Journal of Cellular Biochemistry 86:154–161 (2002)

Activation of Stress Response by Ionomycin in Rat Hepatoma Cells

A.S. Sreedhar and Usha K. Srinivas*

Centre for Cellular and Molecular Biology, Hyderabad 500 007, India

Abstract All living systems respond to a variety of stress conditions by inducing the synthesis of stress or heat shock proteins (HSPs), which transiently protect cells. HSP synthesis was preceded by an increase in intracellular free calcium concentration [$(Ca^{2+})i$]. In this study, we show that Ca^{2+} ionophore, ionomycin, induced an immediate increase in intracellular free Ca^{2+} and examined how this increase affects heat shock response in rat hepatoma cell line H4II-E-C3. Results indicate that incubating H4II-E-C3 cells with 0.3 μM ionomycin at 37°C for 15 min results in the induction of HSP 70 in both Ca^{2+}-containing and Ca^{2+}-free medium. Associated with this increase in free Ca^{2+} is an *in vivo* change in membrane organization and activation of signaling molecules like ERKS and SAPKs/JNK. In Ca^{2+} containing medium HSP 70 induction mediated by HSF–HSE interaction was faster upon ionomycin treatment as compared to heat shock. Our results show that ionomycin, at sub lethal concentration, increases intracellular free Ca^{2+} concentration, activates SAPK/JNK and HSF–HSE interaction, and induces HSP 70 synthesis. J. Cell. Biochem. 86: 154–161, 2002.

Key words: HSP 70; stress response; ionomycin

Stress response is a finely regulated and highly conserved cellular response, which results in the synthesis of a specific set of proteins referred to as heat shock proteins (HSPs) or stress proteins [Lindquist and Craig, 1988]. Induction of HSPs is a universal phenomenon, and HSPs provide cytoprotection when cells/organisms encounter adverse environmental conditions [Lindquist and Craig, 1988; Welch, 1992]. Induction of stress proteins can be modulated and is preceded by a variety of changes in the cells, which include change in membrane organization [Revathi et al., 1994], activation of kinases and phosphatases that function in signal transduction [Kyriakis et al., 1994; Cohen, 1996; Lassle et al., 1997], change in cytoplasmic pH [Srinivas and Revathi, 1993], change in cytoskeletal organization, and disruption of polysomes [Welch and Suhan, 1985; Welch and Mizzen, 1988]. Activation of heat induced signal transduction cascade leads to phosphorylation, multimerization, and translocation of a specific heat shock transcription factor (HSF1) into the nucleus and its binding to a specific heat shock element (HSE) present in the promoters of heat shock genes resulting in the synthesis of HSPs [Morimoto et al., 1992; Morimoto, 1993; Sarge et al., 1993]. Stress or heat shock was shown to increase intracellular Ca^{2+} [Yamamoto et al., 1994; Itagaki et al., 1998], and this increase in [$(Ca^{2+})i$] appears to be essential for the multistep activation of heat shock factor in permeabilized cells [Price and Calderwood, 1991]. Perturbations in intracellular calcium was reported to directly modulate induction of HSP 26 synthesis [Evans et al., 1991]. Also, a calcium dependent metabolic process was shown to be involved in the generation of heat shock signal [Lamarche et al., 1985], and an increase in intracellular calcium was shown to stimulate HSF1 translocation into the nucleus resulting in HSP 70 expression [Ding et al., 1996]. Cell injuries leading to increased [$(Ca^{2+})i$] in rat proximal tubule epithelium was shown to result in the induction of c-fos, c-jun, c-myc, and HSP 70 probably by activating intracellular Ca^{2+} dependent protein kinases [Yamamoto et al., 1993]. It was also reported that heat preconditioning confers

Usha K. Srinivas is a scientist and project leader at Centre for Cellular and Molecular Biology.

*Correspondence to: Dr. Usha K. Srinivas, Centre for Cellular and Molecular Biology, Hyderabad 500 007, India. E-mail: ushaks@gene.ccmbindia.org

Received 22 January 2002; Accepted 21 March 2002

DOI 10.1002/jcb.10204

protection from Ca^{2+}-mediated cell injury [Kuhlmann et al., 1997]. In human erythroleukemia cells calcium ionophore, A23187, was reported to alter heat induced HSF1 phosphorylation and inhibit HSP 70 mRNA transcription and thereby HSP 70 expression [Elia et al., 1996]. These studies clearly demonstrate a role for Ca^{2+} in stress response, but the precise function of increase in intracellular Ca^{2+} during heat shock response is not clearly understood.

Ionomycin, a calcium ionophore has multiple effects on cellular growth and apoptosis [Liu and Harmann, 1978; Orrenius and Nicotera, 1994]. Ionomycin was reported to increase intracellular Ca^{2+} and in the presence of other chemicals was shown to induce HSP synthesis [Khanna et al., 1995]. In comparison to another Ca^{2+} ionophore A23187, ionomycin is more effective as a mobile ion carrier for Ca^{2+} and is more useful for measuring cytoplasmic free Ca^{2+} [Lee, 1993].

In the present study, we investigated the effect of ionomycin on rat hepatoma cells specifically to understand its role in stress response. Our results show that exposure of hepatoma cells to 0.3 μM ionomycin for 15 min was sufficient to induce stress response and HSP 70 synthesis. We also demonstrate that there is an immediate change in intracellular free Ca^{2+} and an in vivo change in membrane order following ionomycin treatment and report activation of stress activated protein kinase (SAPK), which probably results in the activation of signal transducers leading to HSF–HSE interaction and HSP 70 synthesis.

MATERIALS AND METHODS

Cell Culture

H4II-E-C3, rat hepatoma cells were grown in DMEM containing 10% FCS at 37°C in the presence of penicillin (100 μg/ml) and streptamycin (50 μg/ml), unless and otherwise indicated, in 5% CO_2 atmosphere as described earlier [Swamynathan et al., 1996]. To understand the effect of ionomycin on H4II-E-C3 cells in Ca^{2+}-free medium, cells were adapted to grow in Ca^{2+}-free medium for 16 h prior to ionomycin treatment or heat shock.

Heat Shock and Ionomycin Treatment

Exponentially growing cells (1×10^6 cells/ml) were treated with different concentrations of ionomycin (Calbiochem) from 0.1 to 2 μM for different time periods. It was found that ionomycin at a concentration of 0.3 μM for 15 min at 37°C was ideal for 100% cell viability and induction of HSP 70 synthesis. All subsequent experiments were done under these conditions. Also cells were given heat shock at 42°C for 45 min in a water bath [Swamynathan et al., 1996]. Ionomycin treated and heat shock cells were either processed immediately for analysis or were allowed to recover at 37°C in 5% CO_2 atmosphere for an appropriate time before analysis.

Western Blotting

Cell lysates of 3×10^5 cells/ml per lane were run on 10% SDS–PAGE, transferred onto nitrocellulose membrane (Amersham Biotech) and probed with anti-HSP 70, or anti-gp96 (Stressgen, Canada), or anti-ERK-1&2 (Santhacruz, CA), or SAPK/JNK (New England Biolabs, Canada). The bands were visualized using HRPO conjugated secondary antibody for ERK-1 & 2, HSP 70, and gp96 and biotin conjugated secondary antibody for SAPK/JNK using ECL kit from Amersham Pharma.

Electrophoretic Mobility Shift Assay (EMSA)

Whole cell extracts were prepared after different treatments, and HSF–HSE interaction was monitored using a specific oligonucleotide containing multiple HSEs as described earlier [Swamynathan et al., 1996]. Binding of HSF to HSE can be seen in the autoradiogram as a change in the mobility of the labeled oligo in 4% non-denaturing polyacrylamide gels.

Measurement of Intracellular Calcium [$(Ca^{2+})i$]

For measuring [$(Ca^{2+})i$], ionomycin treated cells were washed free of the ionophore, resuspended in HEPES buffer containing 1 μM Indo-I AM (Molecular Probes, Inc.) in triplicate and incubated for 30 min at room temperature. Cells were washed to remove free dye, and fluorescence emission spectra of samples was recorded with excitation at 338 nm. The relative shift of the peak from 475 to 400 nm and relative increase in peak height at 400 nm, which is a measure of free Ca^{2+} bound to Indo-1 AM, was recorded.

Membrane Polarization and Membrane Organization

Rat hepatoma cells (2×10^6/ml), after treatment with ionomycin or immediately after heat

shock, were incubated with 1 mM DPH (1,6 diphenyl-1,3,5 hexatriene) (Molecular Probes, Inc.) in triplicate for 15 min as described earlier [Revathi et al., 1994]. The cells were washed free of DPH, resuspended in 2 ml of PBS and the fluorescence was immediately measured using a Hitachi F-400 steady state spectrophotometer using 1 cm path length quartz cuvettes with an excitation at 358 nm and emission at 438 nm. Fluorescence polarization values were recorded after adapting the cells in dark chamber each time. The polarization values reported in Table I represent an average of 12 measurements in each case.

RESULTS

Induction of HSP 70 Synthesis Upon Ionomycin Treatment

To investigate the effect of Ca^{2+} mobilizing ionophore, ionomycin, on rat hepatoma cells with reference to HSP 70 synthesis, total cell lysate of samples collected at different time intervals after ionomycin treatment or heat shock in Ca^{2+}-containing medium and in Ca^{2+}-free medium were analysed on Western blot with anti-HSP 70 antibody. Results presented in Figure 1 along with densitometric scans show induction of HSP 70 synthesis upon ionomycin treatment in both Ca^{2+}-containing and Ca^{2+}-free medium (Fig. 1C,D). However, synthesis of HSP 70 was faster in ionomycin treated samples as compared to heat shock in Ca^{2+}-containing medium. The pattern of HSP 70 synthesis appears similar under both conditions in Ca^{2+}-free medium.

GP96, another cellular protein, did not show any change in expression under the same conditions. Experiment was repeated several times with similar results.

Transcriptional activation of heat shock genes upon ionomycin treatment was further demonstrated by EMSA done with ^{32}P labeled canonical HSEs with cell extracts prepared from ionomycin treated and heat shocked hepatoma cells. Results presented in Figure 2 shows faster activation of HSF and stronger binding to HSEs in cell extracts from ionomycin treated cells (compare Fig. 2A,B). Also HSF–HSE binding increased with time in ionomycin treated cell extracts till 45 min as compared to their binding with cell extracts from heat shocked samples confirming transcriptional activation of HSP 70 gene upon ionomycin treatment. Appearance of HSF–HSE complex immediately upon ionomycin treatment, and its increase till 45 min was consistently seen in several experiments. Figure 2A2, B2 are densitometric scans of 2A1 & 2B1.

TABLE I. Fluorescence Polarization of DPH Incorporated Into H4II-E-C3 Cells

		Polarization value (P)	ΔP
Heat shock	37°C	0.321 ± 0.010	0.022
	42°C	0.343 ± 0.001	
Ionomycin	Control	0.303 ± 0.010	0.020
	Inonomycin	0.281 ± 0.002	

Polarization values presented are an average of 12 measurements in each case.

Activation of SAPK/JNK by Ionomycin

The stress activated protein kinases, SAPKs and a subclass of MAP kinase family of protein kinases P44/42 MAP kinases (ERK1 and ERK2), were shown to be activated during heat induced signal transduction [Kyriakis et al., 1994; Cohen, 1996; Lassle et al., 1997]. To study whether a similar signaling cascade is activated upon ionomycin treatment leading to the induction of HSP 70, cell extracts from ionomycin treated and heat shocked cells were immediately analyzed on Western blots with anti-ERK1 and ERK2 and anti-SAPK antibodies. Activation of ERK1 and ERK2 was seen (Fig. 3A,B) in Ca^{2+}-containing and in Ca^{2+}-free medium upon heat shock as well as upon ionomycin treatment. At the same time, cross reactivity with anti-phospho SAPK was seen only upon ionomycin treatment but not upon heat shock (Fig. 3D). Cross reactivity with anti-nonphospho SAPK was not seen under either conditions (Fig. 3C).

Increase in Intracellular Free Calcium Upon Ionomycin Treatment

To understand whether there really is an increase in $[(Ca^{2+})i]$ upon ionomycin treatment, $[(Ca^{2+})i]$ was measured using Indo-1 AM. Results of relative increase in peak fluorescence intensity at 400 nm, presented in Figure 4, clearly indicate a rise in free intracellular Ca^{2+} in both Ca^{2+}-containing medium as well as in Ca^{2+}-free medium. $[(Ca^{++})i]$ in Ca^{2+}-free medium is more than that in Ca^{2+}-containing medium. Triplicate readings from three different experiments gave similar results.

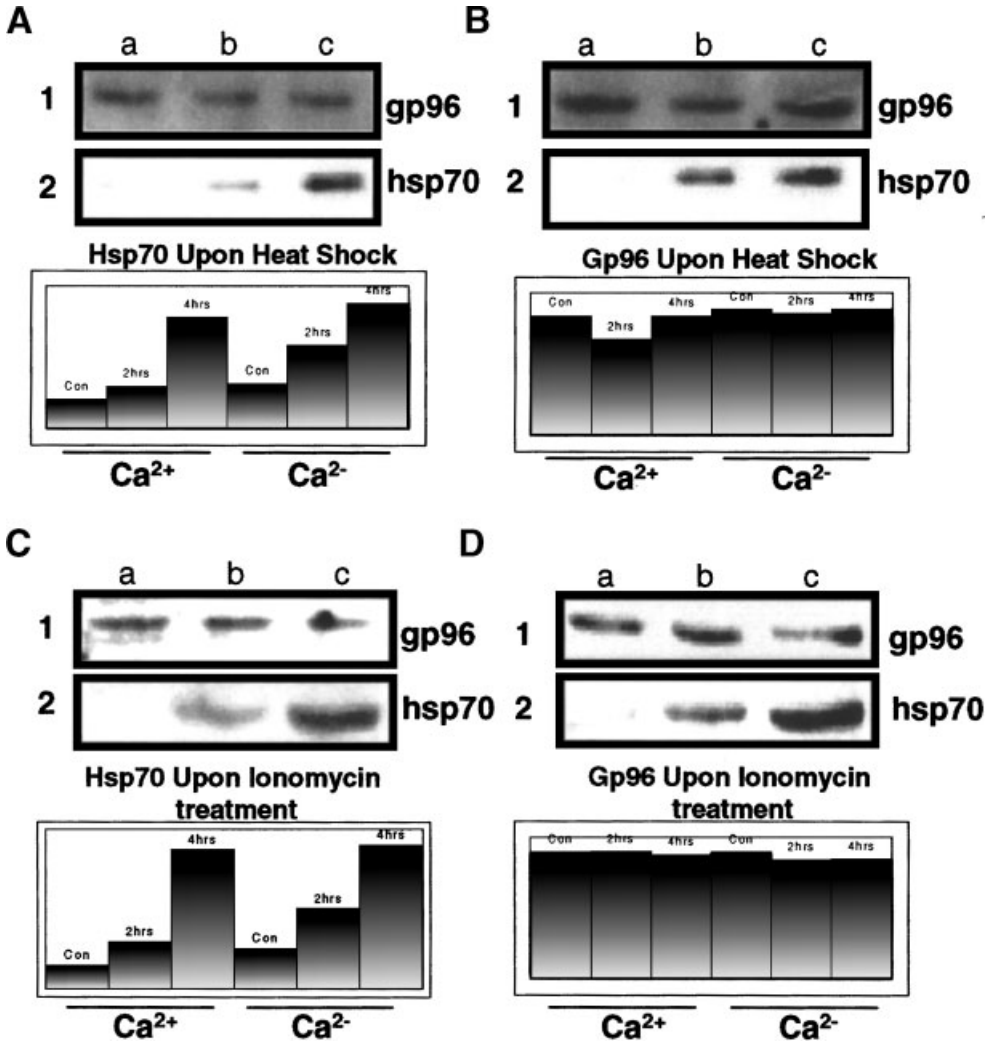

Fig. 1. Expression of HSP 70 upon ionomycin treatment. H4II-E-C3 cells were treated with 0.3 μM ionomycin for 15 min or given heat shock for 45 min at 42°C. Total cell lysates made from control (**a**) and heat shocked cells after 2 h (**b**) and 4 h (**c**) of recovery at 37°C were processed for immunoblot analysis using HSP 70 monoclonal antibody (blots A2, B2, C2, and D2) or gp96 antibody (blots A1, B1, C1, and D1). A1, heat shock in Ca^{2+} containing medium; B1, heat shock in Ca^{2+}-free medium; C1, ionomycin treatment in Ca^{2+}-containing medium; D1, ionomycin treatment in Ca^{2+}-free medium. **Lanes**: **a**, control; **b** & **c**, 2 and 4 h after heat shock or ionomycin treatment. Figures with bars are the densitometric scans of blots.

Ionomycin Induced Change in Membrane Organization

Heat stress has been shown to induce a change in membrane organization [Revathi et al., 1994]. To study whether ionomycin also causes a similar change in membrane organization, fluorescence probe DPH was incorporated into the membranes after ionomycin treatment or heat shock, and fluorescence spectra and fluorescence polarization were recorded. Figure 5 shows the corrected fluorescence spectra of DPH incorporated into hepatoma cells. These spectra are characteristic of membrane-bound DPH molecules with emission maxima around 428 nm. There is a decrease in fluorescence intensity of DPH in ionomycin treated samples, whereas heat stressed sample shows an increase in fluorescence intensity though the amount of probe incorporated (as checked by OD of samples at 350 nm) was the same.

The change in membrane organization seen under heat shock and ionomycin treatment was further characterized by measuring the fluorescence polarization of DPH incorporated into the cells. Fluorescence polarization values presented in Table I show a decrease in membrane

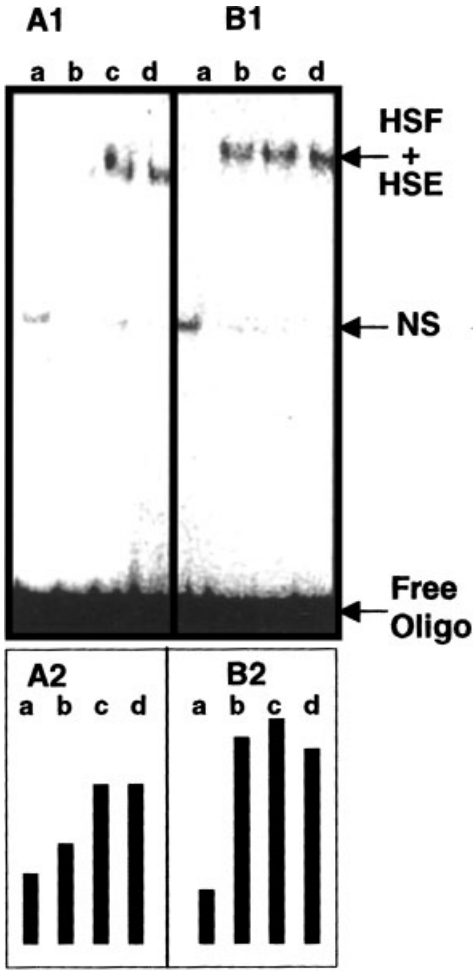

Fig. 2. Activation of HSF by ionomycin. Hepatoma cells were treated as described in the legend of Figure 1. Whole cell extracts of samples collected from control and after heat shock or ionomycin treatment were analyzed for HSF–HSE interaction by EMSA using canonical HSE. Arrow indicates HSF–HSE complex; NS, non-specific binding. **Panel A**: heat shock; **Panel B**: ionomycin treatment. **Lanes**: **a**, control; **b–d**, heat shock and ionomycin treatment and recovery at 37°C for 15, 30, and 40 min, respectively. A2 and B2 are densitometric scans of the blots.

polarization upon ionomycin treatment and an increase in polarization upon heat shock suggesting a change in orientation of the fluorophore incorporated into the membrane due to rotational motion during the lifetime of the excited state.

DISCUSSION

HSPs are induced under a variety of external stimuli. Stress was reported to increase intracellular Ca^{2+} concentration and induce stress protein synthesis [Kiang et al., 1998a]. Data

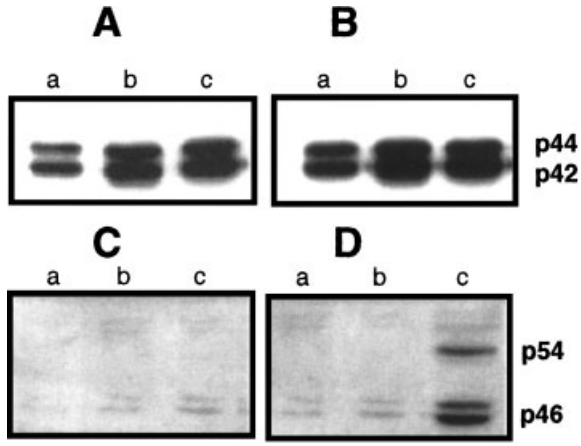

Fig. 3. Activation of SAPKs. H4II-E-C3 cell lysates prepared immediately upon ionomycin treatment or heat shock were run on SDS–PAGE and immunoblotted with antibody to ERKs or SAPK. A and B lysates from cells cultured in Ca^{2+}-containing and Ca^{2+}-free medium, blotted with ERK1 and ERK2 antibodies. C and D lystes from cells cultured in Ca^{2+}-containing medium blotted with nonphospho and phospho SAPK. **Lanes**: **a**, control; **b**, heat shock; **c**, ionomycin treatment.

presented here show that calcium ionophore, ionomycin causes an increase in cytoplasmic free Ca^{2+} both in Ca^{2+}-containing and Ca^{2+}-free medium, suggesting release of Ca^{2+} into the cytoplasm from membrane bound sources within the cell.

Ionomycin also caused a change in membrane organization as measured by DPH fluorescence. Fluorescence intensity of DPH incorporated into plasma membrane is a reflection of the microenvironment around the DPH molecule and has been used as a method to evaluate the fluidity parameters of plasma membranes [Pap et al.,

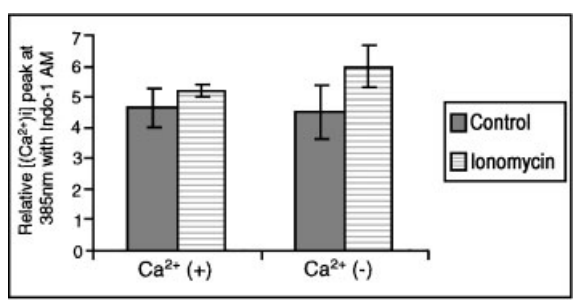

Fig. 4. Measurement of $[(Ca^{2+})i]$. Cells were treated with ionomycin or given heat shock at 42°C for 45 min and resuspended in HEPES with Indo-1 AM as described in Materials and Methods. Free dye were washed off and emission spectra of samples were recorded with excitation at 338 nm. Peak value of relative increase in fluorescence intensity of free Ca^{2+} bound of Indo-1 AM in Ca^{2+}-containing and Ca^{2+}-free medium are presented. Bar indicates SEM.

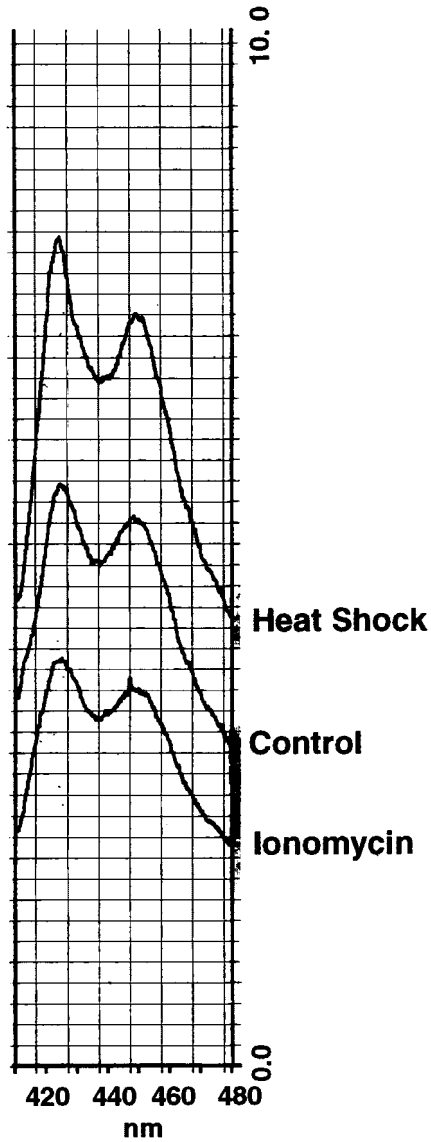

Fig. 5. Corrected fluorescence emission spectra of DPH incorporated into H4II-E-C3 cells, after ionomycin treatment or heat shock, were incubated with 1 mM DPH for 15 min. Cells were washed free of DPH and resuspended in PBS, and fluorescence was measured with excitation at 358 nm.

1994; Illinger et al., 1995]. The differences in the fluorescence intensities of control, heat shocked, and ionomycin treated samples possibly reflect the different environments and locations of this probe in the membrane. We interpret these changes in fluorescence intensity as a result of microenvironmental changes occurring in the immediate vicinity of these fluorophores, which may be due to a change in membrane organization induced by heat shock or ionomycin.

Differences in membrane organization upon heat shock and ionomycin treatment of cells was further characterized by measuring the fluorescence polarization of DPH incorporated into their plasma membrane. The degree of depolarization of the emitted fluorescence from membrane bound probes depends on the rotational mobility of the probe in the time scale of fluorescence (typically in nanoseconds) in the given environment. If the fluorophore undergoes rapid rotational motion leading to a substantial change in its orientation during the lifetime of the excited state, its polarization decreases. The decrease in fluorescence intensity of DPH and a decrease in polarization values upon ionomycin treatment suggest a change in membrane order in a way different from heat shock in these cells upon ionomycin treatment. However, this change in membrane order appears to activate a similar set of signaling molecules like ERK1 and ERK2 upon heat shock and ionomycin treatment. In addition, phosphorylated form of SAPK is activated (Fig. 3C,D) immediately upon ionomycin treatment but not upon heat shock though the significance of this observation cannot be explained fully.

Incubation of rat hepatoma cells with 0.3 μM ionomycin for 15 min appears to activate HSF1 faster as HSF–HSE binding with an upward shift was evident immediately upon ionomycin treatment (Fig. 2B). The upward shift of the HSF–HSE complex in ionomycin treated samples is probably due to the hyperphosphorylation of the transcription factor. Based on the intensity of the band representing HSF–HSE, ionomycin seems to induce faster and stronger interaction between HSF and HSE.

Coincident with this is the rate of synthesis of HSP 70 under these two conditions as the amount of HSP synthesized within 2 h of ionomycin treatment is more than that synthesized in 2 h following heat shock with maximum induction seen after 4 h of heat shock or ionomycin treatment. HSP 70 synthesis per se is not affected by depletion of external Ca^{2+} though the pattern of synthesis is slightly altered in Ca^{2+}-free medium. The absolute amount of free Ca^{2+} inside the cell is more upon ionomycin treatment of H4II-E-C3 cells in Ca^{2+}-free medium than in Ca^{2+}-containing medium, probably due to release of sequestered Ca^{2+} from within the cell.

It was reported that another Ca^{2+} ionophore A23187 inhibits HSP 70 synthesis in K562 human erythroleukemia cells. This inhibition was correlated with ionophore causing alterations in HSF1 phosphorylation, inhibiting the formation of the phosphorylated high molecular mass form of HSF1 in heat treated cells [Elia et al., 1996]. Unlike this, ionomycin appears to activate HSF1 faster leading to its more stable binding to HSE and faster synthesis of HSP 70.

Thus ionomycin induced disturbance in intracellular Ca^{2+} homeostasis appears to activate heat shock transcription factors resulting in HSP 70 synthesis. Heat induced release of Ca^{2+} has been reported in the literature. We report here that release of intracellular Ca^{2+} by ionomycin is enough to activate HSF1 resulting in the induction of HSP 70. Alternatively, cells may sense change in intracellular Ca^{2+} concentration caused by ionomycin as a stressful condition and activate HSF1 leading to the synthesis of HSP 70 for cellular protection. Overexpression of HSP 70 was shown to protect cells from NaCN cytotoxicity probably by attenuating intracellular Ca^{2+} response [Kiang et al., 1998b]. Synthesis of HSP 70 upon ionomycin treatment may also have a similar purpose in rat hepatoma cells.

ACKNOWLEDGMENTS

Authors thank Mr. M.V. Subba Rao for secretarial assistance and P.K. Srivastava for antibody to gp96.

REFERENCES

Cohen P. 1996. Dissection of protein kinase cascades that mediate cellular response to cytokines and cellular stress. Adv Pharmacol 36:15–27.

Ding XZ, Smallridge RC, Galloway RJ, Kiang JG. 1996. Increases in HSF1 translocation and synthesis in human epidermoid A-431 cells: Role of protein kinase C and $[Ca^{2+}]_i$. J Invest Med 44:144–153.

Elia G, Marco AD, Rossi A, Santoro MG. 1996. Inhibition of hsp70 expression by calcium ionophore A23187 in human cells. J Biol Chem 271:16111–16118.

Evans DP, Corbin JR, Tomasovic SP. 1991. Effects of calcium buffering on the synthesis of the 26-kDa heat-shock protein family. Radiat Res 127:261–268.

Illinger D, Duportail G, Mely Y, Poirel-Morales N, Gerard D, Kuhry J-G. 1995. A comparison of the fluorescence properties of TMA-DPH as a probe for plasma membrane and for endocytic membrane. Biochim Biophys Acta 1239:58–66.

Itagaki Y, Akagi K, Uda M, Tanaka Y. 1998. Role of intracellular calcium concentration on tumor cell death from hyperthermia. Oncol Rep 5:139–141.

Khanna A, Aten RF, Behrman HR. 1995. Physiological and pharmacological inhibitors of leutinizing hormone-dependent steroidogenesis induce heat-shock protein-70 in rat isolated cells. Endocrinology 136:1775–1781.

Kiang JG, Gist ID, Tsokos GC. 1998a. Cytoprotection and regulation of heat shock proteins induced by heat shock in human breast cancer T47-D cells: Role of $[Ca^{+}]_i$ and protein kinases. FASEB J 12:1571–1579.

Kiang JG, Ding XZ, Mc Clain DE. 1998b. Over expression of hsp70 attenuates increases in $[Ca^{2+}]_i$ and protects human epidermal A-431 cells after chemical hypoxia. Toxicol Appl Pharmacol 149:185–194.

Kuhlmann MK, Betz R, Hanselmann R, Kohler H. 1997. Heat preconditions confers protection from Ca^{2+}-mediated cell toxicity in renal tubular epithelial cells (BSC-1). Cell Stress Chaperones 2:175–179.

Kyriakis JM, Banerjee P, Nikolakai E, Dai T, Rubie EA, Ahmad MF, Avruch J, Woodgett JR. 1994. The stress-activated protein kinase subfamily of c-Jun kinases. Nature 369:156–160.

Lamarche S, Chretien P, Landry J. 1985. Inhibition of the heat shock response and synthesis of glucose-regulated proteins in Ca^{2+}-deprived rat hepatoma cells. Biochem Biophys Res Commun 131:868–876.

Lassle M, Blatch GL, Kundra V, Takatori T, Zelter BR. 1997. Characterization of binding domains for heat shock proteins and in vitro phosphorylation by different kinases. J Biol Chem 272:1876–1884.

Lee HC. 1993. Potentiation of calcium- and caffeine-induced calcium release by cyclic ADP-ribose. J Biol Chem 268:293–299.

Lindquist S, Craig EA. 1988. The heat shock proteins. Annu Rev Genet 22:631–677.

Liu C, Harmann TE. 1978. Characterization of ionomycin as a calcium ionophore. J Biol Chem 253:5892–5894.

Morimoto RI. 1993. Cells in stress: Transcriptional activation of heat shock genes. Science 259:1409–1410.

Morimoto RI, Sarge KD, Abravaya K. 1992. Transcriptional regulation of heat shock genes. J Biol Chem 267:21987–21990.

Orrenius S, Nicotera P. 1994. The calcium ion and cell death. J Neural Transm Suppl 43:1–11.

Pap EHW, ter Horst JJ, Van Hoek A, Visser AJWG. 1994. Fluorescence dynamics of diphenyl-1,3,5-hexatriene-labeled phospholipids in bilayer membranes. Biophys Chem 48:337–351.

Price BD, Calderwood SK. 1991. Ca^{2+} is essential for multistep activation of the heat shock factor in permeabilized cells. Mol Cell Biol 11:3365–3368.

Revathi CJ, Chattopadhyay A, Srinivas UK. 1994. Change in membrane organization induced by heat shock. Biochem Mol Biol Int 32:941–950.

Sarge KD, Murphy SP, Morimoto RI. 1993. Activation of heat shock gene transcription of HSF1 involves oligomerization, acquisition of DNA binding activity and nuclear localization and can occur in the absence of stress. Mol Cell Biol 13:1392–1407.

Srinivas UK, Revathi CJ. 1993. Changes in cytoplasmic pH upon heat shock in embryonic and adult rat liver cells. J Biosci 18:175–186.

Swamynathan SK, Revathi CJ, Srinivas UK. 1996. Identification and characterization of promoter elements responsible for the induction of the albumin gene by heat shock in early embryonic rat liver. DNA Cell Biol 15:897–905.

Welch WJ. 1992. Mammalian stress response. Cell physiology, structure/function of stress proteins and implications for medicine and disease. Physiol Rev 72:1063–1080.

Welch WJ, Mizzen LA. 1988. Characterization of the thermotolerant cell. II. Effects on the intracellular distribution of heat-shock protein 70, intermediate filaments, and small nuclear ribonucleoprotein complexes. J Cell Biol 106:1117–1130.

Welch WJ, Suhan JP. 1985. Morphological study of the mammalian stress response: Characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. J Cell Biol 101:1198–1211.

Yamamoto N, Maki A, Swann JD, Berezesky IK, Trump BF. 1993. Induction of immediate early and stress genes in rat proximal tubule epithelium following injury: The significance of cytosolic ionized calcium. Ren Fail 15: 163–171.

Yamamoto N, Smith MW, Maki A, Berezesky IK, Trump BF. 1994. Role of cytosolic Ca^{2+} and protein kinases in the induction of the hsp70 gene. Kidney Int 45:1093–1104.