mammalian adenylate cyclases are selectively adenoribosylated by these toxins [10].

The protozoan adenylate cyclase is stimulated by K^+ [7]. This is interesting, in view of the ionic regulation of the swimming behaviour of this unicell. In *Paramecium*,

voltage-gated Ca^{2+} channels are localized in the ciliary membrane and serve as mediators for all excitatory, i.e. depolarizing, stimuli [3,11]. Graded to the magnitude of the receptor potential, a depolarizing inward Ca^{2+} current occurs which is directly coupled to the reversal of the ciliary power stroke [3,12]. Repolarization is accomplished by an outward K^+ current through voltage- and Ca^{2+} -activated K^+ channels [3,11]. Accordingly, we investigated the ionic regulation of the adenylate cyclase in the cilia from *Paramecium*. The enzyme is regulated allosterically by Mg^{2+} , activated by K^+ , and inhibited by Ca^{2+} . Considering the role of Ca^{2+} and K^+ in excitatory processes in *Paramecium*, such a ciliary enzyme may be involved in the biochemical transmission systems governing its swimming behaviour.

EXPERIMENTAL

Materials

[α - ^{32}P]ATP [tetra(triethylammonium) salt; 600 Ci/mmol] and cyclic [8- 3H]AMP (38 Ci/mmol) were purchased from Amersham. Dowex 50WX4 was from Serva, Heidelberg, Germany; ATP (Tris salt) was from Sigma, Munich, Germany, and cyclic AMP (free acid) from Boehringer, Mannheim, Germany. Neutral aluminium oxide (activity grade I) was from Merck, Darmstadt, Germany. All other reagents were from standard commercial sources.

Enzyme preparation

Paramecium tetraurelia wild type, stock 51s, was grown axenically in 20-litre bioreactors [13]. Early stationary cells (about 30000/ml) were deciliated by a calcium shock, and cilia were isolated by several centrifugation steps as reported [14]. Ciliary membranes were separated from axonemes by vigorous shaking on

* This paper is dedicated to the memory of Dr. Roger Eckert, who died 16 June 1986.

a Vortex Whirlmix for 2 min and purified on a sucrose step gradient [15]. The membrane fraction on top of the 45%-sucrose layer was washed by centrifugation (48 000 g, 30 min) in 10 vol. of 5 mM-Tris/Mops, pH 7.4. Extraction and solubilization of the adenylate cyclase from the ciliary membranes was accomplished by a two-step procedure successively using 1% Brij 56 and 0.5% Lubrol PX as detergents and 20% (v/v) glycerol as stabilizer [7]. Adenylate cyclase activity was released into the Lubrol-PX-containing buffer [7]. Ciliary membrane vesicles as source of the particulate adenylate cyclase and the solubilized enzyme were stored in batches at -80°C without loss of activity for at least 6 months.

Determination of adenylate cyclase activity

Adenylate cyclase activity was assayed for 8 min at 37°C in a total volume of 100 μl with 30% (v/v) glycerol, 25 mM-Tris/Mops, pH 7.6, 2 mM-cyclic $[8\text{-}^3\text{H}]\text{AMP}$ (5 nCi), 0.5–20 mM- MgSO_4 , 0.2–2 mM-ATP/Tris, 3–10 μg of soluble or 10–30 μg of particulate enzyme, and test substances as specified. Linearity of the reaction with respect to time and protein concentration was ascertained for all incubation conditions. Interference in the assays by phosphodiesterase or ATPase activities was not detected. Furthermore, an excess of up to 4 mM-ATP over $[\text{Mg}^{2+}]$ or $[\text{Ca}^{2+}]$ did not affect adenylate cyclase activity, virtually excluding inhibitory effects of HATP^{3-} or ATP^{4-} (see also [16]). The reaction was started by addition of 0.5 μCi of $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ and terminated with 150 μl of a solution containing 1.5% SDS, 2.8 mM-ATP and 2.8 mM-cyclic AMP, pH 7.5. Cyclic $[^{32}\text{P}]\text{AMP}$ was purified by sequential chromatography on Dowex 50WX4 and aluminium oxide [17]. Recovery as monitored by determination of cyclic $[^3\text{H}]\text{AMP}$ was about 75%. The product was identified by t.l.c. on polyethyleneimine-cellulose F plates with 0.5 M-LiCl as solvent, followed by autoradiography, and by cyclic AMP phosphodiesterase digestion before and after isolation of cyclic $[^{32}\text{P}]\text{AMP}$ and rechromatography. All experiments were performed with at least two different enzyme preparations, and values reported are the means of duplicates. The maximal differences between two determinations never exceeded 5%.

Miscellaneous methods

The concentrations of Mg^{2+} , Ca^{2+} and MgATP in the incubations were calculated with the program of Fabiato & Fabiato [18], by using the stability constants K given therein: $\log K(\text{MgATP}) = 4.324$ and $\log K(\text{CaATP}) = 3.982$.

All straight lines and their respective correlation coefficients were calculated with a linear-regression program. From the correlation coefficients and the number of points/line t values were calculated and converted into P values by using standard statistical procedures. For secondary graphs only the computed intercepts of primary plots were used.

Protein was determined by the method of Peterson [19], with bovine serum albumin as standard.

RESULTS

General properties

With MgATP as substrates for the membrane-bound or solubilized adenylate cyclase of *Paramecium*, we

observed that at 37°C the rate of cyclic AMP formation was linear for only about 2 min. A longer linearity could not be obtained by variation of free Mg^{2+} concentrations, use of higher substrate concentrations, addition of an ATP-regenerating system or phosphodiesterase inhibitors. An investigation of the thermal stability revealed that the activity of the adenylate cyclase rapidly decreased at 37°C . The inactivation could not be prevented by inclusion of a cocktail of proteinase inhibitors, consisting of 50 μM -7-amino-1-chloro-3-(*p*-tosylamido)heptan-2-one hydrochloride ('TLCK'), 7.5 μM -leupeptin, 12 μM -pepstatin, 1 μM -aprotinin and 60 μM -phenylmethanesulphonyl fluoride, or by addition of 2 mg of bovine serum albumin/ml to the incubation. A decrease in the incubation temperature lowered the rate of inactivation. Adenylate cyclase was completely stabilized and activated severalfold by addition of 30% glycerol. With 1 mM free Mg^{2+} , V_{max} was increased 3-fold to 5.4 nmol of cyclic AMP/min per mg, whereas K_m was decreased 6-fold to 0.86 mM-ATP. All further experiments were carried out with 30% glycerol in the assay.

Fig. 1. Effect of $[\text{MgATP}]$ on the rate of cyclic AMP formation by *Paramecium* adenylate cyclase

The incubation mixture contained 5.2 μg of solubilized ciliary membrane protein and either ATP and Mg^{2+} at a molar ratio of 1:1 (●) or an excess of 2 mM free Mg^{2+} above the prevailing MgATP concentration (○). Depicted substrate MgATP concentrations were calculated as described in the Experimental section. (a) Michaelis-Menten graph; (b) Hill plot of the same data; the correlation coefficients for the computed lines of the Hill plot were > 0.988 ($2P < 0.005$).

Irrespective of the presence of glycerol, the pH optimum (pH 8.8) and the activation energy obtained by an Arrhenius plot (115 kJ/mol) were identical.

Kinetic analysis for ATP and Mg^{2+}

Initially, the kinetics of the adenylate cyclase were studied with concentrations of Mg^{2+} and ATP in a molar ratio of 1:1. Plotting only those points where the concentration of substrate MgATP exceeded the amount of Mg^{2+} plus ATP, sigmoid Michaelis–Menten graphs were obtained, indicating an allosterically regulated enzyme (Fig. 1a, ●). The corresponding Hill plot was linear, $h = 1.6$ (Fig. 1b).

When the adenylate cyclase was assayed with increasing MgATP concentrations in the concomitant presence of 2 mM free Mg^{2+} , a hyperbolic Michaelis–Menten plot was obtained (Fig. 1a, ○); the slope of the corresponding Hill plot was 0.9 (Fig. 1b). These data are consonant with a positively co-operatively regulated enzyme.

Under these conditions, determination of Michaelis constants, K_m^A , for MgATP is feasible only in the presence of fixed activator concentrations. We measured the K_m^A values for MgATP at fixed free Mg^{2+} concentrations of 0.55, 1, 2, 3, 4 and 8 mM. The values derived from corresponding Lineweaver–Burk plots were 0.6, 0.54, 0.44, 0.49, 0.42 and 0.41 mM- MgATP (Fig. 2). The K_m^A values were replotted versus $1/[\text{Mg}^{2+}]$ (Fig. 2 inset). The intercept on the abscissa of this secondary graph corresponds to $-1/K_s$, K_s being the dissociation constant of the enzyme and its allosteric activator. K_s was 0.27 mM- Mg^{2+} . From an equation given by Ahlers *et al.* [20], a K_m' value for MgATP of 0.26 mM was obtained.

Next, the number of Mg^{2+} -binding sites on the adenylate cyclase was assessed. We established dose-response curves for free Mg^{2+} in the presence of fixed MgATP concentrations. Free Mg^{2+} concentrations up to 4 mM only were used, since higher concentrations are in all likelihood physiologically not relevant. Using a Dixon-analogous graph [20], we obtained linearity by plotting $1/[\text{Mg}^{2+}]$ versus $1/v$ (Fig. 3). This indicates that only 1 mol of Mg^{2+} binds per mol of enzyme. Linearity of plots of $1/[\text{Mg}^{2+}]^2$ versus $1/v$ would have indicated two binding sites [20].

Effect of Ca^{2+}

The behaviour of *Paramecium* is greatly affected by Ca^{2+} and K^+ , which respectively carry the depolarizing inward and repolarizing outward currents of the action potential [3,11]. Therefore, we examined the effect of Ca^{2+} on the ciliary adenylate cyclase. With MnATP as substrate, Ca^{2+} up to 1 mM has no inhibitory effect [4]. However, with 1 mM- MgATP as substrate we observed a considerable inhibition of adenylate cyclase activity at Ca^{2+} concentrations below 100 μM . To determine the mode of Ca^{2+} inhibition, the enzyme was assayed with MgATP concentrations ranging from 0.2 to 2 mM in the presence of fixed concentrations of free Mg^{2+} and Ca^{2+} . The program of Fabiato & Fabiato [18] was used to calculate the applicable ionic conditions. Using free Ca^{2+} concentrations of 0.4, 27, 60 and 95 μM and free Mg^{2+} concentrations of 0.55, 1 and 2 mM, we consistently obtained parallel lines in the corresponding Lineweaver–Burk plots (Fig. 4). These data allude to a rare case of an uncompetitive inhibition, as previously observed for Ca^{2+} inhibition of an adenylate cyclase from bone and

Fig. 2. Substrate-dependence of *Paramecium* adenylate cyclase as a function of Mg^{2+} concentration

Activity was measured with 5 μg of solubilized ciliary membrane protein. Free Mg^{2+} concentrations in excess of MgATP were (○) 0.55, (●) 1, (△) 2, (□) 3, (■) 4 and (▲) 8 mM. For clarity of presentation, only four different concentrations are shown in the main Figure. The inset is a plot of K_m^A values derived from the Lineweaver–Burk plot versus $1/[\text{Mg}^{2+}]$. All lines were fitted by regression analysis (correlation coefficients > 0.93 , $2P < 0.01$).

Fig. 3. *Paramecium* adenylate cyclase activity as a function of free Mg^{2+} concentration (Dixon-analogous plot)

Enzyme activity was assayed with 5 μg of solubilized membrane protein in the presence of (●) 0.3, (△) 0.6 and (■) 1 mM- MgATP and the indicated concentrations of free Mg^{2+} . Lines were fitted by linear regression ($2P < 0.01$ for all lines).

osteosarcoma cells [16]. Uncompetitive inhibition is characterized by the fact that the inhibitor binds reversibly to the enzyme–substrate complex, but not to the enzyme alone. This enzyme–substrate–inhibitor complex (ESI) is inactive. With increasing concentrations of the inhibitor, both K_m and $V_{\max}$ decrease by the factor $(1 + [I]/K_i)$ [21]. To exclude the possibility of a mixed-type inhibition, we established Ca^{2+} -dose-response curves from 0.4 μM to 3 mM free Ca^{2+} , with 180, 540 and 900 μM - MgATP as substrate concentrations. Free Mg^{2+}

Fig. 4. Effect of Ca^{2+} on the kinetics of *Parametium* adenylate cyclase at various free Mg^{2+} concentrations

The Ca^{2+} concentrations used were (●) 0.4, (△) 27, (■) 60 and (○) 95 μM . Free Mg^{2+} was (a) 0.5, (b) 1 and (c) 2 mM; 4.8 μg of solubilized protein was used per assay. MgATP concentrations were varied as indicated. Linearity of all lines was ascertained by statistical evaluation; $2P$ values ranged from < 0.05 to < 0.001 .

Fig. 5. Determination of K_i values for Ca^{2+} inhibition of *Parametium* adenylate cyclase

The $V_{\text{max},i}$ values derived from Fig. 4 were plotted against the respective Ca^{2+} concentrations. Free Mg^{2+} was 0.55 mM (▲; Fig. 4a), 1 mM (○; Fig. 4b) and 2 mM (■; Fig. 4c). All lines were fitted by linear regression analysis. Statistical significances for linearity were: $2P < 0.025$ (▲); $2P < 0.05$ (○); $P < 0.05$ (■).

was kept constant at 550 μM in these experiments. The Dixon plots of $1/v$ versus $[\text{Ca}^{2+}]$ always yielded straight lines, with correlation coefficients greater than 0.9 (results not shown). This essentially rules out a mixed-type inhibition and demonstrates that the ciliary adenylate cyclase from *Parametium* is uncompetitively inhibited by Ca^{2+} when MgATP is used as substrate.

For an uncompetitive inhibition, a K_i value can only be derived from a secondary graph plotting the concentration of the inhibitor, $[\text{Ca}^{2+}]$, versus $1/V_{\text{max},i}$

(Fig. 5) [21]. The latter values had to be derived from the corresponding Lineweaver-Burk plots of Fig. 4. The K_i values for Ca^{2+} were 4, 14 and 130 μM for free Mg^{2+} concentrations of 0.55, 1 and 2 mM respectively (Fig. 5). These data indicate that Mg^{2+} and Ca^{2+} compete for the same binding site at the enzyme. The inhibition of adenylate cyclase by Ca^{2+} was fully reversible; addition of an excess of the Ca^{2+} chelator EGTA to the assay immediately neutralized the inhibition.

To evaluate the specificity of the Ca^{2+} inhibition, we tested the effect of the alkaline-earth-metal ions Sr^{2+} and Ba^{2+} . With 1 mM- MgATP we observed that Ba^{2+} or Sr^{2+} up to 100 μM inhibited adenylate cyclase activity by not more than 20%. Furthermore, the inhibitory action of Ca^{2+} was unaffected by the concomitant presence of 100 μM - Ba^{2+} . For example, the K_i value of 4 μM - Ca^{2+} in the presence of 0.55 mM free Mg^{2+} was unaltered by 100 μM - Ba^{2+} . The trivalent La^{3+} ion, which has an ionic radius similar to that of Ca^{2+} , often has effects comparable with those of Ca^{2+} . La^{3+} inhibited the adenylate cyclase almost completely at 10 μM . It is conceivable that La^{3+} , with its additional positive charge, binds even tighter to the inhibitory site at the enzyme than does Ca^{2+} .

Effect of K^{+}

Preliminarily, we have reported that the ciliary adenylate cyclase from *Parametium* is activated by K^{+} when assayed with MgATP as substrate. A half-maximal effect was found with 3 mM- K^{+} ; Na^{+} and Li^{+} ions were inactive [7]. We now characterized the effect of K^{+} , in view of the kinetic properties outlined above. K^{+} stimulated adenylate cyclase activity in whole ciliary homogenates, in isolated ciliary membrane vesicles and in the solubilized enzyme preparation. The pH- and temperature-dependence of the ciliary adenylate cyclase were not affected by stimulation with 20 mM- K^{+} . Likewise, the activation energy of 115 kJ/mol was

Fig. 6. Effect of K^+ on *Paramecium* adenylate cyclase as a function of the free Mg^{2+} concentration

Enzyme activity was assayed with 1 mM-MgATP, 5 μ g of solubilized ciliary membrane and the indicated concentrations of free Mg^{2+} in the absence (100%, respectively) and presence of 20 mM-KCl.

Fig. 7. Effect of K^+ on substrate kinetics of *Paramecium* adenylate cyclase

Enzyme activity was determined with 5 μ g of solubilized ciliary adenylate cyclase without ($\circ$, $\triangle$) and with ($\bullet$, $\blacktriangle$) 20 mM-KCl in the presence of 0.5 mM ($\circ$, $\bullet$) and 2 mM ($\triangle$, $\blacktriangle$) free Mg^{2+} . Note that at a given free Mg^{2+} concentration K_m values for MgATP are unaltered by KCl. Lines were fitted by computer ($2P < 0.001$ for all lines).

independent of the presence of stimulatory concentrations of K^+ . In addition, the activation by K^+ was unaltered by the concomitant presence of up to 20 mM-NaCl. The stimulatory potency of K^+ was dependent on the concentration of free Mg^{2+} (Fig. 6): the higher the free Mg^{2+} concentration, the smaller the activation by K^+ . At

1 mM free Mg^{2+} , 20 mM- K^+ approximately doubled adenylate cyclase activity (Fig. 6). The activation was due to an increase in V_{max} , whereas the K_m^A values (depending on the concentrations of Mg^{2+}) were unchanged (Fig. 7). Finally, we examined a possible interaction between inhibitor Ca^{2+} and activating K^+ ions. At 0.55 mM free Mg^{2+} , the K_i for Ca^{2+} in the absence and presence of 20 mM-KCl was identical (4 μ M). This indicates that K^+ and Ca^{2+} bind at different and independent sites at the adenylate cyclase. Thus at a constant physiological concentration of free Mg^{2+} adenylate cyclase could be regulated by the transient ratios of K^+ and Ca^{2+} concentrations in the cilia.

DISCUSSION

The role of cyclic nucleotides in sensory organelles of ciliary origin is attracting much interest, e.g. rod and cone photoreceptor outer segments or olfactory cilia [22–25]. The ciliary membrane of *Paramecium* contains an adenylate cyclase which is different from those found in most eukaryotic cells in several respects. First, the enzyme from *Paramecium* is not regulated by a known hormone, and there is no evidence that it is coupled to guanine-nucleotide-binding proteins, which are common constituents of many hormone-regulated adenylate cyclases in metazoan cells [5,6]. Therefore, the adenylate cyclase from *Paramecium* seems more related to the spermatozoan enzyme [26,27]. Although evidence for the presence in spermatozoa of a G_i protein was reported recently by using detergent-treated membranes, its association with the spermatozoan adenylate cyclase was not reported [8,9]. In *Paramecium*, we failed to detect GTP-binding proteins in the cilia under similar experimental conditions. Furthermore, a radioimmunoassay for the α_o -subunit of the heterotrimer $\alpha\beta\gamma$ of the GTP-binding protein G_o , using antibodies against α_o from rat brain, was negative.

Second, in *Paramecium* a single Mg^{2+} -binding site on the catalyst ($K_s = 0.27$ mM) is responsible for modulation of adenylate cyclase activity. In contrast, most metazoan adenylate cyclases are reported to have two metal-binding sites [16,28–30]. There, addition of GTP and its analogues, e.g. the [$\beta\gamma$ -imido]triphosphate, quite often leads to a linearization (one-side behaviour) of the Mg^{2+} -dependence, i.e. one Mg^{2+} -binding site virtually disappeared [16,28,29]. Therefore, the single Mg^{2+} -binding site of *Paramecium* adenylate cyclase may reflect the lack of interaction with a GTP-binding protein.

Third, the ciliary adenylate cyclase from *Paramecium* is inhibited by low concentrations of Ca^{2+} in an uncompetitive manner relative to MgATP and competitive with respect to Mg^{2+} . In contrast, many mammalian adenylate cyclases seem to be regulated bidirectionally by Ca^{2+} [31–33]. Low Ca^{2+} concentrations stimulate, whereas higher concentrations inhibit, enzymic activity. The stimulatory action of Ca^{2+} is usually mediated by a calmodulin-dependent mechanism [32,34,35]. We did not detect a stimulation by Ca^{2+} of *Paramecium* adenylate cyclase. Furthermore, addition of EGTA, calmodulin or the calmodulin antagonist trifluoperazine did not affect enzymic activity. Usually, Ca^{2+} inhibition of adenylate cyclases from several tissues, e.g. platelets [31], smooth muscle [34], brain [32,36], mouse melanoma cells [37], cardiac muscle [36], bone and osteosarcoma cells [16] has not been related to a

calmodulin-mediated effect, but seems to be a direct Ca^{2+} action on the catalyst. In this respect, *Paramecium* adenylate cyclase may resemble the catalytic moiety of the metazoan enzymes devoid of a calmodulin-stimulatory site.

Fourth, the adenylate cyclase from *Paramecium* is uniquely activated by low, physiologically meaningful, concentrations of K^+ ions. This is not simply an effect of ionic strength, since Na^+ and Li^+ were inactive. It appears that K^+ acts like a stimulatory hormone in other systems, (i) increasing the V_{max} of the enzyme and having no effect on the K_m for MgATP and (ii) being less stimulatory at high Mg^{2+} concentrations. Such a specific effect of K^+ has not yet been reported for any other eukaryotic adenylate cyclase. However, K^+ ions seem to have a direct effect on cyclic AMP accumulation in rat brain cortex [38–41] and in rabbit and rat superior cervical ganglia [42]. These observations from different groups have never been explained satisfactorily. One is tempted to speculate that adenylate cyclases may be found in excitable mammalian tissues which are not coupled to GTP-binding regulatory proteins but subject to an ionic regulation. In fact, evidence for the presence of two distinct adenylate cyclase catalysts in rat brain being subject to different regulation has been published recently [43].

In conclusion, our results show that the ciliary adenylate cyclase from *Paramecium* is regulated by Mg^{2+} , Ca^{2+} and K^+ in a unique manner not known for adenylate cyclases of prokaryotic or eukaryotic origin. Although the actions of cyclic AMP itself are unclear at present, they could involve processes related to either the regulation of ion conductances of the membrane in conjunction with the $\text{Ca}^{2+}/\text{K}^+$ action potentials of *Paramecium*, or to mechanical switching of the ciliary beat. The available battery of behavioural *Paramecium* mutants with defects in ion permeabilities [11] could be used in future studies to initially assess the participation of Mg^{2+} , Ca^{2+} and in particular of K^+ in the regulation *in vivo* of the protozoan adenylate cyclase and to clarify the physiological function of cyclic AMP in this model system.

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