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Lithium ions induce prestalk-associated gene expression and inhibit prespore gene expression in *Dictyostelium discoideum*

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Summary

We investigated the effect of Li⁺ on two types of cyclic AMP-regulated gene expression and on basal and cyclic AMP-stimulated inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃) levels. Li⁺ effectively inhibits cyclic AMP-induced prespore gene expression, half-maximal inhibition occurring at about 2 mM-LiCl. In contrast, Li⁺ (1-3 mM) promotes the cyclic AMP-induced increase of cysteine proteinase-2 mRNA levels, and induces the expression of this prestalk-associated gene in the absence of cyclic AMP stimuli. At concentrations exceeding 4-5 mM, LiCl inhibits cysteine proteinase-2 gene expression. LiCl reduces basal Ins(1,4,5)P₃ levels and decreases the cyclic AMP-induced accumulation of Ins(1,4,5)P₃; both effects occur half-maximally at 2-3 mM-LiCl. These results indicate that the induction of the cysteine proteinase-2 gene by Li⁺ is not due to elevated levels of Ins(1,4,5)P₃. It is, however, possible that inhibition of prespore gene expression by Li⁺ is caused by Li⁺-induced reduction of basal and/or stimulated Ins(1,4,5)P₃ levels.

Key words: lithium, gene expression, *Dictyostelium discoideum*.

Introduction

In the presence of nutrients, the cellular slime mold *Dictyostelium discoideum* lives as a unicellular organism. Upon starvation, cells start to secrete the chemoattractant cyclic AMP in a pulsatile manner. Surrounding cells detect cyclic AMP by means of cell surface receptors and the interaction of cyclic AMP with these receptors evokes a number of rapid responses, such as transient synthesis and secretion of cyclic AMP, which is responsible for relay of the original cyclic AMP pulse through the population, and transient increases in the levels of intracellular inositol 1,4,5-trisphosphate (Ins(1,4,5)P₃), Ca²⁺ and cyclic GMP, as well as increases in actin polymerization and myosin heavy-chain phosphorylation. The second group of responses are considered to result in chemotactic movement towards the cyclic AMP source (for review, see Janssens & Van Haastert, 1987). As a consequence of signal relay and chemotaxis, the cells aggregate to form a multicellular mound, which develops into a slug-shaped structure and finally culminates in a fruiting body, composed of stalk cells and spores. Besides its function as a chemoattractant, extracellular cyclic AMP also acts as a morphogen and regulates gene expression during several stages of development.

The chemotactic signal, i.e. nanomolar cyclic AMP pulses accelerate the synthesis of gene products, which are involved in the aggregation process, such as cell surface cyclic AMP receptors, cyclic AMP phosphodiesterase, and the adhesive contact sites A (Darmon et al. 1975; Gerisch et al. 1975; Franke et al. 1987). Steady-state concentrations of cyclic AMP in the micromolar range, which are considered to accumulate in the multicellular structures after aggregation (Wang et al. 1988), induce the expression of prespore-specific genes as well as the synthesis of a class of gene products, which are preferentially present in the anterior prestalk region of the slug (Kay, 1982; Schaap & Van Driel, 1985; Mehdy et al. 1983; Oyama & Blumberg, 1986).

Earlier studies have shown that the induction of prespore- and prestalk-associated gene expression by cyclic AMP is most likely mediated by the chemotactic cyclic AMP receptor (Schaap & Van Driel, 1985; Oyama & Blumberg, 1986; Haribabu & Dottin, 1986), but the intracellular mechanisms mediating cyclic AMP-induced gene expression are still obscure. In the case of prespore gene expression, it was demonstrated that cyclic AMP-induced adenylate and guanylate cyclase activation are not involved in transduction of the response. This type of gene expression was, however, strongly inhibited by
Ca\(^{2+}\) channel blockers and inhibitors of the interaction of Ca\(^{2+}\) with Ca\(^{2+}\)-binding proteins (Schaap et al. 1986).

In higher eukaryotes, the interaction of certain growth factors and hormones with surface receptors induces hydrolysis of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) into 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3). Ins(1,4,5)P_3 is postulated to induce the release of Ca\(^{2+}\) from intracellular stores, which in turn activates Ca\(^{2+}\)-binding proteins, such as calmodulin, or in combination with DAG, protein kinase C. Tumor-promoting phorbol esters bypass the requirement for the ligand–receptor interaction and activate protein kinase C directly (Downes & Michell, 1985; Nishizuka, 1984).

In Dictyostelium, cyclic AMP also induces Ins(1,4,5)P_3 production (Europe-Finner & Newell, 1987; Van Haastert, unpublished), while Ins(1,4,5)P_3 was shown to induce an increase in intracellular Ca\(^{2+}\) levels (Europe-Finner & Newell, 1986). The sensitivity of prespore induction to Ca\(^{2+}\) antagonists could therefore indicate that these responses to cyclic AMP mediate the transduction of the signal to prespore gene expression. However, attempts to bypass cyclic AMP-induced Ca\(^{2+}\) and DAG production by treatment of permeabilized cells with Ca\(^{2+}\) and phorbol esters have hitherto not been successful (unpublished data).

In this study, we attempt another approach to investigating a possible involvement of cyclic AMP-stimulated Ins(1,4,5)P_3 accumulation in the regulation of gene expression. It was recently found that LiCl inhibits several steps in the dephosphorylation of inositol phosphates in Dictyostelium in vitro (Van Lookeren Campagne et al. 1988) and could significantly alter basal and stimulated inositol phosphate levels in vivo. Early reports showed that LiCl diverts Dictyostelium cells from the spore into the stalk differentiation pathway (Maeda, 1970; Sakai, 1973). We investigated the possibility that LiCl causes this effect by interfering with the intracellular processing of cyclic AMP signals to cell-type-specific gene expression. It was found that LiCl inhibits cyclic AMP-induced prespore gene expression, while it promotes and partially bypasses cyclic AMP induction of prestalk-associated gene expression. These results are discussed in relation to the effects of LiCl on cyclic AMP-activated Ins(1,4,5)P_3 production in Dictyostelium.

Materials and methods

Materials

\([\alpha-\text{32P}]\text{dATP}\) and the Ins(1,4,5)P_3 assay kit were obtained from Amersham (UK). Gene Screen was from New England Nuclear, cyclic AMP from Sigma, LiCl from Merck and beef-heart phosphodiesterase from Boehringer-Mannheim.

Culture and incubation conditions

Dictyostelium discoideum NC4 (H) was cultured on glucose-peptone agar in association with Escherichia coli 281 as described (Schaap & Spek, 1984). Vegetative cells were freed from bacteria, by repeated washing with 10 mM-Na/K phosphate buffer, pH 6.5 (PB) and resuspended in PB to 10^7 cells ml^{-1}. The cell suspension was shaken at 150 revs min^{-1} and 21°C.

To obtain aggregation-competent cells, vegetative cells, freed from bacteria were distributed on non-nutrient agar plates (1·5 % agar in PB) at 2·5 X 10^6 cells cm^{-2} for 16 h at 6°C. The cells were subsequently incubated in PB at 5 X 10^7 cells ml^{-1}. Cells were pulsed with 30 nm-cyclic AMP every 6 min or cyclic AMP was added every hour as a single dose of 10^{-9} M. During incubation of both vegetative and aggregation-competent cells, the cells were collected by centrifugation at 2-h intervals and resuspended in fresh incubation mixture. This was done to avoid excessive accumulation of cellular secretion products.

RNA isolation and analysis

Total cellular RNA was isolated from 5 X 10^7 lysed cells as described previously, purified by phenol/chloroform extraction and ethanol precipitation (Mann & Firtel, 1987). RNA size was fractionated on 1·5 % agarose gels containing 2·2 M-formaldehyde and transferred to Gene Screen membranes. Northern transfers were hybridized in 50 % formamide and 1 % SDS in 5 X SSPE (0·9 M-NaCl and 5 mM-EDTA in 50 mM-NaPO_4, pH 7·4) with 5 X Denhardt's solution (0·25 % Ficoll, 0·25 % polyvinylpyrrolidone, 0·25 % bovine serum albumin (BSA)) to cDNA probes, which were labeled with [\alpha-32P]dATP by means of nick-translation (Rigby et al. 1977). After hybridization, the membranes were rinsed three times with 0·5 X SSPE and washed twice during 30 min at 42°C with 1 % SDS in 5 X SSPE and exposed to X-ray films. The amount of 32P-labeled cDNA probes hybridized to specific mRNAs was semiquantitatively determined by measuring the absorbance on autoradiographs by means of an Ultrascan XL densitometer (LKB).

Determination of LiCl effects on basal and stimulated Ins(1,4,5)P_3 levels

Vegetative cells were starved in PB at 10^7 cells ml^{-1}. After 5 h the cells were washed and incubated at 10^6 cells ml^{-1} with 0 to 10 mM-LiCl for 15 min. To measure LiCl effects on basal Ins(1,4,5)P_3 levels, 100 μl of cell suspension was added to 100 μl of 3·5 % perchloric acid (PCA). To measure LiCl effects on cyclic AMP-stimulated Ins(1,4,5)P_3 levels, the cells were stimulated with 10^{-6} M-cyclic AMP and 100 μl cell suspension was added to 100 μl of 3·5 % PCA at different times after cyclic AMP stimulation. The samples were neutralized with 50 μl of 50 % saturated KHCO_3 and the amount of Ins(1,4,5)P_3 was measured with a commercially available Ins(1,4,5)P_3 isotope dilution assay kit. The kit was tested for the specificity for Ins(1,4,5)P_3 by Van Haastert (1989). The assay was performed as described by the manufacturer, with the following modifications: the incubation mixture contained 20 μl of each [\text{H}] Ins(1,4,5)P_3 (2700 cts min^{-1}), neutralized cell lysate, Ins(1,4,5)P_3-binding protein, and assay buffer. After 15 min of incubation at 0°C, samples were centrifuged for 2 min at 10000 g. The supernatant was aspirated and the radioactivity of the pellet was measured by liquid scintillation counting. Data were converted to pmol of Ins(1,4,5)P_3 by using a standard curve with authentic Ins(1,4,5)P_3.

Results

Effects of Li\(^{+}\) on cyclic AMP-induced prespore gene expression

Prespore gene expression is effectively induced by micro-molar cyclic AMP concentrations in aggregation-competent cells (Schaap & Van Driel, 1985; Oyama & Blumberg, 1986). Fig. 1 shows that D19 prespore mRNA levels increase strongly after 5 h of incubation
Fig. 1. Dose dependency of the effects of LiCl on the cyclic AMP-induced increase of D19 mRNA levels. Aggregation-competent cells were incubated in PB with various LiCl concentrations in the presence of 30 μM-cyclic AMP, added every hour. After 5 h of incubation, RNA was isolated, size fractionated and probed with D19 cDNA. The data represent relative O.D. values of D19 mRNA bands on autoradiograms expressed as percentage of values obtained after cyclic AMP stimulation in the absence of LiCl.

Effects of Li⁺ on cysteine proteinase-2 gene expression in vegetative cells

In aggregation-competent cells, micromolar cyclic AMP concentrations also induce the expression of a class of genes whose products are preferentially, but not exclusively, present in the prestalk region of the slug, such as the prestalk cathepsin or cysteine proteinase-2 (CP-2) gene (Mehdy et al. 1983; Pears et al. 1985). We recently found that CP-2 gene expression can also be induced by nanomolar cyclic AMP pulses instead of micromolar cyclic AMP concentrations. Fig. 2 shows the effect of nanomolar cyclic AMP pulses and micromolar cyclic AMP concentrations on the levels of CP-2 mRNA in vegetative and aggregation-competent cells. In vegetative cells, CP-2 mRNA levels increase strongly after 4–6 h in response to nanomolar cyclic AMP pulses. Micromolar cyclic AMP concentrations also induce an increase in CP-2 mRNA levels, which seems to occur somewhat earlier than induction by pulses (Fig. 2A). In aggregation-competent cells, CP-2 mRNA is already present at a low level and a further increase is induced by nanomolar cyclic AMP pulses. However, in this stage, stimulation with micromolar concentrations increases CP-2 mRNA to higher levels than stimulation with nanomolar pulses (Fig. 2B).
of CP-2 mRNA by cyclic AMP pulses. These experiments demonstrate that the inducing effects of LiCl represent a true bypass of the cyclic AMP receptor and seem to be counteracted by low background cyclic AMP levels.

Effects of LiCl on basal and stimulated Ins(1,4,5)P₃ levels

It was recently shown that LiCl effectively inhibits dephosphorylation of inositol phosphates in *D. discoideum* in vitro (Van Lookeren Campagne et al. 1988), which may result in altered intracellular levels of these compounds in vivo. We investigated the effects of Li⁺ on Ins(1,4,5)P₃ levels in cyclic AMP-stimulated and non-stimulated cells. As shown in Fig. 5A, cyclic AMP induces a transient Ins(1,4,5)P₃ accumulation. Half-maximal inhibition of cyclic AMP-stimulated Ins(1,4,5)P₃ accumulation occurs at about 2–3 mM-LiCl (Fig. 5B). LiCl also causes a 30% reduction of the basal Ins(1,4,5)P₃ levels (Fig. 5B), with a half-maximal effect at 2.5 mM-LiCl. It thus appears that in vivo, LiCl acts to decrease basal and stimulated Ins(1,4,5)P₃ levels.

Discussion

Li⁺ exerts divergent effects on cyclic AMP-induced cell type-specific gene expression in *D. discoideum*. It effectively inhibits cyclic AMP-induced prespore gene expression, half-maximal inhibition occurring at about 2 mM-LiCl. The effects of Li⁺ on the expression of the

![Figure 3](image-url)
CP-2 gene, which is preferentially expressed in prestalk cells, are more complex. It was previously reported that CP-2 gene expression is induced in response to constant micromolar cyclic AMP stimulation (Mehdy et al. 1985; Peers et al. 1985). We show here that in vegetative cells CP-2 expression is also effectively induced by nanomolar cyclic AMP pulses (Fig. 2A). Depending on the concentration, LiCl exerts both stimulatory and inhibitory effects on CP-2 expression (Fig. 3). At concentrations from 1 to 3 mM, LiCl enhances the induction of CP-2 expression by cyclic AMP pulses (Fig. 3A,B,D) and by constant micromolar cyclic AMP levels (Fig. 3C). Furthermore, at these concentrations LiCl can partially replace the requirement for cyclic AMP stimulation to increase CP-2 mRNA levels. The LiCl-induced increase in CP-2 mRNA levels is more pronounced when extracellular cyclic AMP levels are reduced by addition of cyclic AMP-phosphodiesterase (Fig. 4). It is possible that low background cyclic AMP levels cause partial desensitization of the cells (Theibert & Devreotes, 1983), which counteracts the stimulatory effect of LiCl. At concentrations above 4–5 mM, LiCl no longer induces an increase in CP-2 mRNA levels and inhibits the increase induced by cyclic AMP pulses (Figs 3, 4). Possibly, the stimulatory effect of LiCl would be more pronounced if this inhibitory effect was not also present to counteract it.

An obvious explanation of the stimulatory effects of LiCl would be that it increases the levels of the intracellular messenger Ins(1,4,5)P\textsubscript{3}, which in turn results in stimulation of CP-2 gene expression. In D. discoideum homogenates Li\textsuperscript{+} inhibits the dephosphorylation of Ins(1,4,5)P\textsubscript{3} at the 1 position (Van Lookeren Campagne et al. 1988). Thus Ins(1,4,5)P\textsubscript{3} levels may increase in Li\textsuperscript{+}-treated cells. On the other hand, Li\textsuperscript{+} also inhibits the dephosphorylation of inositol monophosphate. Since inositol monophosphate, formed from either glucose 6-phosphate or from inositol polyphosphates, is the endogenous source of inositol, this effect of Li\textsuperscript{+} may lead to reduced levels of inositol and hence of PtdIns(4,5)P\textsubscript{2} and Ins(1,4,5)P\textsubscript{3}. Using the recently introduced isotope dilution assay for Ins(1,4,5)P\textsubscript{3}, the molar levels of this compound were determined in Li\textsuperscript{+}-treated cells (Van Haastert, 1989).

LiCl concentrations of 1 mM and higher were found to decrease intracellular Ins(1,4,5)P\textsubscript{3} levels and the cyclic AMP-stimulated Ins(1,4,5)P\textsubscript{3} accumulation (Fig. 5). It is therefore clear that the stimulatory effects of LiCl on CP-2 gene expression are not due to stimulation of Ins(1,4,5)P\textsubscript{3} production. We recently measured effects of LiCl on cyclic AMP binding and on two other cyclic AMP-activated second messenger systems. Besides inducing a small increase in the affinity of the cell surface cyclic AMP receptor, LiCl does not stimulate the cyclic AMP-induced activation of adenylate or guanylate cyclase activity, which indicates that the stimulatory effect of LiCl on CP-2 gene expression is also not due to stimulation of cyclic AMP-induced cyclic AMP or cyclic GMP production (unpublished data).

The inhibitory effects of LiCl on cyclic AMP-induced Ins(1,4,5)P\textsubscript{3} production argue against induction of CP-2 gene expression by Ins(1,4,5)P\textsubscript{3}, but could be the cause of the inhibition of prespore gene expression by LiCl.

A possible involvement of Ins(1,4,5)P\textsubscript{3}, and Ins(1,4,5)P\textsubscript{3}-induced release of Ca\textsuperscript{2+} from intracellular stores, as intermediates of cyclic AMP-induced prespore

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gene expression, is corroborated by earlier findings that prespore gene expression is effectively inhibited by Ca\(^{2+}\) channel blockers and by antagonists of Ca\(^{2+}\) binding proteins (Schaap et al. 1986).

Although the present study shows that LiCl does interfere with Dictyostelium inositol phosphate metabolism, this process need not be the only target for the effects of Li\(^{+}\). It was shown, for instance, that in the rat cerebral cortex Li\(^{+}\) also interferes with the function of G-proteins (Avissar et al. 1988). The observed LiCl-induced increase of cyclic AMP binding activity could reflect an effect on G-proteins, since the functional interaction between cyclic AMP receptors and G-proteins involves changes in receptor affinity (Janssens et al. 1986; Van Haastert et al. 1986). The identity of the target for the effect of Li\(^{+}\) on CP-2 expression will be further investigated.

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