Vol. 210, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 5, 1995 Pages 21-30

INVOLVEMENT OF PHOSPHOLIPASE C IN HEAT-SHOCK-INDUCED PHOSPHORYLATION OF P-GLYCOPROTEIN IN MULTIDRUG RESISTANT HUMAN BREAST CANCER CELLS[1]

Jin-Ming Yang, Khew-Voon Chin and William N. Hait[2]

Departments of Medicine and Pharmacology, The Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School, 679 Hoes Lane, Piscataway, New Jersey 08854

Received March 27, 1995

The phosphorylation of P-glycoprotein has been appreciated for many years, yet little is known about the factors that initiate this post-translational modification. To determine whether the activation of P-glycoprotein phosphorylation could occur in response to cellular stress and to investigate the possible signal pathways involved, we studied the effect of heat shock on the phosphorylation of P-glycoprotein in sensitive and resistant MCF-7 human breast cancer cells. Treatment of multidrug resistant MCF-7/AdrR cells with heat shock increased the phosphorylation of P-glycoprotein. The response was not seen in the sensitive MCF-7 line, which does not express this drug transporter. Phosphorylation of P-glycoprotein induced by heat shock was not dependent on synthesis of new proteins, since phosphorylation was not inhibited by cycloheximide and the content of P-glycoprotein, as measured by immunoblotting, did not change after heat shock. The activation of P-glycoprotein phosphorylation by heat shock may be initiated through activation of phospholipase C, since heat shock stimulated the activity of this enzyme, as evidenced by increased formation of inositol trisphosphate and diacylglycerol and by phosphorylation of phospholipase C-γ. U-73122, an inhibitor of phospholipase C and staurosporine, an inhibitor of protein kinase C, both decreased the heat-shock-induced phosphorylation of P-glycoprotein. These results suggest that heat shock induces phosphorylation of P-glycoprotein through the activation of the phospholipase C/protein kinase C pathway.

[1]Supported by grants from the US Public Health Service #2 P01-CA 08341, NCI R29CA58452-03, NCI CA43888 and NCI P20CA57140-03.

[2]Address correspondence to William N. Hait, M.D., Ph.D., The Cancer Institute of New Jersey, CABM Building, 679 Hoes Lane, Room 242, Piscataway, New Jersey 08854. FAX:908-235-5475.

The abbreviations used are: MDR, multidrug resistant or multidrug resistance; P-gp, P-glycoprotein; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PLC, phospholipase C; IP3, inositol trisphosphate; DAG, diacylglycerol; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

0006-291X/95 $5.00

Exposure of multidrug resistant (MDR) cancer cells to certain cancer chemotherapeutic drugs or chemosensitizers increases the basal level of phosphorylation of the *MDR*1 gene product, P-gp (1-4). For example, Center and colleagues have shown that exposure of MDR Chinese hamster lung cells to N-ethylmaleimide or trifluoperazine increased the phosphorylation of P-gp (3, 4) and Tsuruo's group found similar results with verapamil (2). More recently, it has been reported that exposure of K562/ADR cells to doxorubicin also produced phosphorylation of this membrane protein (1).

Although the enzyme(s) that phosphorylate P-gp have not been completely defined, PKC is an important candidate. Our group demonstrated that the activity of PKC was increased in certain, but not all, MDR cell lines (5) and since that time numerous reports have demonstrated that PKC activity is increased in MDR cells compared to that of sensitive cells (6-9). P-gp can serve as a substrate for PKC (10,11) and is phosphorylated on serine residues in human and murine MDR gene products (12,13). The role of individual isozyme of PKC in MDR cell lines has been recently investigated. While Gollapudi et al. showed overexpression of PKCα and β in P388/ADR cells (8), Blobe et al. demonstrated that the 10-fold increase in enzymic activity in MCF-7/AdrR cells was due to a selective increase in the expression of PKCα (9). Glazer and colleagues co-transfected MCF-7 cells with cDNA for *MDR*1 and PKCα and found that overexpression of this isozyme increased drug resistance and phosphorylation of P-gp (14). In addition, PKC phosphorylates P-gp in cell membranes (11) and in immunoprecipitates (10), and is translocated to cell membranes in response to PMA in a temporally consistent manner with changes in drug accumulation (10,15).

Despite the intense interest in the enzyme(s) that mediate the phosphorylation of P-gp, the mechanism by which this phosphorylation reaction is initiated remains unknown. We reasoned that phosphorylation of P-gp could represent a response to the cellular stress imposed on cancer cells by a hostile environment and that PLC, an enzyme that regulates the activity of PKC, may initiate this reaction cascade. In fact, environmental stress such as heat shock has been shown to stimulate PLC activity in some tumor cell lines (16). The purpose of this study was to determine whether cellular stress induced by heat shock could activate the phosphorylation of P-gp and if PLC was involved in this process.

MATERIALS AND METHODS

Materials. [^{32}P]orthophosphate and phosphate deficient RPMI 1640 medium were from ICN Biomedicals (Irvine, CA). Polyclonal antibody mdr (Ab-1) recognizing human P-gp was purchased from Oncogene Science (Uniondale, NY) and polyclonal anti-PLC-γ1 antiserum from Upstate Biotechnology Incorporated (Lake Placid, NY). Monoclonal antibody C219 was a gift from Centocor Diagnostic (Malvern, PA). PMA and staurosporine were from Sigma (St. Louis, MO). Protein-A Sepharose CL-4B was from Pharmacia (Piscataway, NJ). U-73122 was obtained from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). [^{14}C]methylated protein standards (M.W. 14,300-200,000), [^{3}H]-*myo*-inositol, DAG assay kit and [^{32}P]γ-ATP were purchased from Amersham (Arlington Heights, IL). High range prestained SDS-PAGE standards (M.W. 49,500-205,000) was from Bio-Rad (Hercules, CA).

Cell Lines. MCF-7 and MCF-7/AdrR cell lines were kindly supplied by Dr. Kenneth Cowan of the National Cancer Institute (Bethesda, MD) and were maintained in RPMI 1640 medium containing 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2/95% air. Cells were passed twice weekly and were replaced from frozen stocks every three months. Under these conditions, the MCF-7/AdrR line retained resistance and P-gp expression. Cells were free from contamination by mycoplasma and fungi.

Phosphorylation of P-gp and PLC-γ. 4 x 10^6 cells were plated in 100-mm petri dishes in RPMI 1640 medium with 10% fetal bovine serum. At 90% confluence, cells were rinsed twice with phosphate deficient RPMI 1640 and then labeled with 1mCi [^{32}P]orthophosphate/2 ml phosphate deficient RPMI 1640 medium containing 0.3% fetal bovine serum for 2 hr at 37°C. The cells were then heat-shocked at 43°C or incubated at 37°C for 30 min in the presence of activators or inhibitors as described below. Radiolabeled lysates were prepared by washing the adherent cells twice with 5 ml of STE buffer (20 mM Tris-HCl, pH 7.2, 0.15 M NaCl, 1 mM EDTA, 10 mM NaF, 2 mM PMSF and 1% aprotinin). Cells were harvested by scraping into 5 ml of STE buffer and were collected by centrifugation (900 x g). Cells were then resuspended in 1 ml RIPA buffer (20 mM Tris-HCl, pH 7.2, 0.15 M NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 10 mM NaF, 2 mM PMSF and 1% aprotinin). The lysates were transferred to Eppendorf tubes and clarified by centrifugation at 16,000 x g for 30 min at 4°C. The precleared lysates were then normalized for [^{32}P] counts for each sample and proteins were immunoprecipitated from the precleared lysates by incubation with mdr (Ab-1) polyclonal antibody or anti-PLC-γ1 antiserum overnight at 4°C. The immune complexes were precipitated using protein A-Sepharose. Immunoprecipitated proteins were released by the addition of 50 μl of Laemmli sample buffer and subjected to electrophoresis in a 7.5% SDS-polyacrylamide gel (17). The gels were fixed with 10% methanol/5% glycerol, dried and exposed to X-ray film (REFLECTION , Dupont) with an intensifying screen at -70°C. Densitometry of autoradiograms was performed using a ScanMaker 600G scanner (Microtek) and data were analyzed using NIH Image 1.55 software.

Preparation of Membrane Proteins and Immunoblotting. Membrane proteins were prepared by the method of Gerlach et al. (18). Proteins were dissolved in sample buffer for 10 min at room temperature before loading, then fractionated by 7.5% SDS-PAGE, transferred to nitrocellulose membranes and probed with a monoclonal anti-P-gp antibody C219. P-gp was visualized with an alkaline phosphatase-conjugated secondary antibody.

Measurement of IP3. Cells were plated in 33 mm dishes and labeled with [^{3}H]-*myo*-inositol (3 μCi/ml) in inositol-free medium for 48 hr at 37°C. After washing, the cells were incubated at 37°C or heat shocked at 43°C. The incubations were terminated by addition of 0.6 ml of 5% perchloric acid. Acid extracts were clarified by centrifugation and applied to Bio-Rad AG-X2 ion exchange columns to separate inositol phosphates as described (19). IP3 was eluted between 0.1 M formic acid/0.4 M ammonium formate and 0.1 M formic acid/0.8 M ammonium formate. A 2-ml portion of each fraction was taken for liquid scintillation counting.

Measurement of DAG. Cells plated in 60 mm dishes were incubated at 37°C or heat shocked at 43°C. The incubations were terminated by aspirating the medium and rapidly washing the cells three times on ice with cold phosphate-buffered saline. Samples were extracted with chloroform/methanol and the chloroform phase was analyzed for DAG by a radioenzymatic assay as described (20). This assay employs DAG kinase which quantitatively converts DAG to phosphatidic acid. Following extraction and separation of [^{32}P]-phosphatidic acid from [^{32}P]γ-ATP and other [^{32}P]-labeled species, the [^{32}P]-phosphatidic acid was quantitated by liquid scintillation counting.

RESULTS

Exposure of MCF-7/AdrR cells to heat for 30 minutes increased the phosphorylation of P-gp (Figure 1A and Table 1). The degree of phosphorylation

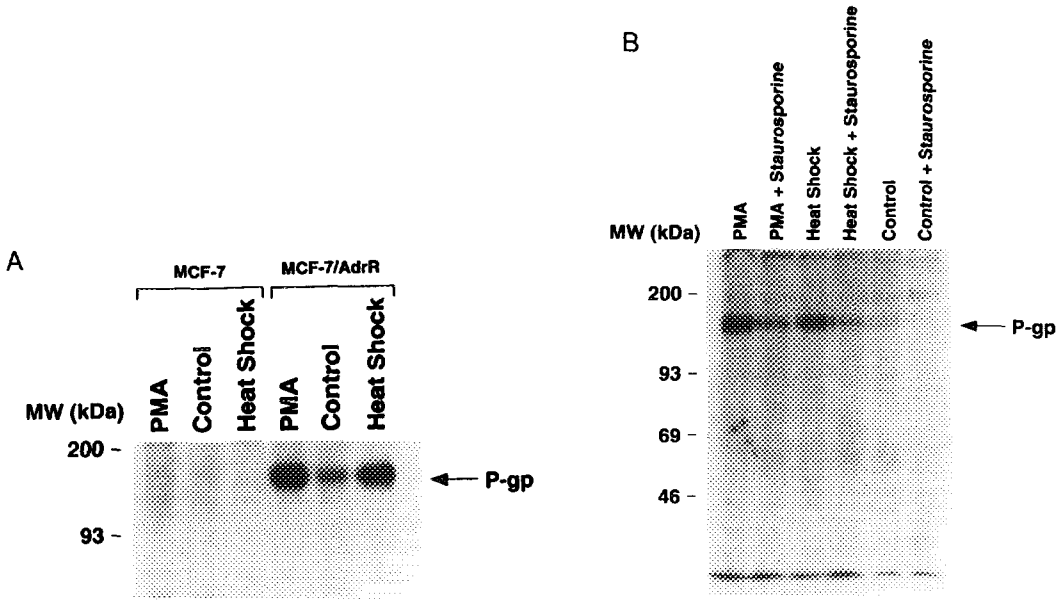

FIGURE 1. (A) Stimulation of phosphorylation of P-gp by heat shock and (B) inhibition of the heat-shock-induced phosphorylation of P-gp by staurosporine in MCF-7/AdrR cells. *(A)* Sensitive MCF-7 or MDR MCF-7/AdrR cells were labeled with [^{32}P]orthophosphate at 37°C for 2 h, then heat-shocked at 43°C or treated with 200 nM PMA or vehicle at 37°C for an additional 30 min. *(B)* MCF-7/AdrR cells were treated as in (A) in the absence or presence of 300 nM staurosporine. P-gp was immunoprecipitated from cell lysates using an anti-P-gp polyclonal antibody, mdr (Ab-1). The samples were analyzed by 7.5% SDS-PAGE, as described in "Materials and Methods". The protein size markers were [^{14}C]methylated protein standards (M.W. 14,300-200,000). Results are representative of three similar experiments.

Table 1 Quantitation of the phosphorylated P-glycoprotein

Figure	Treatment	Phosphorylated P-gp (Integrated Optical Density x 10^{-3})
Fig.1A	Control	10
	Heat Shock	19
	PMA	20
Fig.1B	Control	7
	Heat Shock	20
	PMA	28
	Control + Staurosporine	7
	Heat Shock + Staurosporine	11
	PMA + Staurosporine	13
Fig.2A	Control	7
	Heat Shock	16
	Heat Shock + Cycloheximide	16
Fig.4	Control	7
	Heat Shock	16
	Heat Shock + U-73122	12

Bands of phosphorylated P-glycoprotein in Figures 1A, 1B, 2A and 4 were quantitated using a ScanMaker 600G gel scanner and data were analyzed with NIH Image 1.55 software, as described in "Materials and Methods".

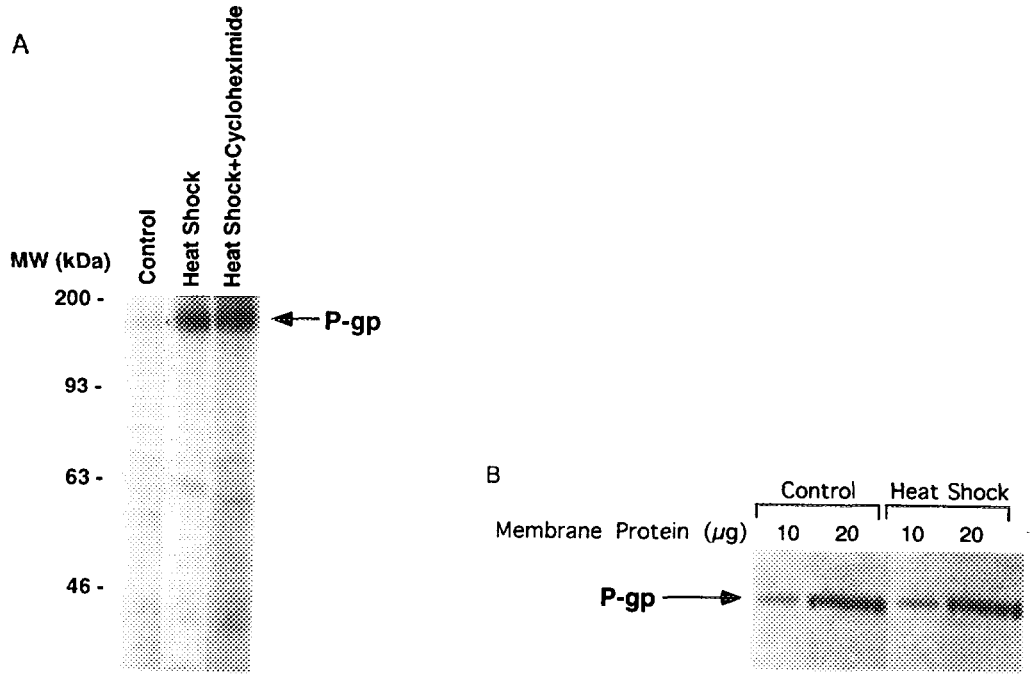

FIGURE 2. Independence of protein synthesis from the phosphorylation of P-gp induced by heat shock. *(A)* Effect of cycloheximide on the phosphorylation of P-gp in MCF-7/AdrR cells. Cells were labeled with [^{32}P]orthophosphate for 2 h, then heat-shocked at 43°C in the presence or absence of 5 μg/ml cycloheximide for an additional 30 min. P-gp was immunoprecipitated and analyzed as described in Figure 1. *(B)* Effect of heat shock on the expression of P-gp in MCF-7/AdrR cells as detected by immunoblotting. Cells were heat-shocked at 43°C or incubated at 37°C for 30 min. Membrane proteins were resolved by SDS-PAGE, transferred to nitrocellulose and probed with the C219 monoclonal antibody as described in "Materials and Methods". Results are representative of three similar experiments.

produced by heat shock was similar to that produced by PMA. In contrast, neither PMA nor heat shock produced phosphorylation of P-gp in sensitive MCF-7 cells (Figure 1A). To determine whether PKC was involved in the phosphorylation of P-gp, we tested the effect of staurosporine, an inhibitor of PKC (21), on the phosphorylation of P-gp induced by heat shock. Figure 1B and Table 1 demonstrate that 300 nM staurosporine inhibited the phosphorylation of P-gp induced by heat shock or by PMA in MCF-7/AdrR cells by 45% and 53%, respectively.

To test whether or not increased expression of P-gp might have produced an apparent increase in phosphorylation of the transporter, we performed the identical experiments in the presence of cycloheximide at a concentration shown to completely inhibit protein synthesis in MCF-7/AdrR cells[3]. Cycloheximide (5 μg/ml) was added to the medium 5 min before the start of heat shock. Figure 2A and Table 1 show that

[3]Dana T. Aftab. (1992) "Development of an Assay and Inhibitors of Protein Kinase C, and Studies on the Role of the Enzyme in the Phosphorylation of P-glycoprotein". Ph.D. Dissertation. Yale University School of Medicine, Department of Pharmacology.

cycloheximide did not inhibit the phosphorylation of P-gp induced by heat shock. Furthermore, the content of membrane P-gp, as determined by immunoblotting, did not change after exposure to 43°C (Figure 2B).

The activity of PKC is regulated by the concentration of membrane DAG. Therefore, we studied whether heat shock activated PLC in MCF-7/AdrR cells as measured by the production of IP3 and DAG in intact cells. Figure 3 demonstrates that treatment with heat shock led to the production of both IP_3 (Figure 3A) and DAG (Figure 3B). The

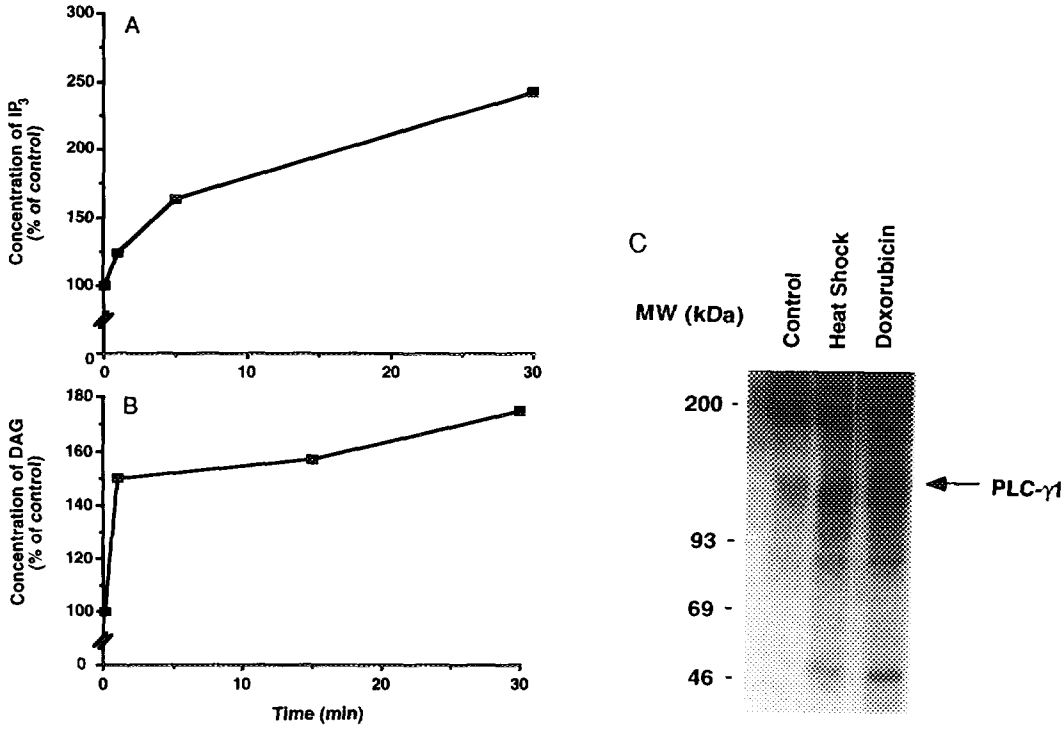

FIGURE 3. Effect of heat shock on the production of IP3 and DAG in MCF-7/AdrR cells. *(A)* Changes of IP3 content following heat shock. Cells were pre-labeled with [^{3}H]-*myo*-inositol (3 μCi/ml) in inositol-free medium for 48 hr at 37°C. After washing, the cells were incubated at 37°C as control or heat-shocked at 43°C for the indicated times. IP_3 was measured as described in "Materials and Methods". Results are expressed as per cent of control for each respective time point and each point represents the mean of duplicate determinations from one of three similar experiments. *(B)* Changes of DAG content following heat shock. Cells were incubated at 37°C as control or heat-shocked at 43°C for the indicated times. The incubations were terminated by aspirating the medium and rapidly washing the cells three times on ice with ice-cold phosphate-buffered saline. DAG was measured by a radioenzymatic assay as described in "Materials and Methods". Each point represents the mean of duplicate determinations from one of three similar experiments. *(C)* Phosphorylation of PLC-γ following heat shock or doxorubicin treatment. MCF-7/AdrR cells were labeled with [^{32}P]-orthophosphate at 37°C for 2 h, then heat-shocked at 43°C, or treated with 10 μM doxorubicin or vehicle at 37°C for an additional 30 min. PLC-γ was immunoprecipitated from cell lysates using an anti-PLC-γ1 polyclonal antiserum. The samples were analyzed by 7.5% SDS-PAGE, as described in "Materials and Methods". The protein size markers were [^{14}C]methylated protein standards (M.W. 14,300-200,000). Results are representative of two similar experiments.

activation of receptor-linked PLC-γ is associated with the phosphorylation of this enzyme (22,23). We found that PLC-γ was expressed in MCF-7/AdrR cells as measured by western blots (data not shown). Figure 3C demonstrates that heat shock increased the phosphorylation of PLC-γ by 100% in MCF-7/AdrR cells. The activation of PLC-γ was readily detectable with 15 minutes of heat shock (data not shown). In addition, doxorubicin, a chemotherapeutic drug that is transported by P-gp, also increased the phosphorylation of the enzyme by 145% (Figure 3C).

To further investigate whether the activation of PKC and subsequent phosphorylation of P-gp was mediated through PLC, we studied the effect of U-73122, a specific PLC inhibitor (24), on the phosphorylation of P-gp following exposure to heat. Figure 4 and Table 1 demonstrate that phosphorylation of P-gp in response to heat was decreased by U-73122 in MCF-7/AdrR cells.

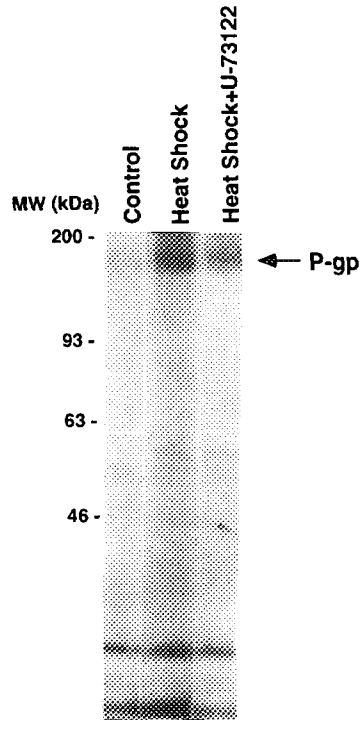

FIGURE 4. Effect of U-73122 on the phosphorylation of P-gp in MCF-7/AdrR cells. MCF-7/AdrR cells were labeled with [^{32}P]orthophosphate at 37°C for 2 h, then heat shocked at 43°C for an additional 30 min in the presence or absence of 5 μM U-73122. P-gp was immunoprecipitated from cell lysates using an anti-P-gp polyclonal antibody, mdr (Ab-1). Samples were analyzed as described in Figure 1. Results are representative of two similar experiments.

DISCUSSION

The regulation of phosphorylation of critical membrane proteins such as P-gp is of considerable interest, yet the mechanisms underlying these reactions remain to be elucidated. The current studies demonstrate that heat shock increases the phosphorylation of P-gp in MDR human breast cancer cells and suggest that PLC is involved in this process. These data support our working hypothesis for how the phosphorylation of P-gp is regulated (25), i.e., the exposure of cells to environmental stress activates PLC which catalyzes the breakdown of phosphatidylinositol-4,5-bisphosphate to IP_3 and DAG. The increase in plasma membrane DAG would result in the translocation of PKC to the plasma membrane where it can phosphorylate P-gp. The phosphorylation of P-gp may then increase its transport function. In fact, as several groups have previously shown, heat shock increased resistance of human kidney carcinoma cells (26) and breast cancer cells (27) to certain chemotherapeutic drugs that are substrates for P-gp.

There were several possible explanations for the increased phosphorylation of P-gp induced by heat shock. For example, Chin and colleagues (26) have shown that heat shock increased *MDR*1 gene expression, raising the possibility that an increased amount of P-gp could have created an apparent increase in membrane phosphoprotein following heat shock. However, the lack of effect of heat shock in sensitive cells which do not express P-gp (Figure 1A), the inability of cycloheximide to decrease the amount of phosphorylated P-gp induced by heat shock (Figure 2A) and the inability to demonstrate increased membrane P-gp by immunoblotting (Figure 2B) exclude the possibility that increased P-gp synthesis was responsible for these results. The lack of an effect of heat shock on *MDR*1 gene expression in our experiments may be related to the timing of the specifics or due to the observation that the effect of heat shock on *MDR*1 gene expression is cell type-specific (26).

Since PKC has been implicated in the phosphorylation of P-gp (10,15,28), this raised the interesting possibility that heat shock induced the phosphorylation of P-gp through the activation of this enzyme. In support of this concept, we found that the phosphorylation of P-gp induced by heat shock is blocked by staurosporine (Figure 1B) at a concentration previously shown by our laboratory to inhibit the activity of PKC and the phosphorylation of P-gp in MCF-7/AdrR cells (10).

Although PKC may be involved in the phosphorylation of P-gp, the mechanism(s) by which PKC is activated in MDR cells is unknown. Heat shock is known to activate PLC in several cell lines, and the activation of PKC through the generation of DAG is believed to be responsible for part of the heat shock response (16). Our current studies also suggest that the activation of PKC by heat shock may be mediated through the activation of PLC. As shown in Figure 3, heat shock stimulates the activity of PLC as measured by the increases in IP_3 and DAG and by the increased phosphorylation of PLC-γ. Phosphorylation of PLC-γ has been shown to correlate with activation of the enzyme in several cell lines (22,23). In addition, when the identical experiments were performed in the presence of U-73122, a specific inhibitor of PLC (24), the phosphorylation of P-gp

was diminished (Figure 4 and Table 1). The levels of both DAG and IP_3 increased following treatment of cells with heat shock. The more rapid rise and plateau of DAG compared to that of IP_3 has been reported with other stimuli (29).

Several lines of evidence suggest that phosphorylation of P-gp may regulate the function of this transporter. For example, phorbol esters increase the phosphorylation of P-gp and increase drug resistance (2,10,28,30). This has been associated with decreased drug accumulation and enhanced drug efflux (10,15,28). To investigate the effect of heat shock-induced phosphorylation of P-gp on the function of the transporter, we performed drug accumulation, uptake and efflux experiments before and after heat shock on sensitive and MDR MCF-7 cells. We found that heat treatment caused a loss of cell membrane integrity as measured by trypan blue exclusion, making accurate measurements of drug transport impossible. However, we have recently found that epidermal growth factor activated PLC, increased phosphorylation of P-gp and also enhanced P-gp function as measured by decreased accumulation of vinblastine in MCF-7/AdrR cells (31).

In summary, our results indicate that PLC may be a component of the signal transduction pathway that mediates the phosphorylation of P-gp in heat shock-treated MDR breast cancer cells, and raise important questions regarding cellular stress mechanisms in drug resistance.

REFERENCES

1. Kato, S., Nishimura, J., Yufu, Y., Ideguchi, H., Umemuru, T., and Nawata, H. (1992) FEBS 308, 175-178.
2. Hamada, H., Hagiwara, K-I., Nakajima, T., and Tsuruo, T. (1987) Cancer Res. 47, 2860-2865.
3. Center, M.S. (1985) Biochem. Pharmacol. 34, 1471-1476.
4. Center, M.S. (1983) Biochem. Biophys. Res. Commun. 115, 159-166.
5. Palayoor, S.T., Stein, J.M., and Hait, W.N. (1987) Biochem. Biophys. Res. Commun. 148, 718-725.
6. Posada, J.A., McKeegan, E.M., Worthington, K.F., Morin, M.J., Jaken, S, and Tritton, T.R. (1989) Cancer Commun. 1: 285-292.
7. Posada, J., Vichi, P., and Tritton, T.R. (1989) Cancer Res. 49: 6634-6639.
8. Gollapudi, S., Patel, K., and Gupta, S. (1992) Cancer Lett. 62: 69-75.
9. Blobe, G.C., Sachs, C.W., Khan, W.A., Fabbro, D., Stable, S., Wetsel, W.C., Obeid, L.M., Fine, R.L., and Hannun, Y.A. (1993) J. Biol. Chem. 268: 658-664.
10. Aftab, D.T., Yang, J.M., and Hait, W.N. (1994) Oncol. Res. 6: 59-70.
11. Chambers, T.C., McAvoy, E.M., Jacobs, J.W., and Eilon, G.J. (1990) Biol. Chem. 265, 7679-7686.
12. Chambers, T.C., Pohl, J., Raynor, R.L., and Kuo, J.F. (1993) J. Biol. Chem. 268: 4592-4595.
13. Orr, G.A., Han, E.K.-H., Browne, P.C., Nieves, E., O'Connor, B.M., Yang, C.-P., and Horwitz, S.B. (1993) J. Biol. Chem. 268: 25054-25062.
14. Yu, G., Ahmad, S., Auino, A., Fairchild, C.R., Trepel, J.B., Ohno, S., Suzuki, K., Tsuruo, T., Cowan, K.H., and Glazer, R.I. (1991) Cancer Commun. 3: 181-189.
15. Chambers, T.C., Chalikonda, I., and Eilon, G. (1990) Biochem. Biophys. Res. Commun. 169, 253-259.
16. Calderwood, S.K., and Stevenson, M.A. (1993) J. Cell. Physiol. 155, 248-256.
17. Laemmli, U.K. (1970) Nature 227, 680-685.
18. Gerlach, J.H., Bell, D.R., Karakousis, C., Slocum, H.K., Kartner, N., Rustum, Y.M., Ling, V., and Baker, R.M. (1987) J. Clin. Oncol. 5, 1452-1460.

19. Downes, P., Hawkins, P.T., and Irvine, R.F. (1986) Biochem. J. 238: 501-506.
20. Preiss, J., Loomis, C.R., Bishop, W.R., Stein, R., Niedel, J. and Bell, R.M. (1986) J. Biol. Chem. 261: 8597- 8600.
21. Sato, W., Yusa,K., Naito, M., and Tsuruo, T. (1990) Biochem. Biophys. Res. Commun. 173: 1252-1257.
22. Nhibe, S., Wahl, M.I., Hernandez-Sotomayor, S.M.T., Tonks, N.K., Rhee, S.G., and Carpenter, G. (1990) Science 250, 1253-1256.
23. Kim, H.K., Kim, J.W., Zilberstein, A., Margolis, B., Kim, J.G., Schlessinger, J., and Rhee, S.G. (1991) Cell 65: 435-441.
24. Yule, D.I., and Williams, J.A. (1992) J. Biol. Chem. 267: 13830-13835.
25. Hait, W.N. and Aftab, D.T. (1992) Biochem. Pharmacol. 43: 103-107.
26. Chin, K.-V., Tanaka, S., Darlington, G., Pastan, I., Gottesman, M.M. (1990) J. Biol. Chem. 265, 221-226.
27. Ciocca, D.R., Fuqua, S.A.W., Lock-Lim, S., Toft, D.O., Welch, W.J., and McGuire, W.L. (1992) Cancer Res. 52, 3648-3654.
28. Chambers, T.C., Zheng, B., and Kuo, J.F. (1992) Mol. Pharmacol. 41, 1008-1015.
29. Rosoff, P.M., Savage, N., and Dinarello, C.A. (1988) Cell 54: 73-81.
30. Fine, R.L., Patel, J., and Chabner, B.A. (1988) Proc. Natl. Acad. Sci. USA 85, 582-586.
31. Yang, J.M., and Hait, W.N. (1995) Proc. Am. Assoc. Cancer Res. 36: 1985.