Cytosolic acidification as a signal mediating hyperosmotic stress responses in Dictyostelium discoideum

ABSTRACT

These findings suggest that hyperosmotic stress, along with a pH signal, induces pleiotropic effects by downregulating cellular activity. Our findings are highly comparable to those of hyperosmolarity and intracellular acidification on receptor-mediated endocytosis in mammalian cells, implying the same mechanism of inhibition by low internal pH.

INTRODUCTION

Background Glucocorticoids exert an impact on many cellular functions by acting on solubility intracellular receptors; unliganded gluco corticoid receptor (GR) are located mainly in the cytoplasm as a heteromeric complex of GR, 90-kDa and 70-KDA heat shock proteins (hsp90 and hcs■70) in most cells. Additional protein components, such as FKBP52, F1B2, and F2B3, have been implicated in the stabilization/assembly of the GR-hosp90-HSP70-complex with a high affinity for agonist. After agonist binding, the complex undergoes modification, and the ligand-bound GR translocates to or from the nucleus in accordance with a nuclear localization sequence (NLS) present in the receptor. The GR acts as a transcriptional stimulator or repressor of primary response genes by binding to glucocorticoid hormone-responsive elements (GRE) present in the promoter regions of steroid-rearing genes, which can either facilitate or inhibit the formation of an active transcriptionally complex. The molecular mechanism that guides steroid receptor movement from the cytoplasm into the nucleus is not well understood, but several investigations suggest that this movement is mediated by reversible phosphorylation. Qi et al.'s research revealed that hormone insensitivity caused by cellular transformation with v-mos is due to both reduced nuclear retention of liganded receptors and decreased reutilization of their GR protein, which cycles back into the cytoplasm. Later, DeFranco et al. discovered that treatment with okadaic acid, a significant inhibitor of ser/thr protein phosphatase, leads to the inefficient nuclear retention of agonist-bound GR and the non-recyclable cytoplasmic "trapping" of highly active GH. Okadaic acid has been used in studies to indicate that phosphorylation alters the high affinity binding of GR to hsp-90, and that an intact cytoskeleton is essential for ligand-activated CR to transfer from the ER to the nucleus. The fact that okadaic acid can alter intracellular partitioning in GR implies that an PPase sensitive to ocasional acid may play a role in the control of GD movement. Okadic acid, on the other hand, acts as hexogenogeneoretic acid (ORG) receptor antagonists, both of which act as active forms of serine/threonine protein phosphatases type 1 (PP1) and 2A (P2A) in vitro. Okadaic acid treatment has been associated with the inhibition of two enzymes, resulting in numerous effects. However, due to limitations in solubility and toxicity, it is challenging to differentiate the actions of PP2A from pp1 using okadic acid in living cells. Also, in humans, there are now four PP1 isoforms (PP, pp and p2a), two structurally related isomegases (PPP2A and Paprika) A fourth of phosphataze systems, known as PD2, are present in the human body. Although no detailed dose-response studies have been conducted on native AP5, CP6, and even DP7, evidence from experiments with both corresponding peptides indicates that they are also sensitive to okadaic acid Despite the lack of specific PP5 substrates, studies have shown that a protein encoding complex called PKD 5 may interact with GRs. Furthermore, PP5 is predominantly inactive and accounts for less than 1% of measurable

peptide phosphatase activity in crude cell homogenates. To identify the cellular functions of this protein, we have developed chimeric antisense 2'-O-(2-methoxy) ethylphosphothioate oligonucleotides that can suppress human p53 expression at nanomolar levels. The use of ISIS 15534, which targets PP5 through RNAase H-mediated degradation, hinders the assessment of rapid changes in mRNA degradation and protein levels due to the preexisting protein's half-life. However, ISIS 15534 effectively suppresses the expression of PP5 in cultured cells for 48-72 hours (IC50 of less than 75 nm), providing a period of approximately 24 to 48 hours during which APK1/2 is destroyed. Secondly, because ISIS 15534 does not modify the structurally related PPases, it can be used to specifically inhibit the actions of 'PP5' by suppressing PI5 protein levels in cultured human cells. In order to evaluate the role of [3H]dexamethasone in regulating GR-mediated events, binding studies were conducted before and after ISIL 15634-stable suppression of PPA expression. These studies indicate, however, that the inhibition of PP5 expression did not seem to have an effect on the binding of dexamethasone, and thus that essentially no mechanism for altering the formation of the high-affinity ligand binding complex (or hormones binding to the GR). On the other hand, mobility gel-shift analysis demonstrated that ISIS 15534 treatment significantly enhances GR association with GRE-containing DNA and that transient transfection studies using a gre-responsive reporter plasmid showed that the inhibition of PP5 expression leads to triggered CR4 transcription (GR-dependent) transcription in the absence of hormone release. Considering the additive effects of ISIS 15534 on A549 cells, (maximum induced luciferase activity is 10 times higher than the maximal dexamethasone-induced response in presence of PP5) it now appears that'some model of post-recombination protein synthesis using pb and dna reducta integris (PP) modulates GR-mediated transcription. Using a GR-GFP fusion protein and fluorescent microscopy, we were able to observe the movement of Grass membranes (GR) in cells treated with dexamethasone and then ISIS 15534 in our study. These studies suggest that this PP5 mediated suppression of [GR]function stems from its ability to suppress the nuclear accumulation of random genes expressed as gram-positive peptide 5.

CONCLUSION

Measuring fertilization-induced calcium transients provides a novel experimental technique for studying C. elegans. Researchers can now use this technique to detect potential calcium defect in many mutants with known fertilizer defects using forward genetic and gene knockout and RNAi methods. It is recommended that elegans permit the identification of proteins that may be involved in this crucial step of embryonic development.