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A Possible Role for a Mammalian Facilitative Hexose Transporter in the Development of Resistance to Drugs

JUAN C. VERA,* GONZALO R. CASTILLO, AND ORA M. ROSEN

Program in Molecular Biology, Memorial Sloan Kettering Cancer Research Center, New York, New York 10021

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We show that D- but not L-hexoses modulate the accumulation of radioactive vinblastine in injected *Xenopus laevis* oocytes expressing the murine Mdr1b P-glycoprotein. We also show that *X. laevis* oocytes injected with RNA encoding the rat erythroid/brain glucose transport protein (GLUT1) and expressing the corresponding functional transporter exhibit a lower accumulation of [^{3}H]vinblastine and show a greater capacity to extrude the drug than do control oocytes not expressing the rat GLUT1 protein. Cytochalasin B and phloretin, two inhibitors of the mammalian facilitative glucose transporters, can overcome the reduced drug accumulation conferred by expression of the rat GLUT1 protein in *Xenopus* oocytes but have no significant effect on the accumulation of drug by *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein. These drugs also increase the accumulation of [^{3}H]vinblastine in multidrug-resistant Chinese hamster ovary cells. Cytochalasin E, an analog of cytochalasin B that does not affect the activity of the facilitative glucose transporter, has no effect on the accumulation of vinblastine by multidrug-resistant Chinese hamster cells or by oocytes expressing either the mouse Mdr1b P-glycoprotein or the GLUT1 protein. In all three cases, the drug verapamil produces a profound effect on the cellular accumulation of vinblastine. Interestingly, although immunological analysis indicated the presence of massive amounts of P-glycoprotein in the multidrug-resistant cells, immunological and functional studies revealed only a minor increase in the expression of a hexose transporter-like protein in resistant versus drug-sensitive cells. Taken together, these results suggest the participation of the mammalian facilitative glucose transporter in the development of drug resistance.

Cultured mammalian cell lines derived in vitro by stepwise selection in the presence of a variety of cytotoxic natural products often develop cross-resistance to a structurally and functionally diverse group of drugs (e.g., colchicine, vinblastine, vincristine, epipodophylotoxins, actinomycin, taxol, and doxorubicin), a phenomenon referred to as multidrug resistance. In general, these cells become highly resistant to the drug used in the selection process but also develop cross-resistance, usually to a lesser degree, to a number of other drugs (19, 25, 39, 67).

While there are a number of different mechanisms which can result in resistance to different drugs, recent studies indicate that multidrug resistance is associated with the overexpression of a family of antigenically related high-molecular-weight membrane phosphoglycoproteins, the P-glycoproteins (4, 11, 14, 30, 31, 38, 40, 54, 55, 58). A number of cDNAs and genomic clones encoding a small family of structurally related P-glycoproteins have been isolated from multidrug-resistant (MDR) mammalian cell lines derived in vitro by stepwise drug selection protocols (8, 17, 24, 27–29, 36, 57, 59, 66, 68, 70). Sequence analysis of these clones suggests that the corresponding proteins from human, hamster, and mouse cells are highly conserved. Each isoform is composed of two homologous halves, each containing six predicted transmembrane domains, with a total of about 1,280 amino acid residues and a predicted M_r of about 140,000. Each duplicated half contains a highly conserved hydrophilic cytoplasmic region containing a putative ATP binding domain (8, 17, 24, 27, 29).

MDR cells, when grown in the presence of drugs, show a lower accumulation of the drugs than do sensitive cells. This decreased drug accumulation is invariably associated with an increased efflux of the drug from these cells, in a process that appears to require ATP (13, 22, 37, 46, 73). Other observations have indicated that P-glycoprotein can bind different drugs related to the MDR phenotype (9–11, 30, 31, 35, 60). These results support the hypothesis that P-glycoprotein acts as an ATP-dependent drug efflux pump and functions directly as a multidrug transporter in MDR cells. cDNA and genomic transfection experiments have provided direct evidence indicating that expression of the human, hamster, or mouse isoforms of P-glycoprotein can mediate the MDR phenotype in otherwise drug-sensitive cells (3, 14, 16, 17, 28, 32, 65). The expression of a single member of the P-glycoprotein gene family results in the transfer of a typical MDR phenotype to the recipient drug-sensitive cells, and different individual members of the *mdr* gene family can provide the transfected cells with a different drug resistance profile (17).

Despite the evidence that links the overproduction of P-glycoprotein to the development of multidrug resistance, there are still important questions remaining to be answered. An important observation is that not every member of the P-glycoprotein family can confer drug resistance when overexpressed in otherwise sensitive cells. Thus, two members of the mouse *mdr* gene family, *mdr1* and *mdr3*, as well as the human *mdr1* gene, can confer multidrug resistance to cDNA-transfected cells overexpressing the corresponding P-glycoproteins (3, 17, 28, 65). However, a cDNA clone of the mouse *mdr2* gene is unable to confer multidrug resistance to transfected cell lines (29, 66). Sequence analysis indicate that the mouse *mdr1* and *mdr2* genes encode proteins that have highly homologous amino acid sequence (71% of identical residues) and predicted secondary structure (29). Interestingly, the *mdr2* gene product is coamplified with the other members of the P-glycoprotein family in independently derived MDR cell lines (36, 56). In addition, little is known

* Corresponding author.

about the possible role of other gene products that are also coamplified and overexpressed in MDR cell lines. For example, a small protein of about 22 kDa, sorcin, is amplified in MDR cells isolated by selection in the presence of a variety of cytotoxic drugs (e.g., colchicine, vinca alkaloids, and dactinomycin) (15, 43, 47, 50, 69, 70). A limited number of proteins other than sorcin also undergo changes in expression during development of multidrug resistance (49, 61). The contribution of these changes to multidrug resistance, if any, remains to be tested.

We have recently described the functional expression of a member of the mouse family of *mdr* gene products (*mdr1b*) (see reference 35 for corresponding nomenclature) in *Xenopus laevis* oocytes (7). We have shown that *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein show a lower accumulation of vinblastine when incubated in the presence of micromolar concentrations of this drug than do control oocytes injected with water. In this report, we provide direct evidence that *Xenopus* oocytes expressing the facilitative rat erythroid/brain glucose transporter also show a lower accumulation of vinblastine than do control oocytes. We also present evidence suggesting that the mammalian facilitative glucose transporter may play a role in the expression of drug resistance in mammalian MDR cell lines.

MATERIALS AND METHODS

Plasmids, in vitro transcription, and RNA injection. The cDNA encoding the mouse Mdr1b P-glycoprotein was provided by Stephen Hsu (36). The cDNA encoding the rat erythroid/brain hexose transporter (GLUT1) has been described elsewhere (5). Plasmids containing the respective cDNAs were linearized with the appropriate restriction enzymes and used for in vitro transcription and capping (7, 71, 72). To obtain a truncated GLUT1 protein, the corresponding cDNA was digested with *Rsa*II, isolated by agarose gel electrophoresis, and used for in vitro transcription and capping (71, 72). The in vitro-synthesized RNA encodes a truncated form of GLUT1 lacking the last C-terminal 51 amino acids. *Xenopus* oocytes were isolated from ovaries from mature frogs by treatment with collagenase, injected in the vegetal pole with RNA or an equivalent volume of distilled water, and incubated for 2 days at 18 to 22°C before being used (7, 71, 72).

Drug accumulation and efflux. [^{3}H]vinblastine accumulation and efflux in *Xenopus* oocytes were assayed as described previously (7). DC-3F (drug sensitive) and DC-3F/AD X (MDR) Chinese hamster cells (kindly provided by Marian Meyers [49]) were grown as monolayer cultures in six-well plates as previously described (49) to a density of approximately 10^6 cells per well. Plates were carefully selected under the microscope to ensure that only plates showing uniformly grown cells were used. Two wells in each plate were used to determine the number of cells, and the four companion wells were used for the drug uptake-release and hexose uptake assays. On average, each well contained 1.1×10^6 cells. For drug uptake studies, fresh grown medium was added to the cells, and then tritiated vinblastine (10 Ci/mol; Amersham, Arlington Heights, Ill.) was added at a concentration of 50 nM. After incubation for 30 min at 22°C, the cells were washed with phosphate-buffered saline, solubilized in 20 mM Tris-HCl (pH 7.8) containing 2% sodium dodecyl sulfate (SDS), and processed for liquid scintillation spectrometry. For drug efflux measurements, cells were incubated for 30 min at 22 or 4°C in the presence of [^{3}H]vinblastine at 100 μM, washed rapidly with culture medium, and incubated in drug-free medium at room temperature for the times indicated in the respective figures (see Results) before solubilization and counting.

Hexose uptake. 2-Deoxy-D-[2,6-^{3}H]glucose (42 Ci/mmol; Amersham) and 3-*O*-methyl-D-[1-^{3}H]glucose (3.67 Ci/mmol; Amersham) uptake by *Xenopus* oocytes was assayed as previously described (71, 72). In both cases, the concentration of hexoses in the assay was varied from 0.1 to 50 mM. Measurement of tritiated 2-deoxy-D-glucose uptake by DC-3F and DC-3F/AD X cells was performed as described by Asano et al. (2).

Immunoblotting and immunoprecipitation. The immunoblotting experiments were performed as described previously (7). Total membranes from DC-3F and DC-3F/AD X Chinese hamster cells were prepared as follows. Cells were scraped off the culture plates, resuspended in 20 mM Tris-HCl (pH 7.6)—5 mM EDTA—0.23 M sucrose containing 20 μg each of aprotinin, leupeptin, and soybean trypsin inhibitor per ml and 0.1 mM phenylmethylsulfonyl fluoride, and homogenized in a glass Dounce homogenizer. The homogenate was centrifugated at $10,000 \times g$ for 5 min, and the supernatant was further centrifuged at $150,000 \times g$ for 1.5 h. The high-speed pellet was resuspended in electrophoresis sample buffer (45) and processed for immunoblotting (7). Membranes were probed with the antipeptide antibody Mdr (Ab-1; Oncogene Science, N.Y.), raised against the peptide CALDTESEKVVQEALDKAREG from the C-terminal region of P-glycoprotein. The blots were also probed with an antipeptide antibody raised against the peptide Ile-386–Ala-405 from the GLUT1 protein (5). This antibody reacts with the GLUT1, GLUT2, and GLUT4 isoforms of the mammalian facilitative glucose transporters. The production and characterization of this antibody will be reported elsewhere (72a). For immunoprecipitation, oocytes were injected with approximately 25 nl of [^{35}S]methionine (>1,000 Ci/mmol; Amersham) 4 h after the injection of the respective RNAs. After 18 h of incubation in Barth' medium (7), groups of 10 oocytes were homogenized in buffer containing detergents and submitted to immunoprecipitation and electrophoresis (71). Two different antisera that recognize the GLUT1 protein were used in these experiments: the antipeptide antibody described above and an antiserum prepared against a synthetic peptide from the C-terminal region of GLUT1 (33).

RESULTS

D-Hexoses modulate the accumulation of vinblastine in *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein, but expression of this protein does not affect the uptake of hexoses by *Xenopus* oocytes. *Xenopus* oocytes injected with in vitro-synthesized RNA encoding the mouse Mdr1b P-glycoprotein expressed a functional P-glycoprotein, as shown by the drug accumulation assay (Fig. 1A). In this assay, groups of oocytes were incubated in Barth medium in the presence of labeled vinblastine, and accumulation of the drug was monitored for up to 60 min. In control oocytes (injected with water), vinblastine appears to enter the cells by simple passive diffusion across the cell membrane (7). On the other hand, no significant accumulation of drug was observed in cells expressing the mouse Mdr1b P-glycoprotein (Fig. 1A). Equivalent results were obtained when the drug accumulation experiments were repeated using Barth medium containing different hexoses (D-glucose, 2-deoxy-D-glucose, or 3-*O*-methyl-D-glucose) at final concentrations of up to 50 mM (data not shown). In other experiments,

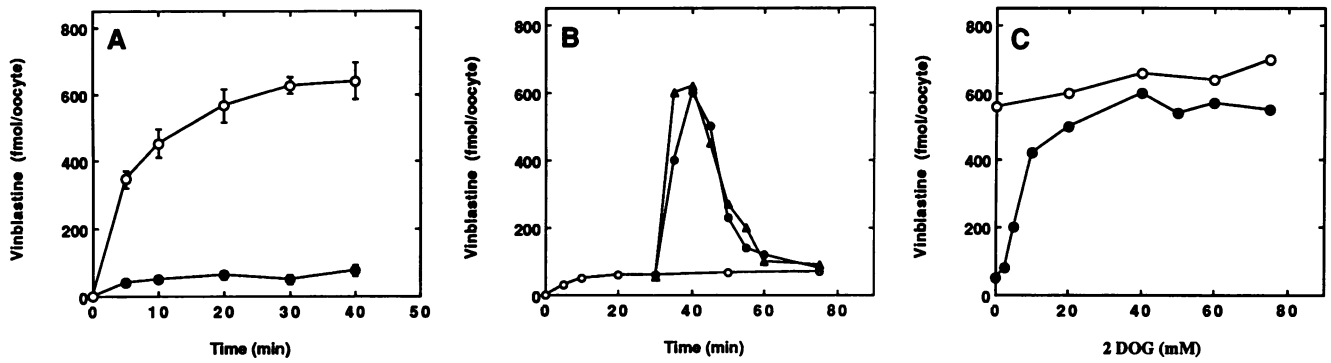

FIG. 1. Effect of hexoses on the accumulation of vinblastine in *X. laevis* oocytes expressing the mouse Mdr1b P-glycoprotein. (A) Accumulation of vinblastine. Oocytes were injected with 25 ng of RNA encoding the mouse Mdr1b P-glycoprotein (●) or with an equivalent volume of distilled water (○), and accumulation of [^{3}H]vinblastine was measured 2 days after the injection. The concentration of vinblastine during the drug accumulation assay was 1 μM. Data represent means ± standard errors of at least three groups of 10 oocytes each. (B) Effect of hexoses on the accumulation of vinblastine. Two days after injection of the RNA encoding the mouse Mdr1b P-glycoprotein, oocytes were incubated for 30 min in Barth medium containing 1 μM [^{3}H]vinblastine (○) before addition (arrow) of 50 mM 2-deoxy-D-glucose (●) or 3-*O*-methyl-D-glucose (▲) to the incubation medium. Accumulation of vinblastine was measured 45 min after addition of the hexoses. Data represent means of two groups of 20 oocytes each. (C) Effects of different concentrations of 2-deoxy-D-glucose on the accumulation of vinblastine. Two days after injection, oocytes were incubated for 30 min in Barth medium containing 1 μM [^{3}H]vinblastine. Different concentrations of 2-deoxy-D-glucose were then added to the incubation medium, and the oocytes were incubated for additional 10 min before the amount of radioactive vinblastine associated with the cells was measured. Symbols: ○, control oocytes injected with water; ●, oocytes injected with RNA encoding the mouse Mdr1b P-glycoprotein. Data represent means of two groups of 20 oocytes each.

oocytes injected with RNA were first incubated for 30 min in the presence of [^{3}H]vinblastine and then challenged with 50 mM 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose. The addition of sugar to the incubation medium produced a dramatic and rapid entry of the drug into the oocytes (Fig. 1B). Vinblastine accumulation increased about 10-fold within 10 min after sugar addition. This was followed by a rapid decrease in the content of vinblastine to the basal levels observed in the absence of sugar (Fig. 1B). The maximal accumulation of vinblastine, induced by the addition of the hexoses to the incubation medium, was roughly equivalent to that observed, in the absence of hexoses, in control oocytes not expressing the mouse Mdr1b P-glycoprotein (Fig. 1A). D-Glucose also induced a rapid and reversible accumulation of vinblastine in oocytes that were expressing the mouse Mdr1b P-glycoprotein. No effect on drug accumulation was observed when 50 mM L-glucose or sucrose was used in these experiments (data not shown). The effect of adding 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose to the incubation medium (a rapid and reversible accumulation of vinblastine) was clearly different from the action of verapamil (100 μM) under similar experimental conditions. Verapamil induced a rapid increase in the accumulation of vinblastine by oocytes expressing the mouse Mdr1b P-glycoprotein, but this was not followed by a decrease in the cellular content of vinblastine to the original basal levels (data not shown).

Figure 1C shows the effect of increasing concentrations of 2-deoxy-D-glucose on the transient accumulation of vinblastine (measured 10 min after addition of the hexose to the incubation medium) by oocytes expressing the mouse Mdr1b P-glycoprotein. Addition of as little as 2.5 mM hexose to the incubation medium resulted in a significant increase in drug accumulation by the oocytes. The maximal level of drug accumulated was observed at 10 mM 2-deoxy-D-glucose; no further accumulation of vinblastine was observed at sugar concentrations of up to 75 mM (Fig. 1C). There was no significant effect on vinblastine accumulation when control oocytes (injected with water) were incubated for 30 min in the presence of vinblastine and then challenged with different concentrations of 2-deoxy-D-glucose (Fig. 1C) or 50 mM 3-*O*-methyl-D-glucose or D-glucose (data not shown).

Interestingly, the rate of accumulation of vinblastine induced by 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose (Fig. 1B) was strikingly similar to the rate of uptake of these sugars by oocytes expressing the mouse Mdr1b P-glycoprotein. In this assay, groups of oocytes were incubated for 30 min in Barth medium containing 1 μM vinblastine and then challenged with cold 50 mM 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose in the presence of traces of 2-deoxy-D-[2,6-^{3}H]glucose or 3-*O*-methyl-D-[1-^{3}H]glucose, respectively. Under these experimental conditions, the accumulation of both hexoses in injected oocytes increased linearly for at least 10 min. Similar results were obtained in control experiments in which preincubation with vinblastine was omitted or with control oocytes (injected with water) that were not expressing the mouse Mdr1b P-glycoprotein (data not shown). Results of the latter experiments suggest that expression of the mouse Mdr1b P-glycoprotein does not affect the transport of hexoses by oocytes. In fact, measurements of the initial rate of uptake of hexoses, using a final sugar concentration of 1 mM during the uptake assay, indicated that *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein accumulated 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose at essentially the same rate (0.60 ± 0.12 or 0.40 ± 0.10 pmol per oocyte per min, respectively) observed in control oocytes injected with water (0.54 ± 0.11 or 0.37 ± 0.11 pmol per oocyte per min, respectively).

Cytochalasin B, cytochalasin E, and phloretin do not affect the accumulation of vinblastine in *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein. The results presented above suggest that the endogenous glucose transporter of the oocyte modulates the accumulation of vinblastine by oocytes expressing the mouse Mdr1b P-glycoprotein. We then investigated whether two inhibitors of mammalian facilitative glucose transporters, cytochalasin B and phloretin, affect the accumulation of vinblastine by oocytes expressing the mouse Mdr1b P-glycoprotein. As shown in Fig.

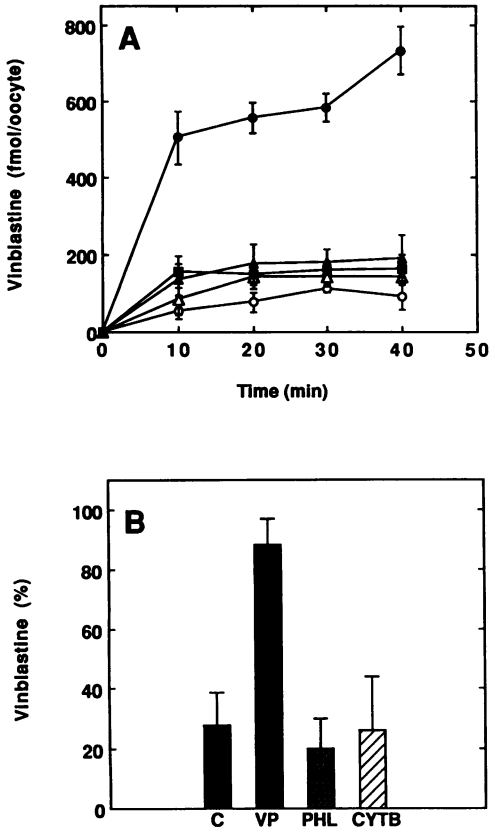

FIG. 2. Effects of verapamil and inhibitors of glucose-facilitated diffusion on the accumulation and efflux of vinblastine in *Xenopus* oocytes expressing the mouse Mdr1b P-glycoprotein. (A) Accumulation of vinblastine in oocytes expressing the mouse Mdr1b P-glycoprotein. Oocytes were injected with 25 ng of RNA encoding the mouse Mdr1b P-glycoprotein, and vinblastine accumulation was measured 2 days after the injection. To measure drug accumulation, oocytes were incubated for the indicated times in Barth medium containing 1 μM [^{3}H]vinblastine, in the absence (○) or presence of 100 μM verapamil (●), phloretin (■), cytochalasin B (▲), or cytochalasin E (△). Data represent means ± standard errors of three groups of 20 oocytes each. (B) Efflux of vinblastine from oocytes expressing the mouse Mdr1b P-glycoprotein. Oocytes were injected with 25 ng of RNA encoding the mouse Mdr1b P-glycoprotein and incubated in Barth medium for 2 days. For the drug efflux assay, oocytes were incubated in Barth medium containing 100 μM [^{3}H]vinblastine for 30 min, washed, and incubated for 15 min in drug-free medium before the amount of radioactive vinblastine remaining associated with the oocytes was measured. The final incubation was carried out in the absence (C) or presence of 100 μM verapamil (VP), phloretin (PHL), or cytochalasin B (CYTB). Results are expressed as percentage of the total radioactivity accumulated in oocytes after the initial 30-min incubation period. Data represent means ± standard errors of three groups of 10 oocytes each.

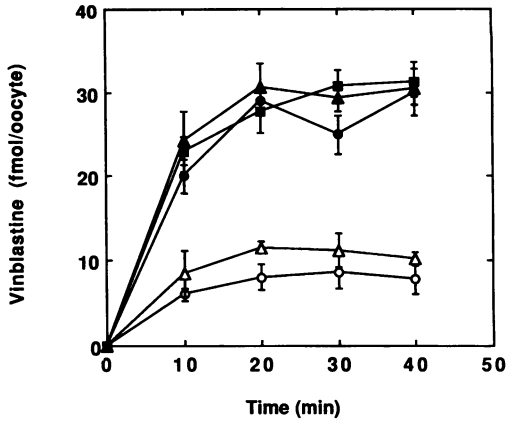

FIG. 3. Effects of verapamil and inhibitors of glucose-facilitated diffusion on the accumulation of vinblastine in *Xenopus* oocytes injected with water. Oocytes were injected with water and incubated for 2 days in Barth medium. For the drug accumulation assay, oocytes were incubated for the indicated times in Barth medium containing 50 nM [^{3}H]vinblastine in the absence (○) or presence of 100 μM verapamil (●), phloretin (■), cytochalasin B (▲), or cytochalasin E (△). Data represent means ± standard errors of three groups of 20 oocytes each.

2A, accumulation of drug by injected oocytes was not significantly affected by either cytochalasin B or vinblastine. Cytochalasin E, an analog of cytochalasin B that does not affect the activity of the facilitative glucose transporter, also had no effect on the accumulation of vinblastine by oocytes expressing the Mdr1b P-glycoprotein. On the other hand, verapamil, a Ca^{2+} channel blocker that can revert the MDR phenotype in MDR cells, had a dramatic effect on the accumulation of vinblastine by injected oocytes (Fig. 2A; 7). In the presence of 100 μM verapamil, the accumulation of vinblastine by injected oocytes was similar to that observed in the respective control oocytes (injected with water) (Fig. 2A and 1A). Similar results were obtained when we studied the effects of these drugs on the efflux of vinblastine from oocytes expressing the mouse Mdr1b P-glycoprotein and loaded with the drug (Fig. 2B; 7). The release of vinblastine by injected oocytes was significantly inhibited by verapamil but not by either cytochalasin B or phloretin (Fig. 2B). The data shown in Fig. 2 were obtained by using each competitor drug at a final concentration of 100 μM. Equivalent results were obtained when the drug accumulation experiments were repeated using the competitor drugs at a concentration of 200 or 20 μM.

The accumulation of low concentrations of vinblastine in uninjected *Xenopus* oocytes is increased by verapamil, cytochalasin B, and phloretin but not by cytochalasin E. Cytochalasin B and phloretin produced a minor (approximately 1.5-fold) but consistent increase in the accumulation of vinblastine in control oocytes injected with water and incubated in the presence of 1 μM vinblastine. The same effect was observed in oocytes challenged with verapamil (data not shown). This result suggests that the oocytes are refractory to the entry of low concentrations of vinblastine. In fact, when these experiments were repeated using 50 nM vinblastine, the expected uptake of drug by control oocytes (injected with water) was not observed (Fig. 3). The data shown in Fig. 3 indicated that not more than 20% of the expected radioactivity was associated with the oocytes, assuming an internal volume of about 0.4 to 0.5 μl for oocytes at stages 5 and 6 (18). On the other hand, oocytes accumulated radioactive vinblastine to the expected levels in the presence of 100 μM verapamil and, most important, also in the presence of 100 μM cytochalasin B or phloretin (Fig. 3). Equivalent results were obtained when each drug was used at a final concentration of 20 μM. Cytochalasin E had no significant effect on the accumulation of vinblastine by these cells. As demonstrated previously (26, 41, 71, 72), both cytochalasin B and phloretin inhibited the activity of the endogenous

glucose transporters of *Xenopus* oocytes. Three different anti-P-glycoprotein antibodies failed to reveal the presence of a cross-reacting protein in control oocytes injected with water (data not shown; 7). Taken together, these results support the hypothesis that the endogenous glucose transporter of oocytes plays a role in the basal resistance to vinblastine accumulation in oocytes lacking (not expressing) the mouse Mdr1b P-glycoprotein and in modulating the accumulation of drugs in oocytes expressing the mouse Mdr1b P-glycoprotein.

***Xenopus* oocytes expressing a functional mammalian facilitative glucose transporter show decreased accumulation of vinblastine and sensitivity to verapamil, cytochalasin B, and phloretin but not to cytochalasin E.** Since functional studies appear to indicate that the endogenous glucose transporters of *Xenopus* oocytes belong to the family of facilitative glucose transporters (71, 72), we examined whether the expression in *Xenopus* oocytes of a mammalian facilitative glucose transporter would alter the resistance of oocytes to the accumulation of vinblastine (as in the case of expression of the mouse Mdr1b P-glycoprotein). As shown in Fig. 4A, the accumulation of vinblastine (1 μM) was markedly decreased in oocytes injected with in vitro-synthesized RNA encoding the rat erythrocyte/brain glucose transporter (the GLUT1 protein). Cytochalasin B and phloretin, two inhibitors of the mammalian facilitative glucose transporters that do not affect the accumulation of vinblastine in oocytes expressing the mouse Mdr1b P-glycoprotein (Fig. 2A), had a dramatic effect on the accumulation of vinblastine in oocytes expressing the rat GLUT1 protein (Fig. 4A). The effects of both drugs, cytochalasin B and phloretin, were similar to the action of verapamil on these cells (Fig. 4A). No significant effect of cytochalasin E on the accumulation of vinblastine by oocytes injected with RNA was observed in these experiments (Fig. 4A). Furthermore, the release of vinblastine by oocytes expressing the rat brain glucose transporter was also significantly inhibited by the presence of either verapamil, cytochalasin B, or phloretin (Fig. 4B). Control experiments demonstrated that the brain glucose transporter was indeed expressed in oocytes injected with the RNA encoding this protein. Immunoblot analysis of oocytes injected with RNA encoding the rat brain glucose transporter indicated the presence of a protein band that migrates with an apparent M_r of about 45,000, the expected position of migration of the rat GLUT1 protein, that is not present in control oocytes injected with water (data not shown).

To evaluate further whether the decreased accumulation of vinblastine in oocytes was related to the RNA-driven expression of the GLUT1 protein, effects of RNA concentration and the functional state of the expressed transporter were studied. Figure 5A shows that the initial rate of 2-deoxy-D-glucose uptake increased with increasing amounts of microinjected RNA (26, 41, 71, 72), with an almost linear dose-response relationship between the amount of microinjected RNA and uptake of 2-deoxy-D-glucose assayed 48 h later. Figure 5B shows the accumulation of vinblastine by oocytes injected with increasing amounts of RNA encoding the rat GLUT1 protein. It is evident that the capacity of the oocytes to maintain a low intracellular concentration of vinblastine when challenged for 30 min with drug concentrations ranging from 0.1 to 100 μM is related to the amount of microinjected RNA.

The next group of experiments was carried out with oocytes injected with increasing amounts of RNA encoding a version of the rat GLUT1 protein lacking the last C-terminal 51 amino acids (see Materials and Methods). It has been

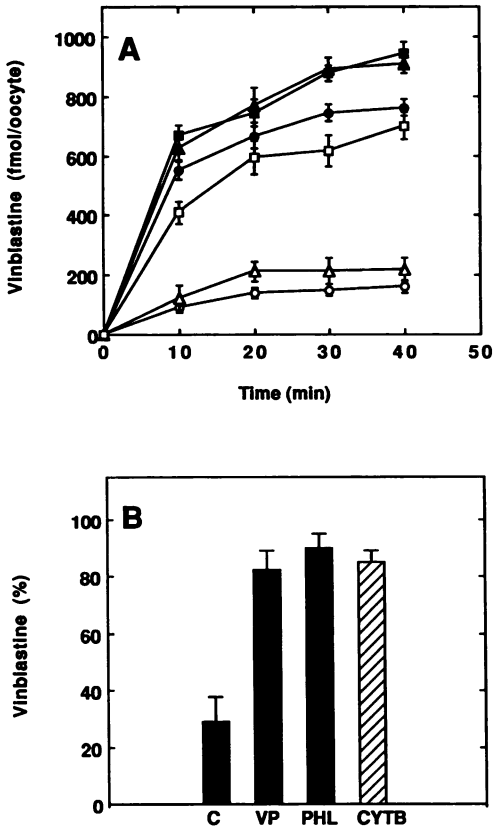

FIG. 4. Effects of verapamil and inhibitors of glucose-facilitated diffusion on the accumulation and efflux of vinblastine in *Xenopus* oocytes expressing the rat GLUT1 protein. (A) Accumulation of vinblastine in *Xenopus* oocytes expressing the rat GLUT1 protein. Oocytes were injected with 20 ng of RNA encoding the rat GLUT1 protein and incubated in Barth medium for 2 days. For the drug uptake assay, oocytes were incubated for the indicated periods of time in Barth medium containing 1 μM [^{3}H]vinblastine in the absence (○) or presence of 100 μM verapamil (●), phloretin (■), cytochalasin B (▲), or cytochalasin E (△). Control oocytes injected with water (□) were incubated under the same conditions in the absence of competitor drugs. Data represent means ± standard errors of at least three groups of 10 oocytes each. (B) Efflux of vinblastine from *Xenopus* oocytes expressing the rat GLUT1 protein. Oocytes were injected with 20 ng of RNA encoding the rat GLUT1 protein and incubated in Barth medium for 2 days. For the drug efflux experiments, injected oocytes were incubated in Barth medium containing 100 μM [^{3}H]vinblastine for 30 min, washed, and incubated for 20 min in Barth medium lacking vinblastine before the amount of [^{3}H]vinblastine remaining associated with the oocytes was measured. The final incubation was carried out in the absence (C) or presence of 100 μM verapamil (VP), phloretin (PHL), or cytochalasin B (CYTB). Results are expressed as percentage of the total radioactivity accumulated in oocytes after the initial 30-min incubation period.

shown that a similar truncated form of the rat GLUT1 protein has no functional activity as a hexose transporter (53). Immunoprecipitation studies using an antipeptide antibody raised in rabbits against a synthetic peptide corresponding to the sequence Ile-386–Ala-405 in GLUT1 were carried out to confirm the expression of the truncated form of the rat GLUT1 protein in oocytes injected with the respective RNAs. The antiserum immunoprecipitated a protein that migrated with an apparent M_r of about 45,000 from oocytes injected with RNA encoding the full-length rat

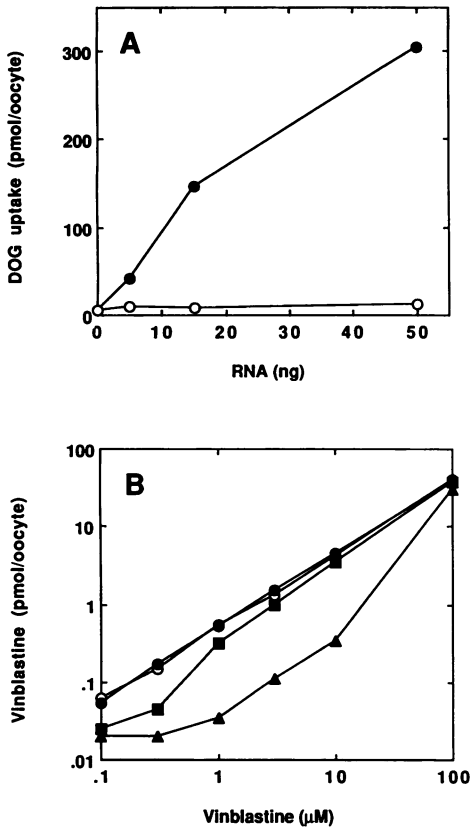

FIG. 5. Effects of the amount of RNA injected and the functional state of the expressed hexose transporter on the uptake of 2-deoxy-D-glucose and the accumulation of vinblastine by injected *Xenopus* oocytes. (A) Uptake of 2-deoxy-D-glucose. Oocytes were injected with increasing amounts of RNA encoding the full-length (●) or a truncated form of (○) the rat GLUT1 protein (see Materials and Methods), and 2-deoxy-D-glucose uptake was determined 2 days after injection. Control oocytes (point with no RNA) were injected with water. For the uptake assay, oocytes were incubated for 10 min in Barth medium containing 1 mM 2-deoxy-D-[^{3}H]glucose. Data represent averages of three groups of 10 oocytes each. (B) Accumulation of vinblastine. Oocytes were injected with water (○), with approximately 50 ng of RNA encoding the truncated form of the rat GLUT1 protein (●), or with approximately 5 (■) or 50 (▲) ng of RNA encoding the full-length rat GLUT1 protein. Accumulation of vinblastine was determined 2 days after injection. For the drug accumulation assay, oocytes were incubated for 30 min in Barth medium containing from 0.1 to 100 μM [^{3}H]vinblastine. Data represent averages of two groups of 10 oocytes each.

GLUT1 protein. On the other hand, a radioactive band with an apparent M_r of about 40,000 was immunoprecipitated from oocytes injected with RNA encoding the truncated form of the rat GLUT1 protein (data not shown). This decrease in size is congruent with this protein lacking the last 51 C-terminal amino acids. These experiments were repeated using an antiserum prepared against a synthetic peptide from the C-terminal region of the rat GLUT1 protein (33). The antiserum immunoprecipitated a radioactive band that migrated with an apparent M_r of about 45,000 from oocytes injected with RNA encoding the full-length rat GLUT1 protein. On the other hand, no labeled material was immunoprecipitated from oocytes injected with RNA encoding the truncated form of the rat GLUT1 protein (data not shown). As shown in Fig. 5, the rate of uptake of 2-deoxy-D-glucose and the accumulation of vinblastine by oocytes injected with increasing amounts of RNA encoding the truncated form of the rat GLUT1 protein were not differ from the corresponding values measured for control oocytes injected with water.

Uptake of 2-deoxy-D-glucose by oocytes expressing the rat GLUT1 protein is sensitive to vinblastine and verapamil. Oocytes expressing the rat GLUT1 protein and challenged with 0.1 mM 2-deoxy-D-glucose accumulated the hexose at an initial rate about 15-fold greater than that of oocytes injected with water (3.2 ± 0.7 and 0.13 ± 0.04 pmol per oocyte per min, respectively) (71, 72). Further analysis showed that both cytochalasin B and phloretin at 100 μM inhibited the uptake of 2-deoxy-D-glucose by oocytes expressing the rat GLUT1 protein by more than 90%. As expected, cytochalasin E at 100 μM had no significant effect on the uptake of 2-deoxy-D-glucose by oocytes expressing the rat GLUT1 protein (less than 10% inhibition). Interestingly, verapamil at 100 μM also inhibited the uptake of 2-deoxy-D-glucose in these injected oocytes, to about 40% of the value for controls without inhibitors. On the other hand, the presence of 100 μM vinblastine during the assay increased by at least 50% the uptake of 2-deoxy-D-glucose in oocytes expressing the rat GLUT1 protein.

MDR and drug-sensitive Chinese hamster cells are sensitive to verapamil, cytochalasin B, and phloretin but not to cytochalasin E. The results presented above provide direct evidence that expression of the rat GLUT1 protein in *X. laevis* oocytes confers a drug-resistant phenotype (as defined by the drug accumulation assay) to these cells. This phenotype can be functionally distinguished from the similar phenotype produced by expression of the mouse Mdr1b P-glycoprotein in *Xenopus* oocytes because cytochalsin B and phloretin inhibit only the decreased accumulation of vinblastine produced by expression of the glucose transporter. Given these observations, we studied whether inhibitors of the facilitative glucose transport affected the accumulation of vinblastine in Chinese hamster MDR cells selected by resistance to dactinomycin (49). In preliminary experiments, we measured the accumulation of vinblastine in drug-resistant (DC-3F/AD X) and drug-sensitive (DC-3F) Chinese hamster cells after incubation in the presence of vinblastine concentrations ranging from 10 to 150 nM. Depending on the concentration of drug in the incubation medium, approximately 3 to 15 times more vinblastine accumulated in sensitive versus resistant cells after 30 min of incubation in the presence of the drug (data not shown). Verapamil significantly increased (about sevenfold) vinblastine accumulation in resistant DC-3F/AD X cells incubated in medium containing 50 nM vinblastine, to levels similar to those observed in sensitive DC-3F cells (compare Fig. 6A and 7). Cytochalasin B and phloretin also increased the accumulation of vinblastine in resistant cells, four- and fivefold, respectively, in comparison with cells incubated only in the presence of vinblastine (Fig. 6A). No significant effect of cytochalasin E on vinblastine accumulation was observed in these experiments (Fig. 6A). An additional control experiment was done to rule out the possibility that the increased accumulation of vinblastine observed in the presence of the these drugs could have resulted from nonspecific interference with the physiology of the cells. Resistant DC-3F/AD X cells were incubated in the presence of either verapamil, cytochalasin B, or phloretin for 30 min, washed extensively, and then challenged with radioactive vinblastine for 30 min. As shown in Fig. 6B, no significant increase in vinblastine accumulation was observed in these cells compared with the cells preincubated in

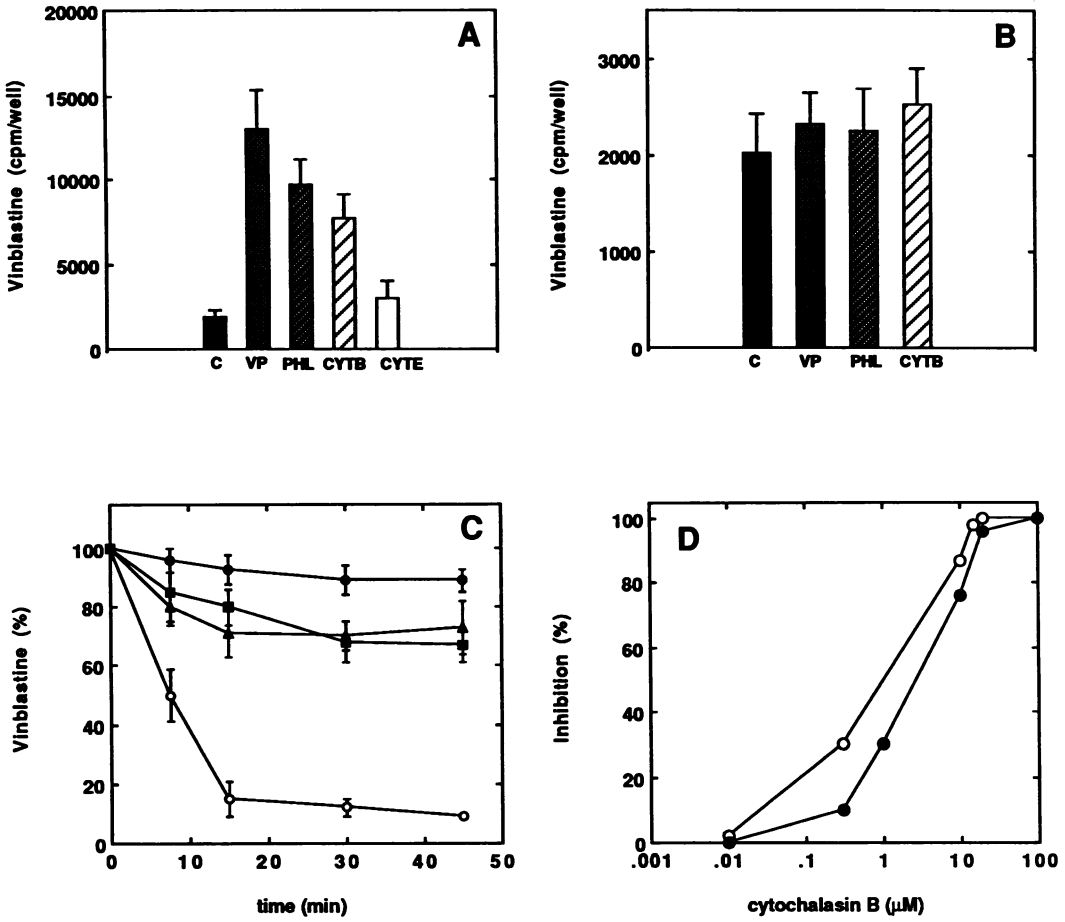

FIG. 6. Effects of verapamil and inhibitors of glucose-facilitated diffusion on the accumulation and efflux of vinblastine and 2-deoxy-D-glucose in MDR Chinese hamster cells. (A) Accumulation of vinblastine in drug-resistant Chinese hamster cells. DC-3F/AD X cells were incubated with 50 nM [^{3}H]vinblastine for 30 min in the absence (C) or presence of 100 μM verapamil (VP), phloretin (PHL), cytochalasin B (CYTB), or cytochalasin E (CYTE). Drug accumulation was determined as indicated in Materials and Methods. Data represent means ± standard errors of four to six determinations. (B) Reversibility of the effects of verapamil and inhibitors of glucose-facilitated diffusion on the accumulation of vinblastine in drug-resistant Chinese hamster cells. DC-3F/AD X cells were incubated in the absence (C) or presence of 100 μM verapamil (VP), phloretin (PHL), or cytochalasin B (CYTB), thoroughly washed, and then assayed for vinblastine accumulation after a further incubation in the presence of 50 nM [^{3}H]vinblastine for 30 min at room temperature. Data represent means ± standard error of four determinations. (C) Efflux of vinblastine from drug-resistant Chinese hamster cells. DC-3F/AD X cells were incubated in the presence of 100 μM [^{3}H]vinblastine for 30 min, washed, and then incubated for the indicated times in drug-free medium before the amount of vinblastine remaining associated with the cells was measured. The final incubation was carried out in the absence (○) or presence of 100 μM verapamil (●), phloretin (■), or cytochalasin B (▲). Results are expressed as percentage of the total radioactivity associated with the cells after the initial 30-min incubation period in the presence of 100 μM vinblastine. Data represent means ± standard errors of six determinations. (D) Effects of increasing concentrations of cytochalasin B on the uptake of 2-deoxy-D-glucose (○) and the efflux of vinblastine (●) by drug-resistant DC-3F/AD X cells. For the hexose uptake assay, cells were incubated at room temperature with medium containing the indicated concentrations of cytochalasin B for 10 min before measurement of the uptake of 0.1 mM radioactive 2-deoxy-D-glucose in a 5-min uptake assay (see Materials and Methods). Results are expressed as percentage of the inhibition in uptake observed in the presence of 100 μM cytochalasin B. For the drug efflux assay, cells were incubated for 60 min at 4°C in medium containing 1 μM radioactive vinblastine and the indicated concentrations of cytochalasin B, washed at the same temperature in vinblastine-free medium (containing cytochalasin B), and then incubated for 10 min at room temperature in medium containing the appropriate concentrations of cytochalasin B before the amount of vinblastine remaining associated with the cells was measured. Results are expressed as percentage of the maximal inhibition of efflux observed in the presence of 100 μM cytochalasin B.

the absence of any drug. In other experiments, resistant DC-3F/AD X cells were loaded with vinblastine and then the efflux of vinblastine was measured in the presence of either verapamil, cytochalasin B, or phloretin. Vinblastine was released rapidly from DC-3F/AD X cells incubated in drug-free medium (Fig. 6C); almost 90% of the drug was released in less than 20 min. On the other hand, the release of vinblastine was almost completely inhibited by the presence of 100 μM verapamil in the incubation medium, and a significant inhibition was also observed when cytochalasin B or phloretin was added (Fig. 6C). To evaluate further the effect of cytochalasin B on the resistant DC-3F/AD X cells, the concentration dependence of cytochalasin B inhibition of 2-deoxy-D-glucose uptake and vinblastine efflux was examined. In a 5-min uptake assay (under conditions of initial velocity), cytochalasin B inhibited the uptake of 2-deoxy-D-glucose by the drug-resistant cells with an apparent K_i of less than 1 μM, and a maximum degree of inhibition was attained

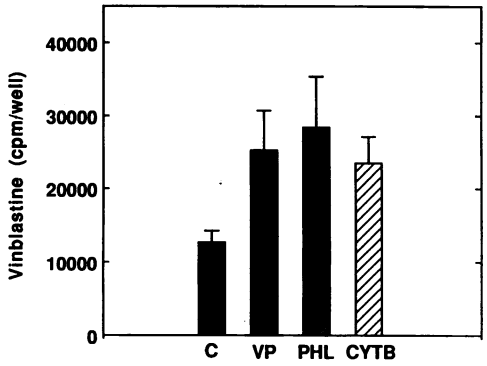

FIG. 7. Effects of inhibitors of glucose-facilitated diffusion on the accumulation of vinblastine in drug-sensitive Chinese hamster cells. For the drug accumulation assay, DC-3F cells were incubated with 50 nM [^{3}H]vinblastine for 30 min in the absence (C) or presence of 100 μM verapamil (VP), phloretin (PHL), or cytochalasin B (CYTB). Data represent means ± standard error of four determinations.

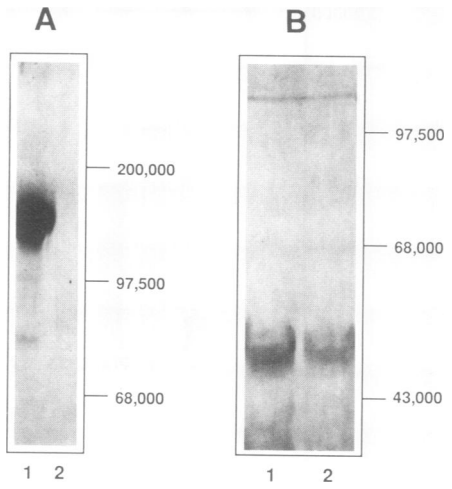

FIG. 8. Immunoblot analysis of membrane-enriched fractions from DC-3F (drug sensitive) and DC-3F/AD X (drug resistant) Chinese hamster cells. Membrane-enriched fractions were solubilized in gel sample buffer and submitted to SDS-polyacrylamide gel electrophoresis. After transfer to nitrocellulose, the membranes were probed with an antipeptide antibody prepared against P-glycoprotein (A) or an antipeptide antibody against the glucose transporter (B). About 30 and 120 μg of membrane protein were loaded in each lane for panels A (7.5% acrylamide gel) and B (12% acrylamide gel), respectively. Lanes: 1, membranes from DC-3F/AD X cells; 2, membranes from DC-3F cells. The blots were developed by using ^{125}I-protein A (Amersham) (A) and AuroProbe (Janssen) (B). Positions of molecular weight markers are shown at the left.

at 20 μM (Fig. 6D). In parallel experiments, resistant DC-3F/AD X cells were loaded with vinblastine and the efflux of the drug was measured in the presence of different concentrations of cytochalasin B. Using a 10-min incubation assay (to remain in the linear part of the efflux curve; Fig. 6C), the concentration dependence of cytochalasin B inhibition of the efflux of vinblastine was similar to its effect on 2-deoxy-D-glucose uptake. Again, maximal inhibition was attained at 20 μM cytochalasin B (Fig. 6D).

To determine whether inhibitors of facilitated glucose diffusion also increased the accumulation of vinblastine in drug-sensitive DC-3F cells (as they do in uninjected *Xenopus* oocytes), we measured the accumulation of radioactive vinblastine in DC-3F cells in the presence of verapamil, cytochalasin B, and phloretin. Each of the three drugs increased the accumulation of vinblastine 1.8- to 2.2-fold (Fig. 7). Thus, as is the case in *Xenopus* oocytes, Chinese hamster DC-3F cells express a basal level of drug resistance which is antagonized by cytochalasin B and phloretin. Taken together, these results suggest the participation of the facilitative glucose transporter in the modulation of drug resistance in Chinese hamster cells.

Overexpression of P-glycoprotein but not of glucose transporters in MDR Chinese hamster cells. To confirm the overexpression of the P-glycoprotein in drug-resistant DC-3F/AD X cells versus drug-sensitive DC-3F cells, membrane fractions from these cells were analyzed for the presence of P-glycoprotein. We used an affinity-purified antipeptide antibody directed against the oligopeptide CALDTESEKV VQEALDKAREG, a sequence conserved within the C-terminal region of all known members of the high-molecular-weight family of P-glycoproteins (17, 36). As expected, the antibody recognized one major band of protein (with an apparent M_r of about 170,000) that was overexpressed in drug-resistant DC-3F/AD X cells (Fig. 8A, lane 1). On the other hand, this protein band was barely detectable in membranes prepared from drug-sensitive DC-3F cells (Fig. 8A, lane 2).

Because drug resistance in DC-3F/AD X cells appears to be sensitive to inhibitors of facilitated glucose diffusion, we investigated the possibility that a glucose transport protein is also overexpressed in DC-3F/AD X versus DC-3F cells. For these experiments, we used an antipeptide antibody raised in rabbits against the peptide IPWFIVAELFSQGPRPAAVA. This antibody reacts with three major members of the family of mammalian facilitative glucose transporters, the GLUT1, GLUT2, and GLUT4 proteins (72a). When this antibody was used, one broad protein band of apparent M_r 45,000 was identified in both cell lines, DC-3F/AD X (Fig. 8B, lane 1) and DC-3F (Fig. 8B, lane 2). Although higher amounts of the immunoreactive protein of M_r 45,000 appear to be present in the drug-resistant cell line DC-3F/AD X than in the drug-sensitive cell line DC-3F (compare lanes 1 and 2 in Fig. 8B), this increase was by no means comparable to the increased overexpression of P-glycoprotein in the drug-resistant cells. Similar results were obtained when the immunoblotting experiments were repeated using plasma membrane-enriched fractions prepared from a murine cell line selected for its resistance to colchicine (data not shown). We next examined the capacity of drug-resistant (DC-3F/AD X) and drug-sensitive (DC-3F) cells to accumulate 2-deoxy-D-glucose. Initial experiments indicated that the uptake of 0.1 mM 2-deoxy-D-glucose by both cells increased linearly with time for at least 5 min. Using short incubation periods, less than a twofold increase in uptake of 2-deoxy-D-glucose was observed in drug-resistant DC-3F/AC X cells (70 pmol/min per 10^6 cells) versus drug-sensitive DC-3F cells (40 pmol/min per 10^6 cells). These results suggest that there is only a minor increase in the expression of a glucose transporter-like protein in drug-resistant versus drug-sensitive Chinese hamster cells.

DISCUSSION

We have demonstrated that *X. laevis* oocytes injected with RNA encoding the mammalian erythrocyte/brain facilitative glucose transporter (the rat GLUT1 protein) show a lower

accumulation of vinblastine and a greater capacity to extrude the drug than do oocytes injected with water in a drug accumulation assay. The expression of this phenotype was dependent on the amount of RNA injected into the oocytes and also on the synthesis of a protein that was functionally active as a hexose transporter. Hexose uptake assays and immunoblot analysis demonstrated that the rat GLUT1 protein was expressed in a functional form in oocytes injected with RNA transcribed form the respective cloned cDNA (this study; 71, 72). Results of hexose uptake experiments using oocytes expressing the rat GLUT1 protein show that verapamil inhibits and vinblastine stimulates hexose uptake. These results support the idea that the expressed mammalian facilitative hexose transporter participates in modulating the drug sensitivity of the injected oocytes. This conclusion is reinforced by the competition experiments which indicate that the accumulation of vinblastine in oocytes expressing the rat GLUT1 protein is sensitive to specific drugs, in comparison with oocytes expressing the mouse Mdr1b P-glycoprotein. Although the accumulation of vinblastine is sensitive to verapamil in both cases (7), two inhibitors of facilitated glucose diffusion, cytochalasin B and phloretin, affect the accumulation of vinblastine in oocytes expressing the rat GLUT1 protein but have no significant effect on oocytes expressing the mouse Mdr1b P-glycoprotein. We (71, 72), and others (26, 41) have shown that different mammalian facilitative glucose transporters expressed in *Xenopus* oocytes show functional properties similar to those of the transporters present in the tissues from which they were originally cloned. These properties include stereospecificity for D-hexoses and inhibition by cytochalasin B and phloretin. Identical conclusions apply to the Mdr1b P-glycoprotein expressed in *Xenopus* oocytes. Thus, oocytes expressing the Mdr1b P-glycoprotein show a decreased accumulation of vinblastine when assayed in a drug accumulation assay, phenotype that is reverted by verapamil but not by colchicine (7).

The differential sensitivity to cytochalasin B and phloretin suggests the possible participation of an endogenous facilitative glucose transporter-like protein in uninjected oocytes that are refractory to the entry of low concentrations of vinblastine (measured in a drug accumulation assay). The activity of this transporter is sensitive to cytochalasin B and phloretin (21, 26, 41, 71, 72). We have shown that 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, and D-glucose, but not L-glucose, induce a rapid and reversible increase in the level of vinblastine accumulated by oocytes expressing the mouse Mdr1b P-glycoprotein. The specificity of the effect of D-hexoses, the temporal correlation of the initial burst of vinblastine influx with the linear phase of the uptake of hexoses by oocytes, the fact that the maximal level of drug accumulation was observed at about 10 mM 2-deoxy-D-glucose, the K_m of the *Xenopus* oocyte endogenous glucose transporter of about 1.5 mM (71), and the observation that the expression of the mouse Mdr1b P-glycoprotein had no effect on the transport of hexoses by *Xenopus* oocytes all suggest that the putative endogenous facilitative glucose transporters in *Xenopus* oocytes modulate the accumulation of vinblastine in oocytes expressing the mouse Mdr1b P-glycoprotein. A plausible alternative explanation is that the effect of the hexoses on drug accumulation is indirectly mediated through the modulation of the activity of Ca^{2+} channels by the hexoses (63). This hypothesis seems plausible given the dramatic effect of verapamil, a Ca^{2+} channel blocker, on the accumulation of vinblastine in uninjected oocytes and on oocytes expressing the rat GLUT1 protein or the mouse Mdr1b P-glycoprotein (7). However, the metabolism of glucose is required for modulation of Ca^{2+} channel activity (63). Clearly this is not the case for the action of hexoses on the accumulation of vinblastine by *Xenopus* oocytes because 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose cause the same effect as D-glucose in this system.

Evidence supporting the validity of the results obtained with the *Xenopus* oocyte system is provided by our observations indicating that the glucose transporter may also be involved in modulating drug resistance in mammalian cell lines. The results obtained in drug accumulation assays using the drug-sensitive DC-3F cells in the presence of verapamil, cytochalasin B, or phloretin suggest that a facilitative glucose transport-like protein may be involved in the basal resistance to the accumulation of vinblastine observed in this cell line. Moreover, the sensitivity of drug accumulation and efflux by MDR DC-3F/AD X cells to cytochalasin B and phloretin gives additional support to the idea of a role for the facilitative glucose transporter in the drug-resistant phenotype. The lack of effect of cytochalasin E and the reversibility of the effects of verapamil, cytochalasin B, and phloretin on drug accumulation rule out the possibility of an artifact originated by the irreversible disruption of some important physiological process by these drugs in DC-3F/AD X cells. An intriguing observation is that drug-resistant cells (DC-3F/AD) show only a minor increase (in comparison with DC-3F drug-sensitive cells) in the amount of a protein cross-reactive with antibodies directed against the mammalian facilitative glucose transporters and in their capacity to take 2-deoxy-D-glucose, suggesting that the development of resistance to drugs is not accompanied by a significant increase in the expression of a facilitative glucose transport-like protein. On the other hand, the correlation observed in the sensitivity of these resistant cell lines to cytochalasin B is consistent with a possible role for the facilitative glucose transporter in the development of drug resistance. This conclusion is supported by the work of Tsuruo and Iida (64) demonstrating an increased accumulation of vincristine and daunomycin in several tumor cells (both drug resistant and drug sensitive) incubated in the presence of either cytochalasin B or verapamil.

There is additional evidence in the literature compatible with the suggested role for the glucose transporter in the development of multidrug resistance. Chinese hamster cells develop resistance to drugs when cultured under conditions of glucose deprivation; this resistance developed rapidly and was totally reversible within 24 h of refeeding (62). A number of well-characterized cell lines grown under conditions of glucose deprivation characteristically show increased expression of a facilitative glucose transporter (12, 34). In addition, it has been shown that exposure of drug-sensitive cell lines to phorbol esters rapidly induces a drug-resistant phenotype that is sensitive to verapamil, which is accompanied by a net decrease in intracellular drug accumulation (vincristine and doxorubicin) (20). It is interesting that phorbol esters, when added to cultured cell lines, induce the increased expression of a facilitative glucose transporter (42, 51, 75).

Our results indicate that two polypeptides, the rat GLUT1 protein and the Mdr1b P-glycoprotein, which appear to be unrelated in terms of primary structure, can confer similar phenotypes (as defined by the drug accumulation assay) when expressed in *X. laevis* oocytes. These two polypeptides are similar only in that they are highly hydrophobic and show a striking similarity in predicted secondary structure that includes the presence of 12 putative transmembrane

domains (27, 52). On the other hand, the mammalian facilitative glucose transporters appear to contain putative ATP binding sites not related in sequence to those described in P-glycoprotein (6). Thus, although these proteins can be related from a functional point of view, with respect to sequence homology the glucose transporter appears not to be a member of the superfamily of nucleotide-binding proteins involved in transport processes. This superfamily comprises the high-molecular-weight membrane proteins involved in the development of multidrug resistance (19, 23, 25, 39, 67, 74), the *Saccharomyces cerevisiae STE6* gene product involved in protein export (44, 48), and a group of well-characterized ATP-dependent proteins involved in several bacterial transport systems (1).

How the facilitative glucose transporter might contribute to drug resistance remains unclear. It is possible that the glucose transporter acts directly as a drug efflux pump, the functional hallmark that defines the activity of mammalian P-glycoproteins. Our results obtained for the *X. laevis* oocyte expression system can be interpreted as giving some support to this hypothesis. The fact that the human erythrocyte hexose transporter contains a putative ATP binding site which is conserved in the different members of the mammalian family of facilitative glucose transporters is also consistent with this hypothesis (6). On the other hand, immunological and functional studies appear to indicate that there is only a minor increase in the expression of a hexose transport-like protein in drug-resistant versus drug-sensitive Chinese hamster cells. We do not know whether the glucose transporter is able to interact with P-glycoprotein in the membrane of drug-resistant cells and whether an interaction of this kind can modulate the functional activity of P-glycoprotein. Given the considerations noted above, this is an intriguing possibility worth exploring.

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