Expression of P-glycoprotein by tumor cells confers resistance to multiple natural product drugs because of its ability to export these compounds. This transporter is a substrate for several protein kinases; however, the functional significance of its phosphorylation is not defined. We examined the effects of many activators and inhibitors of protein kinases on the activity of P-glycoprotein in drug-resistant human breast carcinoma cells (MCF-7/ADR). Several phorbol esters sensitized these cells to P-glycoprotein substrate drugs; however, there was no correlation with activation of protein kinase C. The 4α- and 4β-isomers of phorbol 12-myristate 13-acetate were equally potent in sensitizing the cells to actinomycin D and daunomycin and in increasing the intracellular accumulation of \[^{3}H\]vinblastine. These effects of 4α-phorbol myristate acetate required much higher concentrations than were needed to increase P-glycoprotein phosphorylation and were not antagonized by staurosporine. Similar to verapamil, the phorbol esters did not sensitize MCF-7/ADR cells to cisplatin, nor parental MCF-7 cells to any of the anticancer drugs. Mezerein, K-252a, and H-89 sensitized MCF-7/ADR cells, increased intracellular accumulation of \[^{3}H\]vinblastine, and antagonized photo-labeling of P-glycoprotein by \[^{3}H\]azidopine. Therefore, phosphorylation does not appear to play a significant role in regulating P-glycoprotein activity in MCF-7/ADR cells.

The term multiple drug resistance (MDR)\(^{†}\) refers to the phenomenon in which tumor cells which survive an initial round of chemotherapy subsequently demonstrate decreased sensitivity to both the original therapeutic agent and other seemingly unrelated drugs (reviewed in Refs. 1-5). This is commonly mediated by overexpression of Pgp, a transmembrane protein (\(M_r = \sim 170,000\)) which acts as an energy-dependent drug efflux pump. This transporter actively removes a variety of structurally diverse compounds, including anthracyclines, Vinca alkaloids, epipodophyllotoxins, actinomycin D, and paclitaxel. Enhanced efflux of these compounds reduces their intracellular accumulation and so reduces their cytotoxicity. In contrast, Pgp does not export small hydrophilic drugs such as cisplatin, 5-fluorouracil and melphanal. Certain agents, such as verapamil, have been shown to reverse MDR by competing with the cytotoxic drugs for binding to Pgp, thereby promoting drug accumulation and cytotoxicity (reviewed in Ref. 6). Because of their potential usefulness as anticancer agents, the identification of compounds with this ability to reverse MDR is an area of research receiving high priority in both academic and pharmaceutical settings.

Another approach to the development of MDR reversing compounds involves characterization of the molecular mechanisms which regulate Pgp function and expression. Many cellular activities are regulated by the phosphorylation and dephosphorylation of specific proteins. Shortly after its discovery, Pgp was shown to be phosphorylated on serine residues in resting cells (7-11), most likely at consensus recognition sites for PKC and PKA (12) present in the deduced amino acid sequence of mdr genes. Recently, convincing data that Pgp is a substrate for both PKC and PKA in vitro and in intact cells has been provided (13-15). These kinases phosphorylate serine residues (669 and 681, respectively) in the linker region of Pgp (13, 16). Additional studies demonstrated that treatment of MDR cells with PKC-activating phorbol esters enhances Pgp phosphorylation (9, 17, 18), while the nonspecific protein kinase inhibitor staurosporine reduces Pgp labeling (19, 20). However, the effects of phosphorylation on Pgp activity remain controversial since both stimulation (17, 21-23) and inhibition (9, 24) of drug transport have been reported. Additionally, phorbol esters have been found to decrease the drug sensitivities of cells which do not express Pgp (25, 26), casting doubt on a specific role of Pgp in these responses.

The combined information indicates that multiple protein kinases are able to phosphorylate Pgp; however, the significance of these reactions in regulating Pgp activity remains undefined. To address this ambiguity, we have characterized the effects of a battery of pharmacological inhibitors and activators of several protein kinases on Pgp activity in human breast carcinoma cells which overexpress Pgp (MCF-7/ADR). In these cells, reduction of Pgp activity is manifested as increased cytotoxicity of substrate drugs such as actinomycin D, daunomycin, and vinblastine (27-29). If phosphorylation is important in regulating the function of Pgp, phosphorylation modulators would be expected to demonstrate patterns of reversal of MDR consistent with their effects on specific types of protein kinases.

**EXPERIMENTAL PROCEDURES**

Materials—4α-PMA and 4α-PDBu were purchased from LC Laboratories (Woburn, MA). 4α- and 4β-Phorbol, 4α- and 4β-PDD, and K-252a were from Calbiochem-Novabiochem, while all other drugs and reagents were purchased from the Sigma. These compounds were dissolved in absolute ethanol and stored at \(-20^\circ C\). \[^{3}H\]Vinblastine sul-
RESULTS

Cytotoxities of Phosphorylation-modulating Compounds toward Drug-sensitive and -resistant Cells—To assess the effects of potential modulators on drug sensitivity, it is necessary to first determine the intrinsic cytotoxicity of the compounds. Therefore, we have determined IC50 values (drug concentrations which reduce cell proliferation by 50%) for a battery of protein phosphorylation modulators toward MCF-7 and MCF-7/ADR cells. Compounds which are subject to transport by Pgp usually demonstrate significant resistance factors2 in the MCF-7/ADR (versus MCF-7) cells (29). As indicated in Fig. 1 (for several phorbolesters) and Table I (for other phosphorylation modulators), many of these compounds are very weak cytotoxins, i.e. IC50 > 100 μM. Of the compounds tested, only staurosporine and calcin A demonstrated IC50 values below 1 μM for either MCF-7 or MCF-7/ADR cells. Interestingly, the 4α-isomers of PDBu and PDD were more cytotoxic than the 4β compounds. Resistance factors for MCF-7/ADR cells were generally between 0.5 and 2, indicating that expression of Pgp confers neither resistance nor sensitivity to most of these compounds. However, these cells were 7- and 6-fold resistant to calcin A and A23187, respectively, suggesting that these compounds may be transported by Pgp.

Effects of Phosphorylation-modulating Compounds on Drug Resistance—Reversal of MDR is manifested by the ability of compound to increase cell killing by daunomycin and/or actinomycin D without modulating the cytotoxicity of cisplatin (27, 28). As summarized in Table I, most compounds did not reverse MDR at 100 μM or doses which approached the IC50 for that compound. For example, sn-1,2-dioctanoylglycerol was ineffective at 100 μM, and staurosporine did not increase the cytotoxicities of the drugs at its IC50 dose of 0.04 μM. Several phosphorylation modulators (e.g. calphostin C, K-252a, chlorpromazine, and trifluoperazine) did significantly sensitize MCF-7/ADR cells to the natural product drugs, although these effects were observed only at doses which substantially inhibited cell proliferation. A measure of the potential usefulness of a compound as an MDR reversing agent is the “efficacy index,” calculated as the IC50 for cell growth/the minimum dose for reversal of MDR (29). This index for the above modulators is below 3 suggesting a narrow therapeutic window; however, much larger efficacy indices were observed for the selective PKA inhibitor, H-89 (>20, Table I) and PDBu (>10, Figs. 1 and 4).

Several phorbolesters were tested to explore structure-function relationships among these compounds for reversal of MDR. Dose-response curves for the inhibition of cell proliferation by actinomycin D (Fig. 2A) indicate that the 80-fold resistance of the MCF-7/ADR cells can be fully eliminated by 10 μM verapamil. Similarly, resistance was completely reversed by 5 μM mezerein and slightly reversed by 0.5 μM mezerein. The resistance of MCF-7/ADR cells was reduced 30-fold by 10 μM of either 4α- or 4β-PMA (Fig. 2B), while a 100 nM concentration of either compound was ineffective. High concentrations (50 μM) of 4α- and 4β-PDBu reduced the resistance factor of MCF-7/ADR cells by 20- and 80-fold, respectively, whereas 500 nM doses had no effects on the IC50 for actinomycin D (Fig. 2C). Similar studies with vinblastine demonstrated that high, but not low, doses of

2 Resistance factor = IC50 of resistant cell line/IC50 of parental cell line.
PMA and PDBu sensitized MCF-7/ADR cells to this drug regardless of the stereochemistry at the 4-position of the phorbol ester (data not shown). None of the compounds tested, i.e. verapamil, mezerein, or the 4α- and 4β-isomers of PMA and PDBu, affected the sensitivity of parental MCF-7 cells to either actinomycin D or vinblastine (data not shown).

The effects of multiple concentrations of mezerein and 11 phorbol compounds on the cytotoxicities of daunomycin, actinomycin D, and cisplatin toward MCF-7/ADR cells were examined. As indicated in Fig. 3, 5 μM mezerein strongly enhanced...
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MCF-7/ADR cells were treated with the indicated concentrations of actinomycin D (Fig. 4 28148
A 28148), or 2 μM cisplatin ( ). Cell survival was assayed as indicated under “Experimental Procedures.” Values represent the mean ± S.D. of triplicate samples in one of three virtually identical experiments.

Fig. 2. Chemosensitization of MCF-7/ADR cells by verapamil, mezerein, and phorbol esters. MCF-7/ADR cells were treated with the indicated concentrations of actinomycin D with the following additions. A, none, ; 10 μM verapamil, ○: 0.5 μM mezerein, ★: or 5 μM mezerein, ■. The effects of actinomycin alone on MCF-7 cells are also indicated ( ). B, none, □: 0.1 μM 4α-PMA, □: 10 μM 4α-PMA, ○: 0.1 μM 4β-PMA, ■; or 10 μM 4β-PMA, ★. C, none, ○: 0.5 μM 4α-PDBu, □: 50 μM 4α-PDBu, ○: 0.5 μM 4β-PDBu, ■, or 50 μM 4β-PDBu, ★. The inhibition of cell proliferation was quantitated as indicated under “Experimental Procedures.” Values represent the mean ± S.D. of triplicate samples in one of three virtually identical experiments.

Fig. 3. Chemosensitization of MCF-7/ADR cells by mezerein. MCF-7/ADR cells were treated with the indicated concentrations of mezerein in the presence of 5 μM daunomycin (■), 50 nM actinomycin D (★), or 2 μM cisplatin ( ). Cell survival was assayed as indicated under “Experimental Procedures.” Values represent the mean ± S.D. of triplicate samples in one of three virtually identical experiments.

the abilities of daunomycin and actinomycin D to kill these cells, but did not modulate the cytotoxicity of cisplatin. Similar studies indicated that neither the 4α- nor the 4β-isomers of phorbol or PDD, at doses up to 100 μM, modulated the toxicities of actinomycin D (Fig. 4A). 4β-PDBu was slightly more potent than 4α-PDBu in enhancing the cytotoxicities of actinomycin D (Fig. 4B), while the monobutyrated phorbol was ineffective. 4β-PMA increased the cytotoxicities of actinomycin D at 10 μM (Fig. 4C); however, its IC50 of ~20 μM precluded analysis of its effects on MDR at higher doses. The 4α-isomer of PMA and 4β-phorbol 12-myristate were substantially less effective at reversing MDR, while 4β-phorbol 13-acetate was essentially inactive even at 100 μM. Virtually identical results were obtained when these phorbol compounds were tested in combina-

tion with daunomycin, whereas none of these compounds modulated the cytotoxicity of cisplatin (data not shown).

Staurosporine, which inhibits PKC with a Ki of approximately 1 nM (33), was tested for its effects on reversal of MDR by mezerein and phorbol esters. As demonstrated in Fig. 5A, pretreatment of MCF-7/ADR cells with 50 nM staurosporine did not reduce cell killing by combinations of daunomycin and mezerein, 4β-PDBu, 4α-PMA, or 4β-PMA. Similarly, staurosporine did not antagonize the effects of these compounds on the cytotoxicity of actinomycin D (Fig. 5B).

Effects of Phosphorylation-modulating Compounds on Pgp Activity—Levels of intracellular drug accumulation in Pgp-overexpressing cells can be used as a convenient measure of Pgp activity. For example, “classical” Pgp antagonists such as verapamil promote 5- to 8-fold increases in the accumulation of [3H]vinblastine and [3H]taxol by MCF-7/ADR cells (34). To assess the effects of the phosphorylation modulators on the intracellular accumulation of [3H]vinblastine, MCF-7/ADR cells were treated with doses of the modulators up to either their IC50 or to 100 μM. The results for non-phorbol compounds are summarized in Table I. Several compounds demonstrated good ability to enhance [3H]vinblastine accumulation. For example, mezerein, K-252a, H-89, and trifluoperazine demonstrated efficacies at least as great as that of verapamil. Conversely, many kinase activators and inhibitors, as well as two phosphoprotein phosphatase inhibitors, had no effect on Pgp activity.

The dose dependences for modulation of the intracellular accumulation of [3H]vinblastine by mezerein and 5 phorbol esters were tested. None of these compounds significantly altered the accumulation of [3H]vinblastine by MCF-7 cells at doses up to at least 100 μM (50 μM for mezerein) (data not shown). In contrast, mezerein caused dose-dependent and very marked (up to 12-fold) increases in [3H]vinblastine accumulation in MCF-7/ADR cells (Fig. 6), with doses as low as 50 nM being effective. 4β-PMA modestly increased [3H]vinblastine accumulation at very low doses (10 nM), followed by much more substantial accumulations at 100 μM, 4α-PMA, 4α-PDBu, 4β-PDBu, and 4β-phorbol 12-myristate all enhanced [3H]vinblastine accumulation only at 100 μM.

Pretreatment of the cells with 50 nM staurosporine did not
reduce the abilities of mezerein or the phorbol esters to increase intracellular accumulation of $[^3H]$vinblastine (Fig. 7). Staurosporine, K-252a, and calphostin C promoted modest dose-dependent increases in $[^3H]$vinblastine accumulation (Fig. 8); however, responses were seen only at concentrations of these kinase inhibitors which were very close to their IC$_{50}$ values. In contrast, the PKA inhibitor H-89 caused very marked accumulation of $[^3H]$vinblastine at doses much lower than its IC$_{50}$ for either MCF-7 or MCF-7/ADR cells.

Effects of Phosphorylation-modulating Compounds on PgpPhosphorylation and Photolabeling—To ensure that PKC-activating phorbol esters increase the phosphorylation state of Pgp, MCF-7/ADR cells were labeled with $[^32P]$PO$_4$ and then exposed to 4a- or 4b-PMA with or without pretreatment with staurosporine. Immunoprecipitation of Pgp (Fig. 9) demonstrated that Pgp is phosphorylated to a modest extent in unstimulated cells. Exposure to either 100 nM or 10 $\mu M$ 4a-PMA did not significantly enhance the phosphorylation state of Pgp (Fig. 9, lanes 2-5). In contrast, 100 nM and 10 $\mu M$ 4b-PMA increased Pgp phosphorylation by approximately 3-fold (lanes 6 and 8). Pretreatment of the cells with 200 nM staurosporine blocked the ability of either dose of 4b-PMA to increase the level of phosphorylation of Pgp (lanes 7 and 9), indicating that PKC is strongly inhibited by this compound. $\alpha$-glycoprotein in membranes isolated from 4b-PMA-treated cells, with or without pretreatment with 200 nM staurosporine, was equivalently photolabeled with $[^3H]$azidopine, indicating that phosphorylation does not strongly affect the drug binding affinity of Pgp (data not shown).

Interaction of a compound with the drug binding site(s) of Pgp can be inferred if the compound antagonizes the ability of Pgp to bind and become photolabeled by $[^3H]$azidopine (27, 28, 35). As demonstrated in Fig. 10, lanes 1, $[^3H]$azidopine can be cross-linked to Pgp in membranes isolated from MCF-7/ADR cells, whereas no such protein in membranes from MCF-7 cells is photolabeled with $[^3H]$azidopine (data not shown). As expected, photolabeling of Pgp by $[^3H]$azidopine was substantially reduced by including verapamil in the binding buffer (A, lane 2). Photolabeling was very strongly suppressed by H-89 and A23187 (lanes 6 and 7), while calyculin A, dibutyryl-cAMP, dibutyryl-cGMP, W-7, and genistein caused more modest decreases in $[^3H]$azidopine binding. The effects of PKC modulators were also tested (Fig. 10B). Photolabeling was inhibited by 4a-PMA, 4b-PMA, 4a-PDBu, or 4b-PDBu, indicating that these compounds which reversed MDR can directly interact with Pgp. K252-a and H-7 were more effective than staurosporine in reducing photolabeling by $[^3H]$azidopine. Calphostin C also strongly inhibited Pgp labeling by $[^3H]$azidopine, while both the 4a- and 4b-isomers of phorbol and PDBu were ineffective (data not shown).

Role of Pgp in Transporting Phorbol Esters—Since both PMA and PDBu appear to bind to the drug binding site of Pgp, we tested the ability of Pgp to transport these compounds. MCF-7/ADR cells accumulated twice as much $[^3H]$PMA as did MCF-7 cells, while the accumulation of $[^3H]$PDBu by MCF-7 cells was nearly 4-fold greater than that by MCF-7/ADR cells. The addition of verapamil, at doses up to 80 $\mu M$, did not significantly alter the accumulation of either $[^3H]$phorbol ester by MCF-7/ADR cells, indicating that Pgp does not directly transport PMA or PDBu.

**DISCUSSION**

Despite several years of effort, there have been no definitive demonstrations of reversal of MDR due to phosphorylation or dephosphorylation of Pgp. We sought to assess the roles of protein kinases in regulating Pgp function using a pharmacological approach. To this end, a panel of compounds which activate or inhibit protein kinases were tested for their effects on Pgp activity in MCF-7/ADR cells. These compounds included both general protein kinase inhibitors, such as staurosporine, and quite selective protein kinase inhibitors and activators, e.g., calphostin C, H-89, and phorbol esters.

Consideration of the effects of this large panel of phosphorylation modulators indicated that there were no patterns of differential cytotoxicity of inhibitors or activators of any particular class of protein kinase or phosphatase toward the two cell lines. Only calyculin A and A23187 demonstrated significantly different toxicities for the two cell lines. Therefore, PKC, PKA, PKG, and Ca$^{2+}$/calmodulin-dependent protein kinases appear to be similarly regulated and functional in the two cell
types, even though their actual levels may be different (15, 25).
Cell cycle times and cell phase distributions were similar in
wild-type MCF-7 and drug-resistant MCF-7/ADR cells (data
not shown), indicating that pathways involved in regulating
cell proliferation have not been grossly altered in the selected
cells.
Recently, we have identified several novel natural products
which overcome MDR by acting as antagonists for Pgp (e.g., 27,
28). Using the same methods, we have assessed the ability of
the phosphorylation modulators to overcome Pgp-mediated
MDR in MCF-7/ADR cells. While certain of these compounds
inhibited Pgp activity, there are no correlations with the acti-
vation or inhibition of any class of protein kinase. Similarly,
phosphoprotein phosphatase inhibitors, which have more glo-
bal effects on protein phosphorylation, did not modulate MDR
at subcytotoxic doses.
Because of the interest in the potential role of PKC in regu-
lating MDR, we conducted an extensive characterization of the
effects of activators and inhibitors of this family of kinases.
Overall, our data argues against a substantial role of PKC in
regulating Pgp activity for the following reasons.

1. Quite specific activators and inhibitors of PKC do not
consistently affect MDR or drug accumulation by MCF-7/ADR
cells. For example, chelerythrine chloride and calphostin C are
both potent and selective inhibitors of PKC; however, the latter
compound demonstrated much greater ability to reverse MDR
and promote the intracellular accumulation of \[3H\]vinblastine.
Previous studies have shown that calphostin C increases drug
accumulation in MDR cells (36, 37), but this effect is not me-
diated by inhibition of PKC (37).

2. Drug resistance was substantially reversed by micromolar
concentrations of several phorbol esters, including both the \(\alpha\)-
and \(\beta\)-isomers of PMA and PDBu. The high doses required and
the lack of specificity for the \(\beta\)-isomers suggest that these
The abilities of mezerein and phorbol esters to reverse MDR and to potentiate intracellular drug accumulation were not altered by pretreatment of the cells with staurosporine, even though this compound very effectively blocked PKC-mediated phosphorylation of Pgp.

5. All of the "PKC modulators" which reversed MDR inhibited the photolabeling of Pgp by \(^{3}H\)jazidopine. Therefore, it is very likely that the reversing effects of these compounds reflect their abilities to interact directly with Pgp rather than alteration of Pgp phosphorylation by PKC.

Similar conclusions can be drawn from data on PKA modulators. For example, H-89, which is \(-650\)-fold more active toward PKA than PKC (38), effectively inhibited drug transport by Pgp and reversed MDR. However, this was associated with high efficiency of inhibiting Pgp photolabeling by \(^{3}H\)jazidopine, indicating that the activity of H-89 is due to direct interaction with Pgp rather than through alteration of transport activity via the phosphorylation state of Pgp. Notably, the efficacy index of H-89 (i.e. 10–20) is superior to many previously described Pgp antagonists, suggesting that this compound may be therapeutically useful for reversal of MDR. A structurally related PKA inhibitor, H-87, has been shown to partially reverse resistance in MDR cells (39).

Antagonism of Pgp by staurosporine (40–42), H-7 (43), chlorpromazine (44), and trifluoperazine (45) have been described previously, and these compounds demonstrated moderate-to-good inhibition of Pgp in our model system. However, these agents simultaneously inhibit more than one protein kinase, making it difficult to assess the involvement of individual kinases. With the exception of staurosporine, all of these agents markedly inhibited Pgp photolabeling by \(^{3}H\)jazidopine (Fig. 10), again suggesting that they act by direct antagonism of the drug binding site of Pgp rather than by altering its phosphorylation state.

The phorbol ester family represents a new class of Pgp antagonists which demonstrate interaction at low micromolar concentrations. These compounds are of moderate size and are composed of multiple ring systems, similar to several other Pgp antagonists (6); however, their lack of ionizable functional groups is somewhat unusual. The hydrophobicity of these compounds appears to determine interaction with Pgp such that log P values of >2, but <7, effectively block drug transport. While PMA and PDBu are able to bind to Pgp, our results indicate that they are not transported out of the cells by this protein. In this regard, they resemble estramustine (32, 34).

In certain model systems, reversal of MDR is correlated with down-regulation of the expression of Pgp (24, 46–48). Additional studies have suggested that PKA activity may be necessary for the expression of the mdr1 gene (39, 46, 49). However, the relevance of study of the regulation of expression of highly amplified genes, e.g. mdr1 in MCF-7/ADR cells, is open to debate. Therefore, we have not shown the effects of phosphorylation modulators on levels of Pgp in these cells. We have recently found that A-498 kidney adenocarcinoma cells have significant levels of Pgp and are moderately resistant to Pgp.

### Fig. 8. Effects of phosphorylation inhibitors on \(^{3}H\)vinblastine accumulation in MCF-7/ADR cells. MCF-7/ADR cells were incubated with the indicated concentrations of staurosporine ( ), K-252a ( ), calphostin C ( ), or H-89 ( ) for 30 min as indicated under "Experimental Procedures." \(^{3}H\)vinblastine was then added and its intracellular accumulation after 60 min was determined. Values represent the mean ± S.D. accumulation of \(^{3}H\)vinblastine (solvent control = 100%) in one of two similar experiments.

### Fig. 9. Phosphorylation of Pgp. \(^{32}P\)O\(_4\)-labeled MCF-7/ADR cells were incubated for 5 min with 0 (even-numbered lanes) or 200 nM staurosporine (odd-numbered lanes), followed by incubation for 30 min with ethanol (lane 1), 0.1 \(\mu M\) 4\(\alpha\)-PMA (lanes 2 and 3), 10 \(\mu M\) 4\(\alpha\)-PMA (lanes 4 and 5), 0.1 \(\mu M\) 4\(\beta\)-PMA (lanes 6 and 7), or 10 \(\mu M\) 4\(\beta\)-PMA (lanes 8 and 9). Pgp was then immunoprecipitated using the Oncogene Science antibody, subjected to SDS-PAGE, and dried gels were analyzed by autoradiography and scintillation counting as indicated under "Experimental Procedures." The positions of the following prestained molecular mass markers are indicated: myosin, 217 kDa, and \(\beta\)-galactosidase, 130 kDa.

Effects do not involve protein kinase C. Furthermore, 1,2-di-octanoylglycerol, another PKC activator, did not enhance the cytotoxicities of the drugs. 4\(\beta\)-PDBu was particularly interesting since it demonstrated MDR reversing activity at doses at least 12-fold lower than its IC\(_{50}\). This efficacy index of 12 is at least as good as the index for well-characterized Pgp antagonists, e.g. verapamil.

3. The phosphorylation state of Pgp was significantly increased by 4\(\beta\)-PMA, but was unaffected by 4\(\alpha\)-PMA, even at 10 \(\mu M\), indicating that the effects of the latter compound are not mediated by phosphorylation.

4. The abilities of mezerein and phorbol esters to reverse MDR and to potentiate intracellular drug accumulation were not altered by pretreatment of the cells with staurosporine, even though this compound very effectively blocked PKC-mediated phosphorylation of Pgp.

5. All of the "PKC modulators" which reversed MDR inhibited the photolabeling of Pgp by \(^{3}H\)jazidopine. Therefore, it is very likely that the reversing effects of these compounds reflect
substrate drugs. Thus, it would seem appropriate to analyze mdrr gene regulation in this nonamplified system as well.

In conclusion, it is apparent that several compounds which are commonly used as phosphorylation modulators, i.e. are commonly used as phosphorylation modulators, are able to interact directly with Pgp. This results in inhibition of drug transport and reversal of the MDR phenotype independent of the involvement of protein kinases. There is little doubt that several protein kinases, including PKC and PAK, are able to phosphorylate Pgp, but this does not appear to significantly alter its activity in MCF-7/ADR cells. Protein phosphorylation may play a role in regulating Pgp expression; however, additional studies are needed to further explore such possibilities.

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