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PHOTOSENSORY TRANSDUCTION IN THE FLAGELLATED ALGA, *EUGLENA GRACILIS*

III. INDUCTION OF Ca^{2+}-DEPENDENT RESPONSES BY MONOVALENT CATION IONOPHORES

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(1) The effects of monovalent cation ionophores (valinomycin and gramicidin), a protonophore (nigericin) and extracellular pH change on the motility and blue light-induced photobehavior (step-down photophobic response) of *Euglena* were investigated. (2) Monovalent cation ionophores, but not the protonophore, can both partially suppress photobehavior and, under appropriate conditions, induce a change in flagellar activity (and thus cell movement) that appears identical to that associated with the photobehavior. (3) Valinomycin, at low extracellular KCl, delays the induction of photobehavior and also induces a light-independent elevation in the frequency of directional changes in the cells' swimming path. Both effects are suppressed by elevation in extracellular KCl. (4) Gramicidin, in the presence of the anion tetraphenylborate, suppresses photobehavior. The same combination, if applied in the presence of elevated extracellular NaCl, induces a light-independent cell tumbling and elevation in the frequency of directional changes in the cells' swimming path. The induced behavior is dependent on the extracellular Na concentration, requires the presence of extracellular Ca^{2+} and is blocked by La^{3+}. (5) Photobehavior is observed over the pH range 3.5–8.2 and fluence/response relationships for photobehavior are not significantly different over the pH range 5.5–8.2. (6) The results provide a link between the previously reported effects of Ca^{2+} ionophores, and the effects of monovalent cations and monovalent cation-transport inhibitor on motility and photobehavior.

1. Introduction

The flagellated alga, *Euglena gracilis*, responds to reduction in incident blue light intensity by changing its swimming behavior from that of straight-path swimming behavior to that of a discontinuous cell-tumbling response (the step-down photophobic response [1]). The flagellar reorientation, which mediates the photophobic response, can also be induced without change in incident light intensity by application of the ionophore A23187 and suitable concentrations of Ca^{2+} [1]. Since elevation in extracellular Ca^{2+} augments the duration of the photophobic response [1], light-dependent flagellar reorientation would appear to be a consequence of an inward Ca^{2+} flux across the flagellar-photoreceptor membrane. The step-down

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Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N'*-bis(2-ethanesulfonic acid); Taps, tris(hydroxymethyl)methylaminopropanesulfonic acid.

stimulus-induced flagellar reorientation thus appears to be due to a temporary increase in intraflagellar free Ca^{2+}, as is the case in other motile ciliary and flagellar axonemes [2].

The step-down photophobic response is, however, markedly affected by the extracellular concentrations of monovalent cations and by a recognized inhibitor of coupled Na^+/K^+ active transport (Na^+/K^+ pump), ouabain [3]. Such sensitivity, combined with a lack of action of a variety of agents generally recognized as antagonists of voltage-dependent cation fluxes across biological membranes [3], indicates that light does not simply act as an on-off switch in *Euglena* to promote Ca^{2+} influx and suggested that changes in Na^+/K^+ permeability could be, at least in part, responsible for control of Ca^{2+} fluxes. We have therefore attempted to test the latter hypothesis by studying the effects of monovalent cation ionophores on motility and photobehavior of *Euglena*.

Materials and Methods

E. gracilis Z was cultured, harvested and prepared for experimentation as previously detailed [3]. The cells were suspended in 'adaptation buffers' (see below) at $2-4 \cdot 10^5$ cells/ml buffer and adapted for 12–18 h under dim red light at 25°C to the adaptation buffer containing 0.5 mM $CaCl_2$, 0.125 mM $MgCl_2$ and 1 mM KCl adjusted to pH 7.1 with either 1 mM Tris-HCl or 2.5 mM Hepes plus 2.5 mM Pipes-NaOH. In some experiments, the pH was adjusted to 5.5 with 5 mM Mes-NaOH or to pH 8.2 with 5 mM Taps-NaOH or a Ca-EGTA buffer system was used at pH 6.9 [1].

Determination of blue light-induced step-down photophobic responses and the rates of photoaccumulation of cells into a blue light trap (a response mediated by step-down photophobic responses) and video analyses of cell swimming behavior were performed essentially as previously described [1]. Video analyses were carried out on a random selection of swimming tracks from a total of approx. 50 cells/field of view (equivalent to 10^5 cells/ml at the magnification used). Chemicals were added by introducing small volumes (usually 1–10 µl) into aliquots (2.5 or 5 ml) of cell suspensions. Chemicals were prepared on the day of use either in adaptation buffers or organic solvents (ethanol or methanol) and appropriate solvent or dilution controls carried out as required. To facilitate comparisons with other studies, we present the ionophore concentrations in terms of both $\mu g/10^6$ cells or as molarity. For the gramicidins, we have used an estimated molecular weight value of 1883 (based on gramicidin A which comprises 80–90% of commercial gramicidin mixtures and assuming a 90% valine and 10% isoleucine congener distribution [4,5]. The effects of test chemicals were normally evaluated 2–5 min after addition. Cell protein concentrations were determined by the method of Lowry et al. [6] in the presence of 2% (w/v) sodium dodecyl sulfate and using bovine serum albumin as standard.

Gramicidin D, valinomycin, sodium tetraphenylborate, Mes, Hepes, Pipes and Taps buffers (as their acid form) were purchased from Sigma Chemical Co. (St. Louis, MO). Tetraethylammonium chloride (99%) was from Matheson, Coleman and Bell (Cincinnati, OH) and choline chloride from Merck (Elmestead, NY). Nigericin was a generous gift of Dr. Robert Hammil (Ely Lilly Research Laboratories, Indianapolis, IN) and Gramicidin (Nutritional Biochemicals, Cleveland, OH) was kindly provided by Dr. Keith Garlid (Medical College of Ohio at Toledo). All buffers were prepared in double-distilled water.

Results

Further evaluation of cell motility, cell swimming path character and behavioral responses

In our adaptation buffers, at pH 7.1 and under constant light intensity, *Euglena* normally swim in a straight-path (forward) swimming mode. The motility of the cells can be assessed qualitatively, by microscopic observation, in terms of the speed of the cells (slow, normal or fast), the character of the swimming path and the percentage of the cells in the population that are motile. In these studies, this percentage was 80–90%. The swimming path is relatively straight, i.e., there is only a low frequency of directional changes (change in the direction of the swimming path by greater than 30° but less than 90°). The frequency of directional changes has been denoted as normal, moderate, high and very high.

Quantitative analysis of the cells' swimming

path can be made by video recordings. From these, the velocity of the cells in the straight-path swimming mode can be ascertained, while the frequency of directional changes in the swimming paths of continuously swimming cells can be ascertained from event marker recordings and the frequency, in changes per min, calculated [1]. Under certain conditions, the cells can be induced to change their forward swimming mode into that of a discontinuous tumbling mode (mediated by flagellar reorientation). In between the tumbling responses (the durations of which can be measured), the cells are invariably swimming with a very high frequency of directional changes. Such directional changes appear to occur at random, since analysis of event marker traces indicates no obvious periodicity. In determination of the frequency of directional changes, the cells were studied in between tumble events (defined as a directional change of at least 90° and usually greater than 180°) and thus the mean free path of cells between tumbling events can be estimated.

Effect of valinomycin and K^+ on motility and photobehavior

If changes in the relative K^+ concentrations between the inside of the cells and the extracellular medium (i.e., changes in K^+ concentration gradients) were linked to the expression of photobehavior, then the motility or photobehavior of the cells should be affected by the specific K^+ carrier (ionophore) valinomycin ($K^+ : Na^+$ selectivity ratio of 10000:1 [7]). The specificity of any valinomycin-induced effects can be tested by studying the effects of Na^+ in the presence of valinomycin.

Valinomycin has several effects on *Euglena*. Within a few seconds of ionophore addition (10^{-6} M: 2.8 $\mu g/10^6$ cells), there is a change in the frequency of directional changes in the cells' swimming paths. The change is progressive so that after 30–60 s, the cells are changing their direction about once per s. The response is observable over long periods (10–15 min) for cells in standard adaptation buffers containing 1 mM KCl.

At 1 mM KCl, valinomycin increases the frequency of directional changes from 18.8 ± 1.7 ($n = 32$) to 45.1 ± 4.2 ($n = 37$) at 23°C. The ionophore solvent, ethanol, had no effect (frequency of 19.1 ± 1.6, $n = 27$). The enhancement of the frequency of directional changes induced by valinomycin treatment can be readily observed qualitatively (Table I) and determined quantitatively (Fig. 1) to depend on the extracellular K^+ concentration. Furthermore, the suppression of the

TABLE I

EFFECT OF VALINOMYCIN ON *EUGLENA* MOTILITY AND PHOTOBEHAVIOR: DEPENDENCE ON K^+ CONCENTRATION

Cell motility is assessed in terms of relative speed of swimming (++, normal; +, slow) and character (frequency of directional changes in cells' swimming paths: +, normal; ++, moderate; +++, high; ++++, very high. Step-down photophobic responses induced by blue light removal of either moderate (40 mW/m^2) or relatively high (300 mW/m^2) intensity, 466 nm light. Range of durations (from three to six determinations) of total responses given. Conditions: cells in 1 mM Tris, 0.5 mM $CaCl_2$, 0.125 mM $MgCl_2$, 1 mM KCl, pH 7.1, at 19°C plus additions as indicated. Valinomycin added at 10^{-6} M (2.8 $\mu g/10^6$ cells) in ethanol (0.01 % (v/v) final). n.d., not determined.

Conditions	Motility		Step-down photophobic responses			
	Speed	Character	Delay in onset (s)	Duration (40 mW/m^2) (s)	Delay in onset (s)	Duration (300 mW/m^2) (s)
No additions	++	+	none	27–31	none	55–65
+16 mM KCl	++	+	none	34–40	n.d.	n.d.
+valinomycin	++	++++	3 –4	16–18	n.d.	n.d.
+valinomycin+16 mM KCl	+	++++	1 –2	22–24	none	50–57
+valinomycin+32 mM KCl	+	+++	0.5–2	17–22	none	29–34
+valinomycin+48 mM KCl	+	++	0.5	6– 7	none	19–22
+valinomycin+64 mM KCl	+	+	none	6– 7	none	16–17

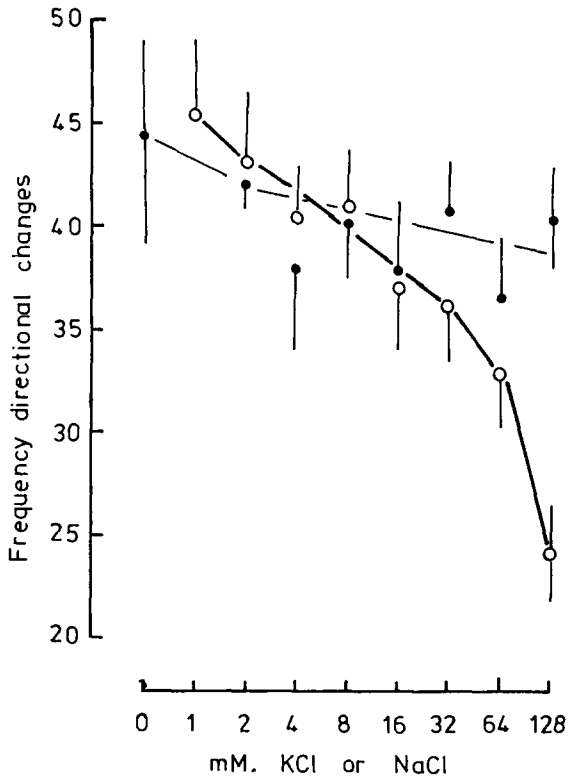

Fig. 1. Effect of extracellular KCl and NaCl concentrations on the valinomycin-induced enhancement in the frequency of directional changes (changes/min) in the cell's swimming paths. Cells treated with 10^6 M valinomycin (2.8 μg/10^6 cells) in 1 mM Tris, 0.5 mM $CaCl_2$, 0.125 mM $MgCl_2$, 1 mM KCl, pH 7.1, at 23°C with additional KCl (○) or NaCl (●) as indicated. Results given as mean ±S.E. (n = 23–46).

valinomycin-induced behavior by elevation in extracellular K^+ is apparently K^+ specific since equivalent elevation in Na^+ has a far lesser effect (Fig. 1). In the absence of valinomycin, similar changes in extracellular KCl concentration have insignificant effects on cell motility.

Valinomycin (10^{-6} M) can also alter the expression of photobehavior, the alteration also depending on the extracellular KCl concentration. At low extracellular K^+ (1 mM KCl), application of valinomycin to the cells, prior to blue light exposure, interferes with the subsequent induction of the step-down photophobic responses. This interference is expressed as a delay between the removal of the light and the appearance of the flagellar reorientation (and thus cell tumbling). Subsequent responses appear to be normal in character (see Ref. 1 for details). However, on pretreating the cells with valinomycin and different concentrations of KCl, it was observed that the lag observed at 1 mM K^+ decreases and the response duration also decreases almost to zero. The response mechanism is, however, not blocked since with larger stimulation (blue light fluence rates of 225 instead of 40 mW/m^2, responses are still observed at KCl-valinomycin combinations that strongly inhibit responses to smaller stimuli (Table I).

Effect of gramicidin on motility and photobehavior

Since previous studies on the effects of Na^+, K^+ and ouabain on the photobehavior of *Euglena* indicated that coupled Na^+/K^+ movements were involved in photobehavior control [3], we tested the effect of an Na^+ ionophore, gramicidin [4]. Addition of methanolic solutions of gramicidin (0.025–140 μg/10^6 cells: 0.004–22 μM, with appropriate solvent controls) to cells in either culture media (pH 3.5–7.2 depending on type or culture age) or adaptation buffers at either pH 5.5 or 7.1 had no obvious or specific effects on either cell motility or photobehavior for many hours. There is, however, a technical problem apparently associated with successful application of the gramicidins to *Euglena* at least under all conditions we have so far tested:

During studies on photoaccumulation behavior of cells into a blue light trap (photoaccumulation being detected by a change in nonactinic transmitted light through a cell suspension [1]), up to a 2-fold increase in the apparent rate of photoaccumulation was sometimes observed for cells treated with gramicidins (14–140 μg/10^6 cells: 2–22 μM) especially in the presence of elevated extracellular NaCl (8–16 mM). Precipitates were present that were of sufficient size to interfere with the nonactinic light transmission through cell-free solutions. These precipitates, possibly of insoluble gramicidin-Na^+ complexes [4], indicated that the gramicidins were simply not penetrating the cells.

5–15 min treatment of concentrated cell suspensions (10^7 cells/ml; higher concentrations cannot be used since flagellar loss occurs) with gramicidins (0.025–0.05 μg/10^6 cells: 0.1–0.2 μM) but not the solvent controls immobilized 60–90% of the cells (evaluated following dilution of the

cells to 10^5/ml), indicating penetration. The remaining motile cells, although slow swimming, still showed definite step-down photobehavior. Preincubation with gramicin and high cell concentrations (cell protein:ionophore weight ratio; estimated 4000:1) appears to be required for any action of gramicidin alone. The gramicidins are neutral molecules and form charged complexes with cations [7]. The ability of neutral ionophores to promote ion exchange is markedly enhanced by the presence of a lipophilic anions such as FCCP or tetraphenylborate [8,9]. Tetraphenylborate should thus facilitate equilibration of the gramicidin-cation complex into the membrane microenvironment and thus promote ion movement/exchange [4,10]. The effects of gramicidins on *Euglena*, in the presence of tetraphenylborate, were therefore tested.

Tetraphenylborate anion (0.125–17.5 $\mu g/10^6$ cells:0.1–20 μM) has only marginal effects on cell motility. Tetraphenylborate (4–18 $\mu g/10^6$ cells:2.6–10 μM) has no significant effects on either the induction, character or duration of the step-down photophobic response to standard stimulation (40 mW/m^2, 477 nm light for 60 s; data not shown). Gramicidins alone at either 28 or 56 $\mu g/10^6$ cells (2.9 or 5.8 μM) likewise have no effects on step-down photobehavior (Table II). However, in the presence of tetraphenylborate, whilst cell motility is only slightly altered, the gramicidins now cause a marked reduction in the duration of the step-down photophobic responses (Table II) and in the percentage of the cells in the population that will show a response, from 90–100% down to 10–15%. The magnitude of this inhibition by gramicidins is dependent on the tetraphenylborate concentration (Table II), indicating that the anion is acting to facilitate ionophore action in the manner proposed above. A similar set of results was obtained with another commercial preparation of gramicidins (gramicidin D from Sigma; see also Table III).

The gramicidin-tetraphenylborate treatments never completely inhibited step-down photobehavior (except under conditions where cell motility was significantly affected). This could have been due to a limiting concentration of monovalent cations for exchange. Therefore, the effects of elevated Na^+ and K^+ on the gramicidin-tetraphenylborate-treated cells were investigated. How-

TABLE II

ACTION OF GRAMICIDINS ON *EUGLENA* MOTILITY AND PHOTOBEHAVIOR: DEPENDENCE ON TETRAPHENYLBORATE

Conditions: cells in 1 mM Tris, 0.5 mM $CaCl_2$, 0.125 mM $MgCl_2$, 1 mM KCl, pH 7.1 at 18–18.5°C. Step-down photophobic responses given as relative durations (%) corrected for solvent and tetraphenylborate (TPB^-) controls. Results are from four to six determinations using two batches of cells. For the initial response (t_{CFR}), the duration was 7.7±0.4 s ($n=8$, =100%) and for the total response duration (t_R), the duration was 26.2±1.2 s ($n=8$, =100%). The number of tumbles is the count of the number of 360° planar tumbles executed by the cells during the initial response. Gramicidins equivalent to 28 or 56 $\mu g/10^6$ cells. TFB^- equivalent to 4, 8 and 16 $\mu g/10^6$ cells.

Conditions	Motility		Photobehavior		
	Speed	Character	Duration of initial response (t_{CFR}) (%)	No. of tumbles in (t_{CFR})	Duration of total response (t_R) (%)
No additions	++	+	100	5–6	100
2.9 μM gramicidins	+++	++	100	5–7	100
2.9 μM gramicidins + 2.6 μM TFB^-	++	++	65	4–5	100
2.9 μM gramicidins + 5.2 μM TPB^-	++	+	37	2	52
5.8 μM gramicidins + 2.6 μM TPB^-	++	+	86	6–8	86
5.8 μM gramicidins + 5.2 μM TPB^-	++	+	52	4	88
5.8 μM gramicidins + 10 μM TPB^-	++	+	30	1–2	50

TABLE III

EFFECT OF NaCl, GRAMICIDIN D AND TERAPHENYLBORATE ON THE SWIMMING PATH CHARACTERISTICS AND BEHAVIOR OF *EUGLENA*

Conditions: cells in 2.5 mM Hepes, 2.5 mM Pipes, 0.5 mM $CaCl_2$, 0.125 mM $MgCl_2$, 1 mM KCl, pH 7.1, at 24.5°C. Ionophore and tetraphenylborate concentrations given in M and as $\mu g/10^6$ cells. With NaCl + gramicidin D + tetraphenylborate, cells now execute a spontaneous, discontinuous tumbling behavior interupted by periods in which the cells are swimming in paths with a very high frequency of directional changes. See text for further details.

Conditions	Mean free path time (s)	Frequency of directional changes	Duration of cell tumble events (s)
No additions	16.7±0.4 (53)	21.2±5.4 (56)	<0.5
2.9 μM (28 μg) gramicidin D	16.6±0.4 (25)	19.9±4.6 (25)	<0.5
4.9 μM (8.75 μg) tetraphenylborate	16.3±0.6 (37)	16.8±3.9 (36)	<0.5
12.5 mM NaCl	15.5±0.6 (36)	21.4±4.6 (41)	<0.5
Gramicidin D + NaCl	14.4±0.5 (40)	17.4±4.4 (34)	<0.5
Tetraphenylborate + NaCl	15.7±0.5 (39)	20.7±4.8 (33)	<0.5
Gramicidin + tetraphenylborate + NaCl	12.7±0.4 (36)	46.7±6.6 (36)	2.0±0.6 (70)

ever, treatment of cells with gramicidin-tetraphenylborate in the presence of elevated extracellular NaCl induced a flagellar reorientation, and thus cell-tumbling behavior, without change in incident light intensity.

Characterization of the ion specificity and Na^+ concentration dependence of the gramicidin-tetraphenylborate-induced behavior

The following series of experiments were carried out to determine the specificity and characteristics of the flagellar reorientation response induced by treatment of cells with gramicidin-tetraphenylborate mixtures under constant light intensity and character.

Firstly, the responses require that the three reagents (gramicidin, tetraphenylborate and Na^+) are simultaneously present. As noted above, neither ionophore nor lipophilic anion alone or in combination have any significant effects on cell motility (% motile, swimming speed and path character) either under constant red (Table II) or blue light (40–350 mW/m^2, 466 nm; data not shown). If applied alone, NaCl (8 mM) effects a 3–4-fold enhancement of the duration of the step-down photobehavior response without alteration in motion characteristics (data not shown and see Ref. 3). Essentially the same magnitude of enhancement is seen in the additional presence of either gramicidin (28 $\mu g/10^6$ cells: 2.9 μM) or tetraphenylborate (8.75 $\mu g/10^6$ cells: 4.9 μM) and there is also little effect on cell behavior under constant light intensity (data not shown). In marked contrast, however, the addition of the three reagents together, at the concentrations given above, immediately changes the cells' behavior from the straight-path swimming mode with low frequency of directional changes to that of a discontinuous tumbling mode interupted by swimming paths with a very high frequency of directional changes. The cells execute three to five full rotations in a single plane (tumbles) in any particular tumble event and this tumbling mode continues for at least 10 min. A quantitative evaluation of cell behavior in the presence of these reagents, combinations of any two of the reagents or all three together can be found in Table III (see below for further discussion).

Secondly, the effect is specific for Na^+ with respect to other monovalent inorganic (K^+) or organic (choline or tetraethylammonium) cations tested or for H^+ (see below). In testing for this specificity, we investigated (i) the effect of the test cation alone (NaCl, 8 mM; KCl, 8 mM; choline chloride, 8 mM and tetraethylammonium chloride, 4 mM) on both motility and photobehavior duration to standard stimuli, (ii) the effects of the test cation in the presence of tetraphenylborate (1.65

$\mu g/10^6$ cells:0.9 μM) on motility and photobehavior and (iii) the effects of the test cation in the presence of tetraphenylborate (0.9 μM) and the additional presence of gramicidin (0.9 $\mu g/(0.8$ $\mu g/10^6$ cells:0.18 μM). In this series of experiments, in agreement with a previous report [3], changes in motility and photobehavior were observed.

Similar evaluations of the duration of photobehavior in the presence of a test cation plus tetraphenylborate showed that the responses were not significantly altered by the anion (data not shown). In the additional presence of gramicidin with the test cation and tetraphenylborate, photobehavior duration was essentially the same as that seen in the presence of K^+, choline or tetraethylammonium cations alone. However, for Na^+, the unique direct cell-tumbling mode was always induced. Due to the strength and persistence of this tumbling behavior, no photobehavior could be demonstrated.

Thirdly, the magnitude of this specific, gramicidin-tetraphenylborate-mediated response was found to be dependent on the Na^+ concentration in the extracellular medium. At low extracellular Na^+, no tumbling response is induced with gramicidin-tetraphenylborate treatment. Under these conditions, step-down photobehavior is all but eliminated (see above), thus providing firm evidence that the ionophore treatment is effective. Under these conditions, Na^+ (among other monovalent ions: see below and Discussion) concentrations would be expected to be in equilibrium between the extracellular medium and the inside of the cell. Elevation of Na^+, in the presence of gramicidin D-tetraphenylborate mixtures, now produces an Na^+ concentration-dependent increase in the frequency of directional changes in the cells' swimming paths (Fig. 2a) and a cell-tumbling response (as expressed by the duration of the tumble response: Fig. 2b). In both parameters, a significant change in behavior from control cells is

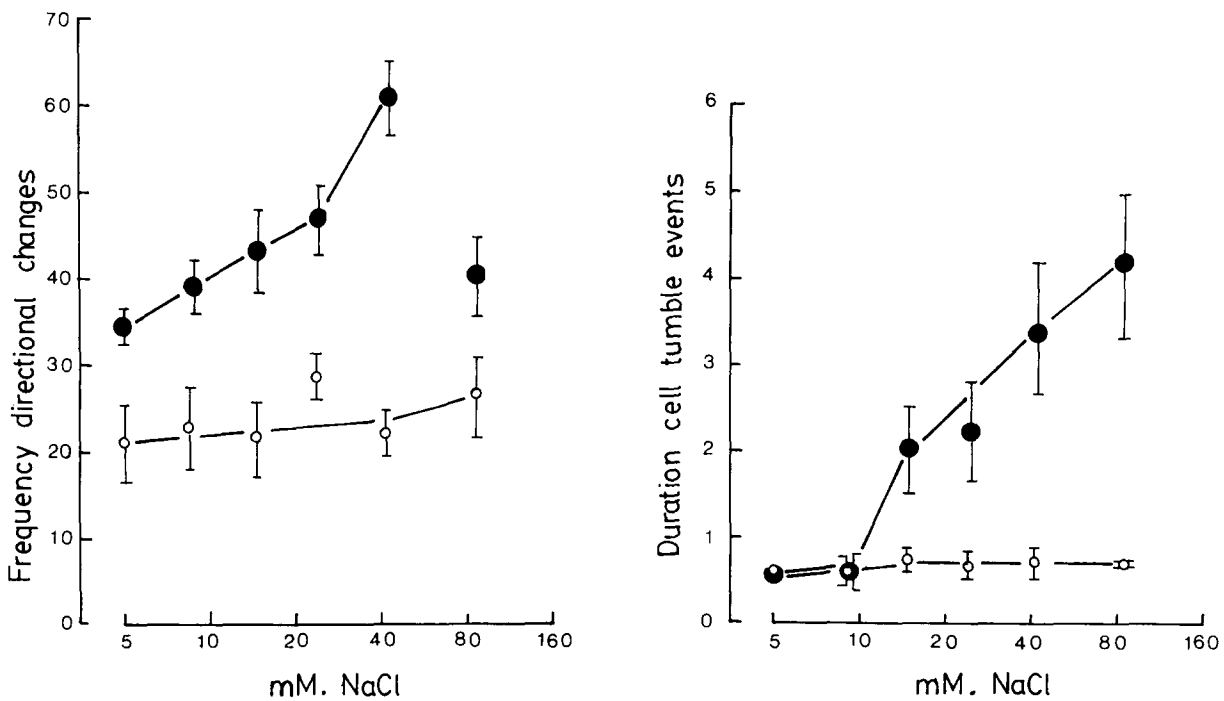

Fig. 2. Na^+ concentration dependence of gramicidin-tetraphenyl-borate-NaCl-induced behaviors. Cells treated with various concentrations of NaCl in Hepes-Pipes adaptation buffers (see text) in either the presence (●) or absence (○) of the ionophore (1.5 μM; 14 $\mu g/10^6$ cells)-tetraphenylborate (2.5 μM; 4.4 $\mu g/10^6$ cells). (A) Frequency of directional changes in changes/min ($n = 17-26 \pm$ S.E.). (B) Duration of cell-tumbling events given in s, $n = 25-70 \pm$ S.E.. 23°C.

seen at greater than 10 mM Na^+ for cells in the Hepes-Pipes adaptation buffers. The Na^+ threshold is slightly lower (greater than or equal to 4 mM) for cells in Tris adaptation buffer – the adaptation buffers differing in their Na^+ concentration.

As a final test of the specificity of the ion study just detailed, possible ionophore-mediated H^+ movement must be considered. Gramicidin-induced alkali metal-H^+ exchange has been reported in other systems [4,8,11]. The gramicidin channel itself is highly selective for monovalent cations and shows a high permeability ratio for H^+/Na^+ [12]. As noted above, the ability of gramicidins to promote ion exchange, i.e., alkali metal/alkali metal or alkali metal/H^+ movement in opposite directions across the membrane, is markedly enhanced by the presence of FCCP with the latter exchange being predominant especially at high ionophore concentrations [4,8]. In addition, the ionophore A23187 that we used in earlier studies [1] catalyses a Ca^{2+}-H^+ electroneutral exchange [1]. As controls for the gramicidin and A23187 studies, i.e., to see if some of the effects due to these ionophores were due to transmembrane H^+ movement, we carried out investigations of the role or magnitude of an H^+ movement in relation to flagellar activity. Such studies are both pertinent to *Euglena* photobehavior control (since earlier hypotheses concerned the role of H^+ in photobehavior [23,24]) and to current concepts on photobehavior control in other microorganisms where evidence for a role of H^+ in sensory transduction has been presented in part using methodology similar to that which we have used (see Discussion).

Euglena swimming velocity was found to be slightly higher at pH 7.1 compared to acid or alkaline pH over the range 3.5–8.2. The enhancement of swimming velocity was accompanied by a reduction in the frequency of directional changes in the cells' swimming path. We have determined that the duration of the step-down photobehavior is not significantly different over the pH range 5.5–8.2 and strong step-down photobehavior is seen for cells in various culture media (pH 3.5–7.2).

These studies indicate a lack of involvement of H^+ movement in photobehavior induction. The rate of photoaccumulation of the cells into a blue light trap was found to be slightly higher at pH 7.1

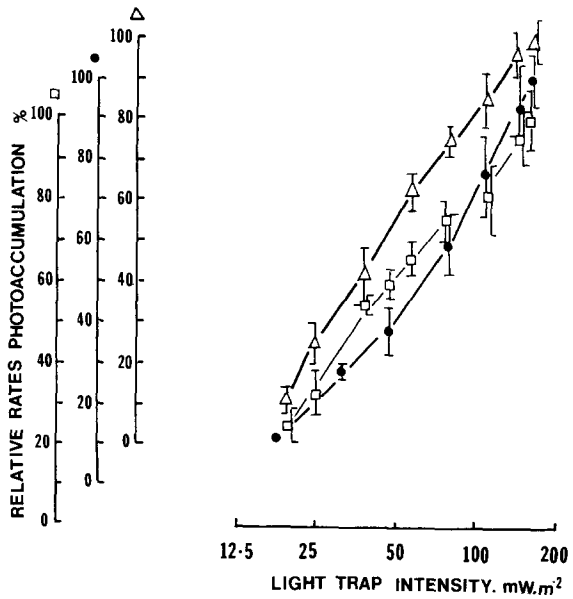

Fig. 3. Effect of extracellular pH on the relative rates of photoaccumulation of cells in blue light traps (475 nm) of different intensities. Fluence-response relationships determined for cells in either Mes (pH 5.5 : □), Hepes-Pipes (pH 7.1 : ●) or Taps (pH 8.2 : △) adaptation buffers (see text). 25°C. Curves slightly displaced for clarity. $n = 4–11 \pm$ S.E. (two to three samples of cells).

compared to pH 5.5 or 8.2. However, fluence rate/relative response plots (light trap intensity vs. relative rate of photoaccumulation) were not found to be significantly different for cells in adaptation buffers at pH 5.5, 7.1 or 8.2 (all other ionic conditions being constant) (Fig. 3). This study clearly suggests that there is no significant H^+ flux involvement in the control of photobehavior since, if transduction involved such an event, changes in such fluence rate-response relationships would be expected. We have also ascertained that the H^+-K^+ exchange ionophore, nigericin (0.3 μg/10^6 cells; 1 μM) has no effect on cell swimming velocity or swimming path character for cells under green light or under blue test illumination either at pH 5.5 or 7.1. Nigericin had no effect on the induction or duration of step-down photobehavior either for cells in standard adaptation buffer or buffer containing 6 mM K^+.

Ca^{2+} dependence of the gramicidin-induced behavior and its blockage by a Ca^{2+} permeability antagonist, La^{3+}

The above studies demonstrate the extreme

sensitivity to and, by implication, the significant role of monovalent cations in the control of photobehavior. By contrast, however, photobehavior and flagellar activity are also altered by Ca^{2+} and the ionophore A23187 [1]. We now present evidence to link the two observations.

The gramicidin-tetraphenylborate-Na^+-induced cell tumbling and elevation in cell swimming path directional change was found to be dependent on the extracellular concentration of Ca^{2+}. No tumbling behavior is induced by the ionophore treatment in the absence of extracellular calcium. The frequency of directional changes increases with extracellular calcium to values far higher than those seen with calcium in the absence of gramicidin [1] and in addition, the duration of the tumble events induced by gramicidin treatment is also related to the extracellular Ca^{2+} concentration (Fig. 4). The Ca^{2+} dependence is further indicated by the inhibitory action of the general Ca^{2+} permeability antagonist, La^{3+} [14]. La^{3+} has an

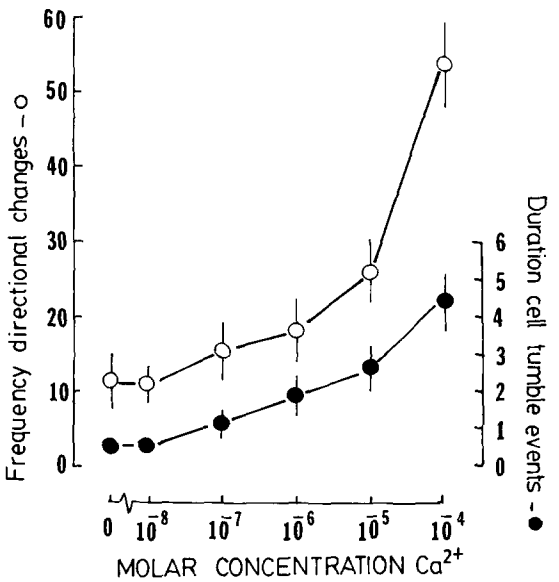

Fig. 4. Ca^{2+} dependence of gramicidin-tetraphenylborate-NaCl-induced behaviors. Cells in Hepes-Pipes-KOH-EGTA buffer containing $CaCl_2$ to give the required free calcium concentrations (see Ref. 1) and treated with ionophore (2.9 μM; 28 $\mu g/10^6$ cells)-tetraphenylborate (4.9 μM; 8.75 $\mu g/10^6$ cells) and NaCl (16 mM) at 23–24°C. Frequency of directional changes given in changes/min; $n = 26–38 \pm$ S.E. Duration of cell-tumbling events given in s; $n = 16–27 \pm$ S.E. Results from two batches of cells.

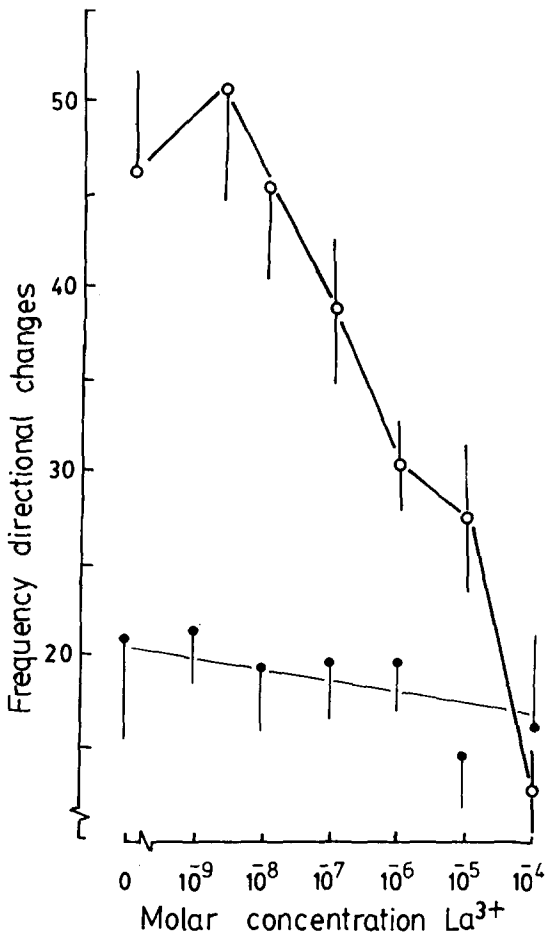

Fig. 5. Inhibition of gramicidin-tetraphenylborate-NaCl-induced behaviors by La^{3+}. Cells treated with various concentrations of $La(NO_3)_3$ in Hepes-Pipes adaptation buffers (see text) in either the absence (●) or presence (○) of ionophore (2.9 μM; 28 $\mu g/10^6$ cells)-tetraphenylborate (4.9 μM; 8.75 $\mu g/10^6$ cells)-NaCl (25 mM) at 23–24°C. $n = 15–31 \pm$ S.E. Results from two batches of cells.

insignificant effect on the frequency of directional changes of the cells in the absence of gramicidin but markedly suppresses the gramicidin-induced elevation in the frequency of directional changes in the cells' swimming paths (Fig. 5). The same concentrations of La^{3+} were also found to suppress step-down photobehavior (Fig. 6), further indicating the relationship of the gramicidin-induced flagellar reorientation to the photobehavior-related flagellar activity. In addition, the previously reported augmentation of photobehavior duration observed on elevation of Na^+ from 0 to 32 mM is

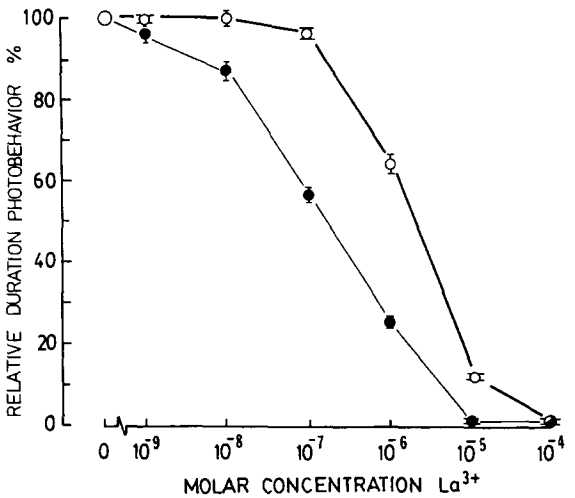

Fig. 6. Inhibition of blue light-induced, step-down photophobic responses by La^{3+}. Cells treated with various concentrations of $La(NO_3)_3$ in Hepes-Pipes adaptation buffers (see text) at 21—22°C. Blue light intensity change, $\Delta I = -225$ mW/m^2. Results given as relative response ±S.E. ($n = 8$–16). ●, initial response (t_{CFR}: 100% = 9.9±0.1 s; ○, total response (t_R): 100% = 40.1 ±0.9 s.

nearly twice as large if the medium contains 2 mM $CaCl_2$ instead of 0.5 mM $CaCl_2$ and is reduced to about 50% of that augmentation seen in the presence of 0.5 mM $CaCl_2$ in the additional presence of 2 mM $CoCl_2$ (data not shown).

Discussion

The present results provide a link between the effects of calcium and calcium ionophore combination on flagellar activity [1] and the effects of monovalent cations and Na^+/K^+ pump inhibitor on photobehavior [3]. The results collectively indicate the presence of monovalent cation gradients either preexistent across the surface (or other) membranes of *Euglena* or generated by blue light. The existence of such gradients and the direction of ion movement (related to flagellar activity and photobehavior at least) in the presence of the ionophores can be rationalized as detailed below:

In the presence of any ionophore, rapid equilibration of relevant ion concentrations across a membrane(s) can be expected due to the high translocation efficiency of most ionophores [4]. The direction of ion flux (influx into or efflux from the cell) to achieve the equilibrium will naturally depend on the relative concentrations in the compartments which the ionophores are linking. For example, if a *Euglena* cell contained a far higher internal concentration of K^+ than that outside the cell then addition of the K^+ ionophore valinomycin would promote K^+ efflux from the cell down its concentration gradient. In *Euglena*, the dominant inorganic cation in the cells has been reported to be K^+ [15], in common with other algae [16]. On ionophore treatment, the initial concentration gradient between the inside of the cells and the extracellular medium will be disipated. The equilibrium concentration of K^+ that will be achieved after ionophore addition will depend on the initial concentrations in the cells and in the extracellular medium. The relative change that has occurred in the chemical concentration gradient for the ion will also change accordingly with change in extracellular K^+ in the short term – where it is assumed that the cells will not have had time to equilibrate (by ionophore-independent mechanisms) to the change in extracellular K^+. Addition of extracellular K^+ to a concentration above that initially present in the cells will result in K^+ influx on ionophore treatment. The valinomycin-induced behavior in *Euglena* (and its suppression by elevation in extracellular K^+) can be readily rationalized on the basis of a valinomycin-induced K^+ efflux at low extracellular K^+ levels. Under this condition, the change in chemical gradient of K^+ that the cells experience will be far larger than the situation in which extracellular K^+ levels are significantly raised. The concentration of extracellular K^+ required to suppress the valinomycin-induced behavior (Fig. 1) indicates that the cells do maintain a significant K^+ electrochemical gradient across some unknown membrane or compartment whose activity is closely coupled to flagellar activity. An equal argument can be presented for the gramicidin-induced behavior and its augmentation by elevation in extracellular Na^+ in the sense that this indicates a greater movement of Na^+ into the cell at the higher extracellular Na^+.

Since photobehavior is expressed in chloroplast-free cells [1] and is unaffected by mitochondrial uncouplers [18,19], the ion-exchange compartment linked to flagellar activity is

either the cell compartment as a whole (with fluxes across the plasma membrane) or the flagellum and flagellar membrane-enclosed photoreceptor unit [1]. Although the ionophores can be expected to equilibrate ions across any compartment, alteration in ion distributions across the two major intracellular compartments (chloroplasts or mitochondria) would appear to be inconsequential to flagellar activity. With no evidence for any plasma membrane ion permeability changes that can be linked to flagellar activity [20,21], we hypothesize that the ionophore effects reflect ion translocation across the flagellar system. The action of gramicidin and tetraphenylborate combinations on photobehavior are thought to reflect general dissipation of monovalent cation gradients. The tetraphenylborate, unless at very high concentrations, is unlikely to significantly alter K^+ levels by complexation on the basis of a K^+ : tetraphenylborate ratio in the insoluble complex of 1 : 10 [22].

We conclude, therefore, that dissipation of cellular K^+ gradients is coupled to change in flagellar activity. Since we have demonstrated the calcium dependence of the Na^+ influx indirectly, (this paper) and the calcium dependence of A23187-induced flagellar reorientation [1], K^+ efflux appears to be coupled to Ca^{2+} influx due to the dependence of flagellar reorientation on internal calcium elevation [1,2]. Direct measurements of the ion fluxes will be required to verify this conclusion. The mechanism of the coupling remains to be elucidated. Finally, we have been unable to obtain any evidence to support previous hypotheses for an H^+ gradient in photobehavior of *Euglena* [23.24]. By similar methods, photobehavior in *Chlamydomonas* and *Stentor* has been shown to be abolished by a small change in extracellular pH [25,26] whereas photobehavior in *Euglena* persists over the pH range 3.5–8.2 (Refs. 1, 19, 27–29 and this study). Photobehavior in *Chlamydomonas* and *Stentor* is significantly altered by protonophorous uncouplers such as 2,4-dinitrophenol, FCCP and nigericin [26,30,31] while 2,4-dinitrophenol, even at pH 3.5 where maximum uptake can be expected [32,33], does not alter induction of step-down photobehavior in *Euglena* [18] although it can have adverse effects on motility [18,19,32] and thus cause apparent inhibition of photoaccumulation behavior [19,34]. Indirect evidence from the pH-dependent uptake of 2,4-dinitrophenol into *Euglena* [32,33] and direct measurements [35] show the existence of a considerable pH gradient between the medium and the inside of *Euglena*. The 2,4-dinitrophenol uptake is cancelled by nigericin [33], indicating that the ionophore can dissipate the pH gradient without affecting photobehavior.

In conclusion, we have provided evidence to support our previous suggestion [1] that intraflagellar free calcium is controlled indirectly by ion gradients of monovalent cations.

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