Cancer Chemother Pharmacol (1991) 29: 127–132

Cancer Chemotherapy and Pharmacology

Multidrug resistance in MCF-7 human breast cancer cells is associated with increased expression of nucleoside transporters and altered uptake of adenosine

Philip F. Morgan[1], Robert L. Fine[2]*, Paula Montgomery[3], and Paul J. Marangos[3]**

[1] Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Building 10, Room 3C-102, Bethesda, MD 20892, USA
[2] Medicine Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
[3] Unit of Neurochemistry, Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, MD 20892, USA

Received 16 January 1991/Accepted 2 July 1991

Summary. The rate of adenosine uptake and the corresponding expression of nucleoside transporters were studied in several MCF-7 human breast-cancer cell lines that express different levels of multidrug resistance (MDR). Kinetic studies of adenosine transport in these cell lines revealed that the mean apparent K_m and V_{max} values for the nucleoside transporters increased with increasing MDR. The apparent K_m and the apparent V_{max} of Adriamycin-resistant (ADR^{10}) cell lines were respectively 3.2- and 1.8-fold those of Adriamycin-sensitive wild-type (WT) cells ($P < 0.001$). A partially revertant cell line (ADR^{10}rev) that was derived from the ADR^{10} line and was partially sensitive to Adriamycin exhibited apparent K_m and V_{max} parameters that lay between those of the ADR^{10} and WT cells ($P < 0.001$ vs ADR^{10} cells; $P < 0.05$ vs WT cells). ADR^{10} cell membranes bound >4 times more of the nucleoside transporter blockers [^{3}H]-nitrobenzylthioinosine ([^{3}H]-NBI) and [^{3}H]-dipyridamole ([^{3}H]-DPR) than did WT cell membranes per unit protein ($P < 0.0001$). Scatchard analysis revealed a 2–3 times greater density for nucleoside transporters in ADR^{10} membranes as compared with those in WT membranes. ADR^{10}rev membranes bound less [^{3}H]-NBI and [^{3}H]-DPR than did ADR^{10} membranes ($P < 0.001$), but they bound more of the blockers than did WT membranes ($P < 0.05$). A 2.5-h exposure to 200 nM phorbol-12,13-dibutyrate (PDBu), which activates protein kinase C (PKC) and induces WT cells to exhibit a 4-fold increased transient MDR phenotype, increased the apparent K_m of WT cells for adenosine transport by >2 times ($P < 0.001$) to a value close to that found for the ADR^{10} cells. An identical exposure of ADR^{10} cells to PDBu produced no significant effect. The apparent K_m of ADR^{10}rev cells was increased 1.4 times by a 2.5-h PDBu exposure. None of the cell lines were affected by a 2.5-h exposure to 200 nM phorbol-13,10-diacetate (PDA), a much less active phorbol, or vehicle. These results suggest that MDR in MCF-7 cells is associated with changes in nucleoside transport, including both the number of transporters and their rate of transport, and that such changes can be partially mimicked by stimulation of PKC.

Introduction

The mechanism underlying multidrug resistance (MDR), whereby cancer cells develop a broad spectrum of resistance to a number of structurally unrelated natural-product antineoplastic drugs, is not completely understood. However, MDR is associated with a net decrease in the intracellular concentration of drug, and the increased expression of a transmembrane transporter, the P-glycoprotein, is thought to be responsible for the MDR cells' accumulation of less antineoplastic drug [1, 4, 19, 27, 29].

Recent evidence presented by Fine et al. [11] suggest that activation of PKC may induce a transient MDR phenotype in drug-sensitive human breast cancer (MCF-7) cells. Activation of PKC has also been associated with other exocytotic or transport phenomena, such as neurotransmitter release [23, 24] and stimulation of cyclic adenosine monophosphate [26, 28], which are also potently affected by the neuromodulator adenosine [7, 17, 18]. We therefore investigated adenosine flux and the expression of adenosine transporters in MDR cell lines and the effect of activation of PKC (using phorbol esters) on nucleoside transport.

Materials and methods

Cell lines. Three related MCF-7 cell lines that differed in their level of MDR were examined [9]. These included a parent wild-type (WT) strain that was sensitive to natural-product antineoplastic agents, a resistant

* *Present Address:* Division of Hematology/Oncology, Duke University Medical Center and V. A. Hospital, Durham, N. C. 27705

** *Present Address:* National Library of Medicine, Bethesda, MD. 20892, USA

Offprint requests to: P. F. Morgan

strain that was capable of growth in 10 μM Adriamycin (ADR^{10}), and a subline of ADR^{10} that had partially reverted (ADR^{10}rev) after passage in drug-free medium for 1 year. All cell lines were passaged in Iscove's minimal essential medium (IMEM) containing 10% heat-inactivated fetal calf serum (FCS) and were split weekly to maintain logarithmic cell growth. The ADR^{10} cell line was passaged in drug-free medium for at least 4 weeks prior to testing.

Clonogenic assays. Clonogenic assays were performed to determine the IC_{50} (see below) so as to demonstrate the degrees of relative resistance between the three lines. Cells from each line were plated in triplicate in IMEM supplemented with 10% FCS according to previously determined plating efficiencies so as to obtain approximately 100 colonies/well. After 24 h, Adriamycin, vinblastine, and vincristine in sterile saline were added at concentrations over a 3-log scale for continuous exposure. At 7–10 days thereafter, the wells were fixed and stained with a methylene blue-ethanol mixture and then counted. The IC_{50} was determined as the mean concentration of drug that reduced the colony count by 50% as compared with control and diluent control values. The experiments were performed three times in triplicate and the IC_{50} was expressed as the mean ± standard error of the mean. Table 1 shows the IC_{50} value obtained in each line for Adriamycin, vinblastine, and vincristine in clonogenic assay.

Drug accumulation assays. To demonstrate further the MDR phenotype of these lines, drug accumulation assays were performed using [^{3}H]-vinblastine according to a previously described method [11]. Briefly, the three lines were grown in 30-cm^2 plastic wells and were tested in the exponential growth phase when they had become approximately 75% confluent. Cells were washed three times in cold phosphate-buffered saline (PBS) and were then incubated with serum-free IMEM supplemented with 20 mM HEPES (pH 7.35). [^{3}H]-Vinblastine (sp. act., 2 Ci/ mM) diluted in unlabeled vinblastine to a concentration of 200 nM was added to the above cells, which were then incubated for 1 h. This time point had been determined to represent the plateau for drug-accumulation changes in previous experiments (data not shown). The plates were then washed three times in cold PBS, solubilized with 1 M NaOH, and neutralized with HCl. Radioactivity was quantitated by the addition of liquid scintillation fluid followed by counting in a scintillation counter. The results were calculated and expressed as picomoles of drug per milligram of protein.

Nucleoside transport. For [^{3}H]-adenosine transport studies, WT, ADR^{10}, and ADR^{10}rev cells were grown in 30-cm^2 plastic wells and were tested in the exponential growth phase when they had become approximately 75% confluent. In studies involving phorbol ester treatment, wells were pretreated with either 200 nM PDBU or 200 nM PDA or vehicle for 2.5 h prior to the uptake studies. Previous studies have obtained maximal stimulation of PKC in MCF-7 cells using the same concentration of PDBu and the same incubation period [11]. Cells were washed with Krebs-Hensleit physiological buffer solution (118.5 mM NaCl, 4.7 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.18 mM KH_2PO_4, 2.49 mM$NaHCO_3$, and 10 mM glucose, aerated with 95% O_2 and 5% CO_2; pH 7.4) maintained at 20° C. They were then immersed in an incubation bath containing [^{3}H]-adenosine (sp. act., 51.3 Ci/mmol, New England Nuclear, Boston, Mass.; diluted 2–160 times with non-radioactive adenosine) dissolved in 500 ml Krebs-Hensleit physiological buffer solution at 20° C.

After a 5-s incubation period, cells were rapidly transferred to a bath containing 500 ml of a "stop" solution consisting of 5 μM nitrobenzylthioinosine (NBI) dissolved in physiological buffer that was maintained at 2° C. Cells were immersed in the stop solution for 5 s, after which they were rapidly washed for 5 s in an additional 500 ml of identical stop solution at 2° C. Cell-culture plates were left inverted for 30–60 min, and the excess buffer was removed. Cells were then collected with a rubber policeman and were disrupted by sonication in 1 ml distilled water.

The radioactivity of samples was determined by adding a 500-μl aliquot to 10 ml scintillation fluid (Beckman Redisolv; Beckman, Fullerton, Calif.) for spectrophotometric analysis. When 5 μM NBI was added to the incubation medium, the accumulation of [^{3}H]-adenosine amounted to 93.1% ± 2.6% of the control value (absence of NBI). The nonspecific component of accumulation (the value obtained in the absence of NBI minus that obtained in the presence of NBI) was assessed individually for each estimate of adenosine accumulation. Each determination was then corrected to reflect only the NBI-sensitive component of accumulation.

Table 1. Clonogenic assay IC_{50} for the three lines

MCF-7	IC_{50} (nM)		
	Adriamycin	Vinblastine	Vincristine
WT	10 ± 2	8 ± 4	14 ± 3
ADR^{10}	850 ± 22	350 ± 14	440 ± 26
ADR^{10}rev	245 ± 18	160 ± 15	200 ± 24

The results were obtained in clonogenic experiments performed 3 times each in triplicate using continuous drug exposure. Data represent mean values ± SEM

For radioligand binding studies of the nucleoside transporter, cells were grown suspended in IMEM containing 10% heat-inactivated FCS in constantly agitated 500-ml plastic conical flasks. Cells were harvested in the exponential growth phase by centrifugation at 30,000 *g* for 20 min at 2° C. The pelleted cells were homogenized in approximately 20 vol. 50 mM TRIS HCl (pH 7.4) at 2° C. The resulting cell membranes were then rewashed twice by repeating this centrifugation/resuspension procedure. The pelleted cell membranes were finally resuspended in TRIS-HCl (pH 7.4) buffer for use in the radioligand binding assays.

The [^{3}H]-NBI binding assay was performed by incubating cell membranes with 0.025–12.5 nM [^{3}H]-NBI (sp. act., 37 Ci/mmol, New England Nuclear, Boston, Mass.) for 30 min at room temperature in the absence and the presence of 1 μM NBI (to assess nonspecific binding) in a total assay volume of 0.5 ml. The [^{3}H]-DPR binding assay was carried out by incubating cell membranes with 0.25–50 nM [^{3}H]-DPR (sp. act., 107 Ci/mmol, Moravek Biochemicals, Brea, Calif.) for 30 min at 2° C in the absence and the presence of 100 μM DPR (to assess nonspecific binding) in a total assay volume of 0.5 ml using plastic tubes. Assays were terminated by vacuum filtration on Whatman GF/B fiberglass filters (Whatman, Clifton, N. J.) that had been prewashed with 0.1% bovine serum albumin and were housed in a Brandel M-24R cell harvester (Brandel, Gaithersburg, Md.). Filters were rapidly washed in 3 × 4 ml ice-cold buffer and the radioactive content of the filters was determined spectrophotometrically in 10 ml Redi-solv scintillant (Beckman Co., Fullerton, Calif.).

Protein determination. Protein concentrations were estimated by the Bradford method as previously described [2].

Statistical analysis. Data were analyzed by analysis of variance with Scheffe post-hoc between cell comparisons.

Results

The MDR phenotype of the cell lines in clonogenic and drug accumulation assays

Table 1 shows the IC_{50} value (nM) obtained in each cell line for Adriamycin, vinblastine, and vincristine. The ADR^{10} line was 85, 44, and 31 times more resistant to Adriamycin, vinblastine, and vincristine, respectively, as compared with the wild-type MCF-7 control cells. The partially revertant line ADR^{10}rev was 24, 20, and 14 times more resistant to the three agents, respectively, as compared with the control line. This represented an approximately 2- to 3-fold decrease in the MDR phenotype as compared with that found in the ADR^{10} cell line, and it has remained stable for >1 year. Under these conditions, the WT control line accumulated approximately 2.2 and 4.4

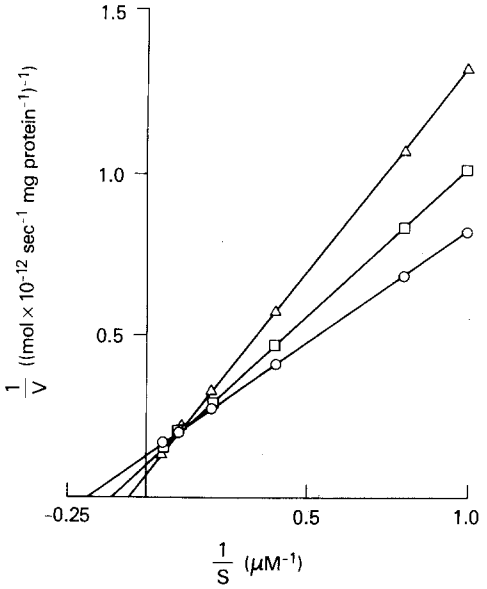

Fig. 1. Uptake of adenosine in WT, MDR ADR^{10}, and the partially revertant ADR^{10}rev human breast cancer cell lines as a function of adenosine concentration. Uptake of [^{3}H]-adenosine in WT cells (○), MDR ADR^{10} cells (△), and partially revertant ADR^{10}rev cells (□). Values represent the mean of 4 experiments, with SEMs being smaller than the symbol size, and are plotted in the form of a linear transformation (Lineweaver-Burke plot). All plots were best fit by a straight line (linear regression correlation coefficients, r^2 >0.98), indicating a single component to uptake. Regression-computed apparent K_m and V_{max} values for these cell lines are shown in Table 2.

times more vinblastine (VLB) than did the ADR^{10}rev and ADR^{10} lines, respectively. Tritiated VLB accumulation studies revealed that the WT cell line accumulated 44.4±4 pmol VLB/mg protein, the ADR^{10} cell line accumulated 10±2 pmol VLB/mg protein, and the ADR^{10}rev line accumulated 20±5 pmol VLB/mg protein (mean values ± SEM of three experiments performed in triplicate). The partially revertant line accumulated 2 times more drug than did the ADR^{10} line, and its drug accumulation represented approximately 45% of that of the sensitive control line.

The MCF-7 cell lines differ in their ability to accumulate adenosine

Kinetic studies of adenosine transport in these MCF-7 cell lines revealed that both the apparent K_m and the apparent V_{max} of nucleoside transport increased with increasing MDR (Fig. 1). Regression-computed apparent K_m and V_{max} values for WT cells were 5.26±0.75 μM and 7.62±0.28 mol × 10^{-12} s^{-1} mg $protein^{-1}$, respectively. Apparent K_m (16.7±1.12 μM) and V_{max} (13.34±0.48 mol × 10^{-12} s^{-1} mg $protein^{-1}$) values for ADR^{10} cells were significantly different (P <0.001) from the corresponding WT values. The partially revertant cell line (ADR^{10}rev) exhibited apparent K_m and V_{max} values that lay between those of the MDR ADR^{10} and the WT lines. Apparent K_m (8.6±0.29 μM) and V_{max} (9.36±0.44 mol × 10^{-12} s^{-1} mg $protein^{-1}$) values for ADR^{10}rev cells were significantly different from those found for the ADR^{10} cells (P <0.001) and the WT cells (P <0.05; Table 2).

Table 2. Apparent K_m and V_{max} values for the three lines

MCF-7	Apparent K_m (μM)	V_{max} (mol × 10^{-12} s^{-1} mg $protein^{-1}$)
WT	5.26±0.75	7.62±0.28
ADR^{10}	16.7 ±1.12	13.34±0.48
ADR^{10}rev	8.6 ±0.29	9.36±0.44

MCF-7 cell lines differ in their ability to bind specifically the nucleoside transporter ligands [^{3}H]-NBI and [^{3}H]-DPR

The MDR ADR^{10} cell membranes bound approximately 4 times more [^{3}H]-NBI and [^{3}H]-DPR per unit protein than did the WT cell membranes (P <0.0001; Fig. 2A). The partially revertant cell line (ADR^{10}rev) bound significantly less [^{3}H]-NBI and [^{3}H]-DPR per unit protein than did the MDR ADR^{10} cell line (P <0.001), but it bound significantly more than did the WT cell line (P <0.05). Scatchard analysis of [^{3}H]-NBI and [^{3}H]-DPR saturation binding to the WT and the ADR^{10} cell lines indicated that the binding of these ligands to the nucleoside transporters was best described by a single binding site, which was labelled equally well by both ligands (Fig. 2B). These analyses suggest that the ability of MDR ADR^{10} cell membranes to bind significantly greater amounts of either [^{3}H]-NBI or [^{3}H]-DPR as compared with WT cell membranes can be largely attributed to a 2- to 3-fold increase in the total number of nucleoside transporters in MDR ADR^{10} cells vs WT cells.

Phorbol esters mimic the increased K_m of nucleoside transport observed with increasing MDR

Previous studies in our laboratory of PKC phosphorylation of histone IIIs revealed that basal PKC activity in MDR ADR^{10} cells was 7-fold that in the parent WT cells and that 200 nM of the active phorbol ester PDBu could stimulate PKC activity in WT and MDR ADR^{10} cells by 2 and 2.5 times, respectively [11], whereas 200 nM of either the inactive phorbol ester PDA or the vehicle produced no effect [11]. Under these PKC-stimulated conditions, WT cells exhibited a 4-fold increase in resistance to Adriamycin and vinblastine over 24-h, thus exhibiting an MDR phenotype. Therefore, the effects of 200 nM PDBu, PDA, or vehicle on adenosine transport in the different MCF-7 cell lines were examined.

The different MCF-7 cell lines differed markedly in their response to phorbol ester exposure with respect to the adenosine transport function. Kinetic studies of adenosine accumulation in the MCF-7 cell lines revealed that a 2.5-h exposure of WT cells to 200 nM PDBu increased the apparent K_m of such cells for adenosine by >2 times to a value close to that found for the untreated MDR ADR^{10}rev

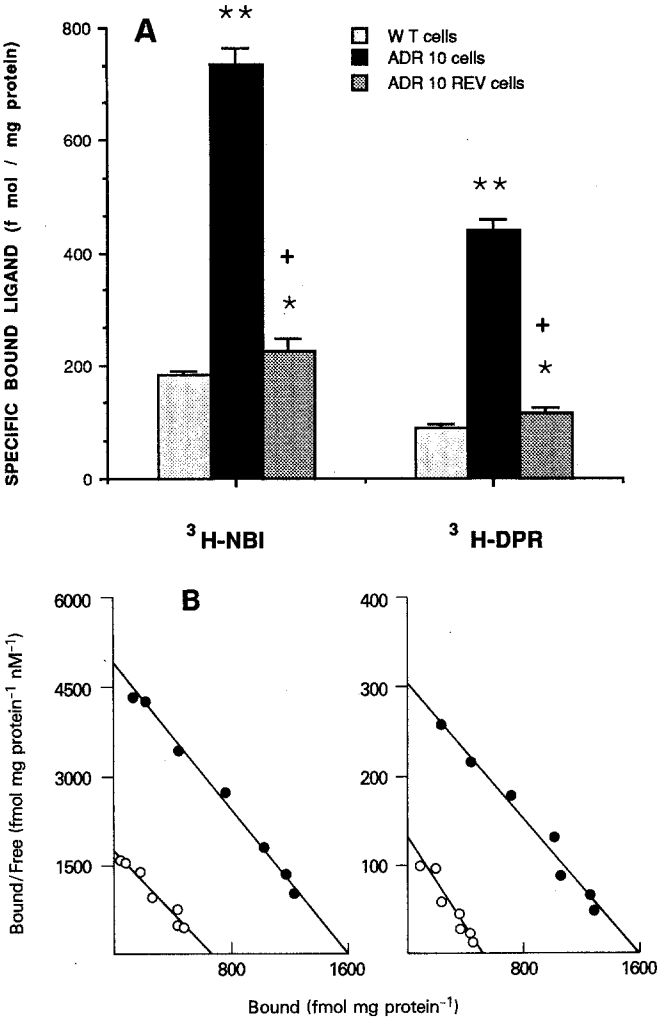

Fig. 2 A, B. [^{3}H]-NBI and [^{3}H]-DPR binding to MCF-7 human breast-cancer cell membranes. **A** Specific binding of [^{3}H]-NBI at a free concentration of 0.3 nM and of [^{3}H]-DPR at a free concentration of 1 nM. Data points represent mean values ± SEM of 4 independent experiments, each performed in triplicate (* P <0.05, ** P <0.001 as compared with binding in WT membranes, + P <0.001 as compared with binding in ADR^{10} membranes). **B** Scatchard analysis of saturation isotherms of [^{3}H]-NBI (*left panel*) and [^{3}H]-DPR (*right panel*) binding to WT (○) and ADR^{10} (●) cell membranes. Data points represent the mean of a single experiment performed in triplicate. Three further experiments yielded similar results. Data were best fit by a straight line (linear regression correlation coefficient, r^2 >0.97) for both [^{3}H]-NBI and [^{3}H]-DPR. [^{3}H]-NBI binding data revealed regression-computed estimates of K_d and B_{max} values to be K_d = 0.38 nM and B_{max} = 670 fmol/mg protein for WT cells and K_d = 0.33 nM and B_{max} = 1596 fmol/mg protein for ADR^{10} cells. [^{3}H]-DPR binding studies revealed regression computed estimates of K_d and B_{max} values to be K_d = 4 nM and B_{max} = 515 fmol/mg protein for WT cells and K_d = 4 nM and B_{max} = 1620 fmol/mg protein for ADR^{10} cells

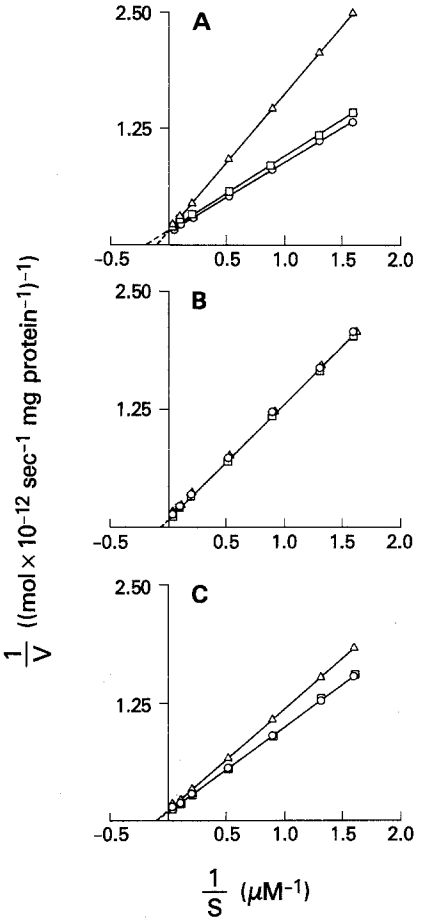

Fig. 3 A–C. The effect of phorbol esters on [^{3}H]-adenosine uptake in MCF-7 cell lines. **A** WT, **B** ADR^{10}, and **C** ADR^{10}rev cells were preincubated for 2.5 h at 37° C with either vehicle (○), 200 nM PDBu (△), or 200 nM PDA (□). Uptake of [^{3}H]-adenosine during a 5-s incubation was then determined as described in Materials and methods. Respective regression-computed apparent K_m and V_{max} values for WT cells were: control, 5.4 ± 0.4 μM and 7.3 ± 0.4 mol × 10^{-12} s^{-1} mg protein^{-1}; 200 nM PDBu, 11.1 ± 0.8 μM and 6.4 ± 0.4 mol × 10^{-12} s^{-1} mg protein^{-1}; and 200 nM PDA, 5.9 ± 0.5 μM and 6.6 ± 0.4 mol × 10^{-12} s^{-1} mg protein^{-1}. Respective K_m and V_{max} values for ADR^{10} cells were: control, 16.7 ± 1.1 μM and 13.1 ± 0.9 mol × 10^{-12} s^{-1} mg protein^{-1}; 200 nM PDBu, 12.5 ± 0.9 μM and 10.1 ± 0.6 mol × 10^{-12} s^{-1} mg protein^{-1}; and 200 nM PDA, 11.6 ± 1.4 μM and 9.8 ± 0.7 mol × 10^{-12} s^{-1} mg protein^{-1}. In ADR^{10}rev cells, K_m and V_{max} values computed were: control, 7.1 ± 0.5 μM and 9.3 ± 0.6 mol × 10^{-12} s^{-1} mg protein^{-1}; 200 nM PDBu, 10.1 ± 0.7 μM and 9 ± 0.5 mol × 10^{-12} s^{-1} mg protein^{-1}; and 200 nM PDA, 8.7 ± 0.7 μM and 10.6 ± 0.7 mol × 10^{-12} s^{-1} mg protein^{-1}. All data are expressed as mean values ± SEM

cell line (Fig. 3 A). A similar exposure of MDR ADR^{10} cells to PDBu produced no significant effect (Fig. 3 B). The apparent K_m of the partially revertant ADR^{10}rev cell line was also increased by a 2.5-h PDBu exposure, although to a lesser extent (1.4 times) than that demonstrated for the WT cell line (Fig. 3 C). None of the cell lines were affected by a 2.5-hour exposure to 200 nM PDA or vehicle.

Discussion

In a wide variety of cell types, MDR is associated with an overexpression of a 170-kDa plasma membrane glycoprotein (P-glycoprotein) [4, 27, 29]. Transfection of drug-sensitive hamster cells with an expression vector containing cDNA encoding a 170-kDa P-glycoprotein has been reported to confer MDR [5, 13]. Computer searches of DNA sequence database reveal a considerable homology between P-glycoprotein cDNA clones and a number of bacterial (principally *Escherichia coli*) genes encoding nucleotide (adenosine triphosphate, ATP)-binding transport

proteins [12, 14], notably hemolysin B. The greatest homology between DNA sequences of P-glycoprotein and those of the transmembrane transport proteins was observed in the regions that are directly involved in nucleotide (ATP) binding. On the basis of this homology, it has been postulated that the 170-kDa P-glycoprotein is a nucleotide-binding transport protein [5, 12, 14]. In view of this homology, it is possible that the 170-kDa P-glycoprotein may share a similar sequence homology with other nucleotide-binding transport proteins whose amino acid sequence is not known (such as the nucleoside transporter) and, consequently, may have some features in common with such transporters.

The present studies revealed that nucleoside transporter expression in MCF-7 cell lines was increased in MDR and that the apparent K_m and V_{max} of nucleoside transport increased with increasing MDR. An increase in V_{max} implies an increase in the maximal velocity at which adenosine and other nucleosides can be transported across the cell membrane. This may be due to an increased number of transporter sites for nucleosides, since Scatchard analysis suggests a 2- to 3-fold increase in the number of nucleoside transporter binding sites in MDR cells. An increase in K_m implies that the initial nucleoside transport velocity is reduced. The effects of increases in apparent K_m and V_{max} values tend to oppose each other. In the case of the present data, the net effect would be that MDR cells exhibit a decreased rate of nucleoside transport at low nucleoside concentrations (<10 μM) but an increased rate of transport at high nucleoside concentrations (>10 μM). Normal plasma adenosine concentrations have been estimated to lie in the range of 1–10 μM, although levels can dramatically increase during anoxia or hypoxia [8].

MDR is a complex phenomenon that may involve a number of known and some unknown modifications. Among these may be an increased production of P-glycoprotein [6, 15, 21, 22], an altered rate of nucleoside transport (which may or may not be associated with or linked to P-glycoprotein), and changes in PKC activity [11]. MDR can be transiently induced in WT MCF-7 drug-sensitive cells by treatment with phorbol esters, presumably via activation of PKC [11]. PKC can exert pleiotropic regulation on a number of cellular processes, particularly transport mechanisms, by phosphorylation [10, 20, 25, 30, 31]. In addition, the P-170 glycoprotein is phosphorylated by PKC [3, 16]. The effect of PDBu on the K_m of the nucleoside transporter for adenosine may reflect a direct or indirect action of PKC on the nucleoside transporter. If increases in nucleoside transporters and adenosine uptake are found to be a general phenomenon in other MDR cell lines, it may be possible to develop strategies directed at obtaining preferential toxicity to MDR cells using adenosine analogues or other nucleosides. In this context, it should be determined whether these cells and other MDR cells are differentially sensitive to adenosine analogues or other substrates for the nucleoside transporter.

Acknowledgements. We would like to thank Dr. K. Cowan, NCI, for kindly providing the cell lines used. We would also like to thank Drs. B. Chabner, NCI, and J. Patel, ICI Americas, for their very useful comments and discussion. The secretarial support of Ms. V. Cameron is much appreciated.

References

1. Beck WT, Mueller TJ, Tanzer LR (1979) Altered surface membrane glycoproteins in vinca alkaloid-resistant human leukemic lymphoblasts. Cancer Res 39: 2070
2. Bradford MW (1980) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248
3. Chambers TC, McAvoy EM, Jacobs JW, Eilon G (1990) Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. J Biol Chem 265: 7679
4. Cornwell MM, Pastan I, Gottesman MM (1987) Certain calcium channel blockers bind specifically to multidrug resistant human KB carcinoma membrane vesicles and inhibit drug binding to P-glycoprotein. J Biol Chem 262: 2166
5. Croop JM, Guild BS, Gros P, Housman DE (1987) Genetics of multidrug resistance: relationship of a cloned gene to the complete multidrug resistant phenotype. Cancer Res 47: 5982
6. Dalton WS, Durie BGM, Alberts DS, Gerlach JH, Cress AE (1986) Characterization of a new drug resistant human myeloma cell line that expresses P-glycoprotein. Cancer Res 46: 5125
7. Dunwiddie TV, Fredholm BB (1985) Adenosine modulation of synaptic responses in rat hippocampus: possible role of inhibition or activation of adenyl cyclase. Adv Cyclic Nucleotide Protein Phosphoryl Res 19: 259
8. Durcan MJ, Morgan PF (1990) Prospective role for adenosine and adenosinergic systems in psychiatric disorders. Psychiatr Med 20: 475
9. Fairchild CR, Ivy SP, Kao-Shan CS, Whang-Peng J, Rosen N, Israel MA, Melera PW, Cowan KH, Goldsmith ME (1987) Isolation of amplified and over-expressed DNA sequences from Adriamycin-resistant human breast cancer cells. Cancer Res 47: 5141
10. Fine RL (1989) Multidrug resistance. In: Pinedo H, Longo D, Chabner B, Eds., Cancer Chemotherapy and Biologic Response Modifiers Annual 10. Elsevier, Amsterdam, p 73
11. Fine RL, Patel J, Chabner BA (1988) Phorbol esters induce multidrug resistance in human breast cancer cells. Proc Natl Acad Sci USA 85: 582
12. Gerlach JH, Endicott JA, Juranka PF, Henderson G, Sarangi F, Deuchars KL, Ling V (1986) Homology between the P-glycoprotein and a bacterial haemolysin transport protein suggests a model for multidrug resistance. Nature 324: 485
13. Gros P, Ben-Neriah YB, Croop JM, Housman DE (1986) Isolation and expression of a complementary DNA that confers multidrug resistance. Nature 323: 728
14. Gros P, Croop J, Housman D (1986) Mammalian multidrug resistance gene: complete cDNA sequence indicates strong homology to bacterial transport proteins. Cell 47: 371
15. Hamada H, Tsuruo T (1986) Functional role for the 170- to 180-kDa glycoprotein specific to drug-resistant tumor cells as revealed by monoclonal antibodies. Proc Natl Acad Sci USA 83: 7785
16. Hamada H, Hagiwara KI, Nakajima T, Tsuruo T (1987) Phosphorylation of the M_r 170,000 to 180,000 glycoprotein specific to multidrug resistant tumor cells: effects of verapamil, trifluoperazine and phorbol esters. Cancer Res 47: 2860
17. Jackisch R, Fehr R, Hertting G (1985) Adenosine: an endogenous modulator of hippocampal noradrenaline release. Neuropharmacology 24: 499
18. Jonzen B, Fredholm BB (1984) Adenosine receptor mediated inhibition of noradrenaline release from slices of the rat hippocampus. Life Sci 35: 1971
19. Juliano RL, Ling V (1976) A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 455: 152
20. Kajikawa N, Kaibuchi K, Matsubara T, Kikkawa V, Takai Y, Nishizuka Y (1983) A possible role of protein kinase C in signal induced lysosomal enzyme release. Biochem Biophys Res Commun 116: 743
21. Kartner N, Riordan JR, Ling V (1983) Cell surface P-glycoprotein associated with multidrug resistance in mammalian cell lines. Science 221: 1285

22. Kartner N, Evernden-Porelle D, Bradley G, Ling V (1985) Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. Nature 316: 820
23. Knight DE, Baker PF (1983) The phorbol ester TPA increases the affinity of exocytosis for calcium in "leaky" adrenal medullary cells. FEBS Lett 160: 98
24. Matthies HJG, Palfrey C, Hirning LD, Miller RJ (1987) Down regulation of protein kinase C in neuronal cells: effect on neurotransmitter release. J Neurosci 7: 1198
25. Meyers MB (1989) Protein phosphorylation in multidrug resistant Chinese hamster cells. Cancer Commun 1: 233
26. Nordstedt C, Fredholm BB (1987) Phorbol-12,13-dibutyrate enhances the cycle AMP accumulation in rat hippocampal slices induced by adenosine analogues. Naunyn-Schmiedeberg's Arch Pharmacol 335: 136
27. Pastan I, Gottesman M (1987) Multidrug resistance in human cancer. N Engl J Med 316: 1388
28. Rebois RV, Patel J (1985) Phorbol ester causes desensitization of gonadotropin-responsive adenyl cyclase in a murine Leydig tumor cell line. J Biol Chem 260: 8026
29. Safa AR, Glover CJ, Meyers MB, Biedler JL, Felsted RL (1986) Vinblastine photo-affinity labeling of a high molecular weight surface membrane glycoprotein specific for multidrug resistant cells. J Biol Chem 261: 6137
30. White JR, Huang CK, Hill JM, Naccache PH, Becker EL, Sha-ati RI (1984) Effect of phorbol-12-myristate-13-acetate and its analogue 4-α-phorbol-12,13-didecanoate on protein phosphorylation and lysosomal enzyme release in rabbit neutrophils. J Biol Chem 259: 8605
31. Wooten MW, Wrenn RW (1985) Redistribution of phospholipid/calcium-dependent protein kinase and altered phosphorylation of its soluble and particulate substrate proteins in phorbol ester-treated rat pancreatic acini. Cancer Res 45: 3912