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Cyclic AMP induces a transient alkalinization in *Dictyostelium*

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In a wide range of cell types, stimulus-response coupling is accompanied by a rise in cytoplasmic pH (pH_i). It is shown that stimulation of developing *Dictyostelium discoideum* cells with the chemoattractant cAMP also results in a rise in pH_i. About 1.5 min after stimulation, pH_i starts increasing from $pH_i \sim 7.45$ to $pH_i \sim 7.60$, as is revealed independently by two different pH null-point methods. The rise in pH_i is transient, also with a persistent stimulus, and effectively inhibited by diethylstilbestrol (DES), strongly suggesting that the rise in pH_i is accomplished by the DES-sensitive plasma membrane proton pump which has been demonstrated in *D. discoideum*.

cytoplasmic pH; cyclic AMP stimulation; Plasma membrane; H^+-ATPase; (*Dictyostelium discoideum*)

1. INTRODUCTION

In *Dictyostelium discoideum* the chemoattractant cyclic AMP (cAMP) induces aggregation and subsequent differentiation of cells [1]. The various receptor-mediated responses induced by cAMP in aggregating *D. discoideum* cells are well documented [2,3] and, interestingly, these responses show a striking similarity to those evoked by chemoattractants and agents that elicit secretion responses in leucocytes, mast cells, platelets and macrophages [4,5]. Thus, stimulation of *D. discoideum* cells by cAMP of human neutrophils by the chemotactic *N*-formylmethionylleucylphenylalanine (FMLP) in both cases results in extracellular acidification [6,7]. In the FMLP-stimulated neutrophils, in addition, a concomitant cytoplasmic alkalinization has been reported [8,9], which seems to be important for the locomotor function of the cells [10]. This, together with the fact that various stimuli have been reported to raise cytoplasmic pH (pH_i) in a variety of cell types (ranging from insulin in frog muscle to growth factors in fibroblasts [11,12]), suggests that the cAMP-induced extracellular acidification in *D. discoideum* might also be accompanied by a concomitant rise in pH_i. Here, this has been investigated.

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2. MATERIALS AND METHODS

2.1. *Cell culture and developmental conditions*

Cells of *D. discoideum* strain Ax-2 were cultured axenically in suspension culture in HL-5 medium at 22°C as in [13]. Cells were harvested, plated on non-nutrient agar for 4 h at 22°C, and resuspended to a density of 10^8 cells/ml in 3.5 mM potassium phosphate buffer. The suspension was aerated and 10 min later used for cAMP stimula-

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tion and subsequent pH_i determination (see below).

2.2. *pH_i measurement by pH null-point determination*

Briefly, with this technique the external pH (pH_e) value is determined at which digitonin or monensin addition to the cell suspension induces no apparent shift in pH_e [14]. This value is taken as a measure of pH_i (see below). The digitonin null-point determinations were performed as described before [15]. Monensin null-point determinations were performed in exactly the same way, except that 30 mM NaCl was added to the suspension before adding monensin (0.06%, w/v; see below).

Because monensin is an Na^+/H^+ ionophore, at $pH_e < pH_i$ the exchange or protons is inhibited by a high extracellular sodium concentration ($[Na^+]_e$), whereas at $pH_e > pH_i$ the exchange of protons is enhanced by high $[Na^+]_e$ (not shown). The best compromise proved to be a $[Na^+]_e$ of 30 mM and this concentration was constantly used in all monensin equilibrations.

It has been shown that digitonin treatment of *D. discoideum* cells selectively permeabilizes the plasma membranes of the cells, while leaving the membranes of endoplasmic reticulum and mitochondria essentially intact [16]. We observed that intracellular ATP levels, measured by HPLC, are not changed after either digitonin or monensin treatment of the cells (now shown), indicating that mitochondrial pH gradients are not disturbed by digitonin or monensin. Furthermore, neutral-red-stained vacuoles do not change their color after either digitonin or monensin addition (not shown), indicating that these acidic organelles are not disrupted. This is also indicated by the fact that after digitonin or monensin treatment, no acid phosphatase activity is detectable in the cell suspension (not shown). After 10 min monensin treatment and subsequent washing, the viability of the cells is not altered because the cells grow and develop like untreated control cells (not shown), indicating that monensin does not disturb intracellular pH gradients. Finally, the pH null-point determined from monensin equilibration increases after treatment of cells with the mitochondrial uncoupler CCCP (not shown), indicating that monensin does not disturb mitochondrial pH gradients. Thus, it is concluded that both digitonin and monensin pH equilibration yields a reliable estimate of pH_i.

3. RESULTS AND DISCUSSION

Addition of cAMP to unbuffered suspensions of developing *D. discoideum* cells results in a transient extracellular acidification [6]. Half-maximal responses occur between 10^{-10} and 10^{-9} M cAMP and from different experiments it was concluded that this acidification is the result of cAMP-induced signal processing via cell-surface receptors [6].

To investigate whether this phenomenon is accompanied by a concomitant rise in pH_i in the cells, developing *D. discoideum* cells were stimulated with 1.5×10^{-7} M cAMP and subsequently changes in pH_i with respect to time were followed by means of two different pH null-point methods. Both methods reveal a transient alkalinization of about 0.15 pH units upon stimulation with cAMP (fig.1); 1.5 min after

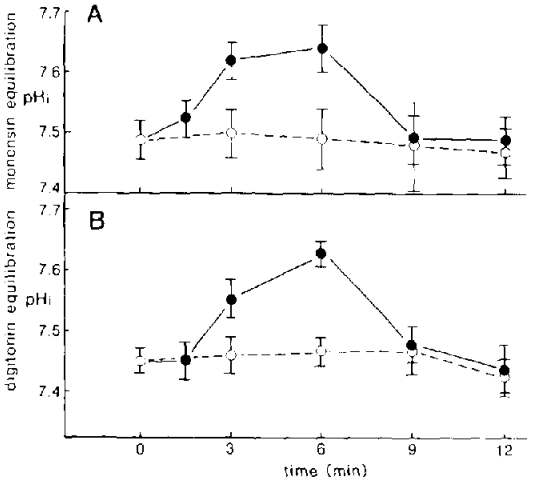

Fig.1. Changes in pH_i after stimulation of *D. discoideum* cells with cAMP. Developing *D. discoideum* cells were either stimulated with 1.5×10^{-7} M cAMP (●) or left unstimulated (○). Subsequently, changes in pH_i were followed by monensin pH null-point determination (A) or digitonin pH null-point determination (B). Means ± SE of 4–10 determinations are shown. For experimental details see Section 2. 6 min after cAMP stimulation the pH_i of the cells is significantly different from that of unstimulated cells ($P < 0.05$ for monensin measurements, $P < 0.001$ for digitonin measurements).

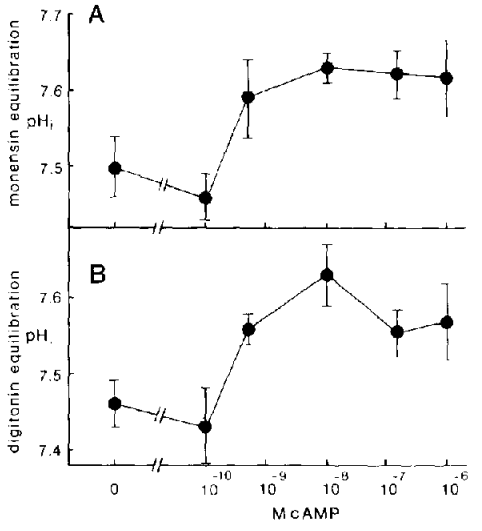

Fig.2. The cAMP-induced rise in pH_i as a function of cAMP concentration. Cells were stimulated with varying concentrations of cAMP and 3 min later pH_i was determined by either monensin pH equilibration (A), or digitonin pH equilibration (B). For experimental details see Section 2 (means ± SE of 3–8 determinations).

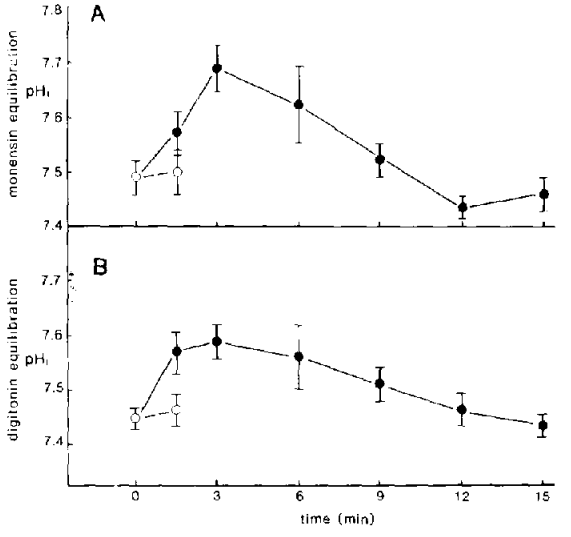

Fig.3. Transient increase in pH_i after stimulation with the cAMP analog cAMPS. (●) Cells were stimulated with 10^{-5} M cAMPS and the subsequent changes in pH_i were followed by either monensin pH equilibration (A) or digitonin pH equilibration (B). (○) Unstimulated control cells (means ± SE of 3–10 determinations).

stimulation pH_i starts increasing from its basal level of pH_i 7.45–7.50 to a value of about pH_i 7.60–7.65, which is reached 3–6 min after stimulation. Thereafter, pH_i decreases until the basal level is reached again about 9 min after stimulation (fig.1). The timing of this rise in pH_i excludes a possible involvement in activation of some of the rapid responses induced by cAMP, such as the accumulation of cAMP and cGMP [2,3].

Fig.2. shows the change in pH_i 3 min after stimulation as a function of the concentration of cAMP applied. Half-maximal responses are obtained between 10^{-10} and 10^{-9} M cAMP, the same concentration range in which half-maximal changes in external pH have been found [6].

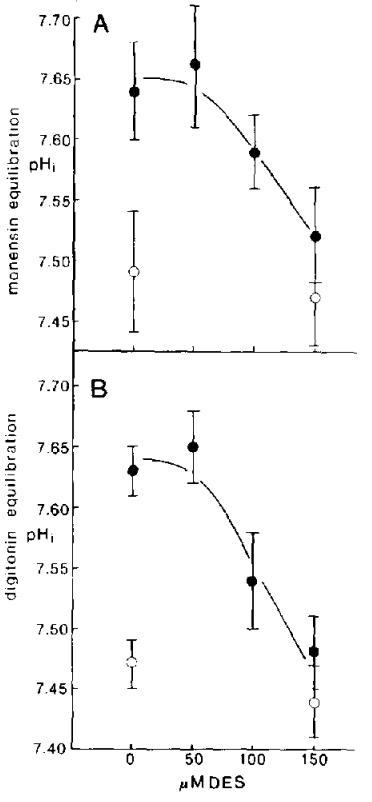

Fig.4. Inhibition of the cAMP-induced rise in pH_i by DES. (●) Cells were preincubated for ~15 min with varying concentrations of DES and subsequently stimulated with 1.5×10^{-7} M cAMP. 6 min later, pH_i was determined by either monensin pH equilibration (A) or digitonin pH equilibration (B). (○) pH_i of unstimulated cells either treated with 150 μM DES of left untreated (means ± SE of 4–7 determinations).

The cAMP analog (Sp)-cyclic adenosine 3′,5′-phosphorothioate (cAMPS) is chemotactically active in *D. discoideum* at concentrations 10–100-fold higher than cAMP, but cAMPS is hydrolysed much more slowly [17,18]. Fig.3 shows that the increase in pH_i after stimulation with 10^{-5} M cAMPS is also transient (cf. [6]), indicating tha the transient character of the cAMP-induced increase in pH_i is not due to hydrolysis of the stimulus.

The induction of differentiation, as judged by enzyme markers or gene expression, is not subjected to adaptation to the cAMP signal [19,20]. Because of its transient character, involvement of the early cAMP-induced pH_i rises in differentiation induction seems unlikely.

In fungi (including *Physarum polycephalum*), algae and higher plants, plasma membrane proton pumps have been demonstrated, which act in an ATP-dependent, electrogenic fashion and which are potently inhibited by the synthetic oestrogen DES [21–24]. Recently, such a DES-sensitive H^+-ATPase has been identified in *D. discoideum* plasma membranes [25,26], and a role of this proton pump in the resistance of acid loads has been suggested [27]. In purified plasma membranes this ATPase activity is half-maximally inhibited by 25–100 μM DES [25,26].

To determine whether the cAMP-induced increase in pH_i (fig.1) might be due to activation of this plasma membrane H^+-ATPase, the effect of DES on the pH_i response was studied. Fig.4 shows that the cAMP-induced cytoplasmic alkalinization can be effectively inhibited by DES; half-maximal inhibition is obtained at about 100 μM DES. The basal pH_i level of unstimulated cells, in contrast, is hardly affected by DES (fig.4). Thus, these data strongly suggest that the cAMP-induced rise in pH_i in *D. discoideum* (fig.1) is accomplished by activation of the plasma membrane H^+-ATPase in these cells.

In conclusion, the present data show that cAMP stimulation of developing *D. discoideum* cells results in cytoplasmic alkalinization (fig.1), which is transient (fig.3), and which is most probably effected by activation of the plasma membrane H^+-ATPase in the cells (fig.4). It will be interesting to study the possible involvement of this pH_i response in the locomotor function of the cells (cf. [10]).

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