Uptake of the Noncytotoxic Transport Probe Procainamide in the Chinese Hamster Ovary Model of Multidrug Resistance

K. V. Speeg, Jr., Catherine deLeon, and William L. McGuire

ABSTRACT

Many of the cytotoxic substrates of the multidrug transporter are organic cations. Cimetidine, procainamide, and tetraethylammonium bromide were used in a Chinese hamster ovary model of multidrug resistance, to study handling of noncytotoxic cationic transport probes. Cimetidine and procainamide, but not tetraethylammonium, accumulated to a greater extent (5-fold) in the sensitive CHOAXB1 (AB) cell line than in the resistant CH *C5 (C5) cell line. Accumulation of both cimetidine and procainamide was significantly increased by verapamil in C5 but not AB. Procainamide accumulation in both AB and C5 was temperature dependent and occurred by passive diffusion. Diltiazem, nifedipine, rifampin, tamoxifen, rhodamine, and ethidium also increased procainamide accumulation in C5 but not AB. Azide in glucose-free medium increased procainamide accumulation in CS, and this was reversed when glucose, but not 3-O-methylglucose, was added. Procainamide efflux rates were similar in AB and C5 and not affected by verapamil or azide. The initial rate of procainamide uptake was higher in AB than in C5, and both verapamil and azide increased the initial rate of procainamide uptake in C5. Thus, differences in accumulation of the noncytotoxic transport probe procainamide in the colchicine-sensitive and colchicine-resistant components of the Chinese hamster ovary cell line mimic the accumulation of known cytotoxic substrates for the multidrug transporter, such as colchicine, vinblastine, and doxorubicin. The differential accumulation of procainamide is due to differences in rates of drug influx, rather than efflux. Since procainamide influx is passive and decreased accumulation in the resistant line appears to parallel M, 170,000 glycoprotein presence and activity, we would speculate that decreased procainamide accumulation may be due to an indirect effect of the M, 170,000 glycoprotein, such as its effect on intracellular pH.

INTRODUCTION

Emergence of drug-resistant cancer cells during the course of chemotherapy is considered a major cause of treatment failure. In in vitro cancer models, MDR occurs following the amplified expression of the mdr gene and the appearance of its product, gp-170, in the plasma membrane (1–3). The drugs to which resistance develops include the organic cations actinomycin, doxorubicin, and vinblastine. Evidence suggests that gp-170 acts as an efflux pump (3). Other organic cations, including calcium channel antagonists, calmodulin inhibitors, rifampin, and fluorescent dyes, have been reported to increase accumulation of cytotoxic drug in multidrug-resistant cells, and some have been demonstrated to increase cytotoxicity, reversing the resistance phenotype (4–6). Since gp-170 has also been found to be a normal constituent of cells in organs known to secrete organic cations (i.e., renal proximal tubule, hepatocyte bile canalculus, and adrenal medulla) (7–9), we used noncytotoxic probes of organic cation secretion (10, 11), which is generally considered to be driven by electrochemical gradients rather than by an ATPase (12), to determine whether these probes might be substrates for gp-170 or whether this secretory mechanism could be characterized in MDR cells.

MATERIALS AND METHODS

Materials. [%H]Cimetidine (specific activity, 20 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL); [%H]procainamide (specific activity, 48 Ci/mmol) and [%H]TEA (specific activity, 16 Ci/mmol) were custom synthesized by Amersham. Verapamil, diltiazem, nifedipine, rifampin, tamoxifen, rhodamine, etidium bromide, procainamide, 3-O-methylglucose, and sodium azide were from Sigma Chemical Co. (St. Louis, MO). GBSS and glucose-free GBSS were prepared in 10-liter batches, filtered through 0.22-μm filters, and stored in sterile bottles. Phosphate-buffered incubation medium contained (in mM): sodium chloride, 139; dibasic potassium phosphate, 2.5; magnesium sulfate, 1.2; calcium chloride, 2.0; glucose, 5.5; L-alanine, 6.0; sodium citrate, 1.0; and sodium lactate, 4.0. The standard pH was 7.4.

Cell Culture. The adenosine-, thymidine-, and glycine-requiring auxotroph AB and its colchicine-resistant mutant C5 were obtained from Dr. Victor Ling of the Ontario Cancer Institute (Toronto, Canada) (13). Cells were maintained at 37°C in 5% CO₂-95% air, in monolayer culture, in α-minimum essential medium (with ribonucleosides and deoxyribonucleosides) supplemented with 10% fetal bovine serum, 10 mM N-(2-hydroxyethyl) piperoxane-N'-(2-ethanesulfonic acid) buffer, pH 7.4, 0.2% sodium bicarbonate, and 40 μg/ml gentamicin. Stock cultures of C5 were maintained in the presence of colchicine (1 μg/ml), to prevent growth of revertants. C5 grown in colchicine-containing medium until the time of experiments gave results identical to C5 maintained in colchicine-free medium. To determine the toxicity of procainamide or cimetidine on AB and C5, cells were grown for 1 week in medium containing either 100 μM procainamide or cimetidine (subculture to confluence). These cultures were without evidence of toxicity.

The human breast cancer line MDA-MB-231, which is sensitive to Adriamycin, and MDA-A1, which is resistant to Adriamycin, were grown as previously described (14). The human cell line KB-3-1, which is sensitive to vinblastine, and KB-V-1, which is resistant to vinblastine, were generously provided by Dr. Dan Von Hoff (University of Texas Health Science Center at San Antonio, TX) and were grown as previously reported (6, 15).

Uptake and Efflux Experiments. Experiments were in 24-well plates (Costar, Cambridge, MA), using confluent cell. Each data point was in triplicate and corrected for an identically treated well without cells. For uptake experiments, medium was removed and the cell sheet was rinsed with GBSS prior to addition of radioactive drug. Following incubation, radioactive medium was rapidly aspirated and the cell sheet was rinsed once with 2 ml of cold 0.9% NaCl. Cells were then dissolved in 1 N NaOH, and radioactivity was quantitated in acidified aqueous counting solution (Amersham), by liquid scintillation counting. Variations for individual experiments are given in "Results" and the figure legends. For efflux experiments, following incubation with radioactive drug to establish equilibrium, medium was rapidly removed, the cell sheet was rinsed with 2 ml of GBSS, and 1 ml of efflux medium (generally GBSS) was added. At the appropriate times, cells were
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Fig. 1. Time course of procainamide accumulation in AB, the cell line sensitive to colchicine (A), or C5, the cell line resistant to colchicine (B). Procainamide (50 nM) was added in the absence or presence of 100 μM verapamil, in Dulbecco's modified Eagle medium, at 23°C. At the times indicated, radioactivity was quantitated by liquid scintillation counting. Each point is the mean of triplicate samples. SD was <5%.

harvested following removal of medium and rinsing with 2 ml of cold 0.9% NaCl. DNA was quantitated fluorometrically (16). Most experiments were performed on at least three occasions.

Statistics. Where appropriate, statistical significance was determined by analysis of variance, with P < 0.05 being considered statistically significant.

Procainamide Metabolism. Following incubation with [3H]procainamide (90 μCi/3 ml/flask) at 37°C for 3 h, AB and C5 cells in radioactive medium were scraped from the plastic flasks and briefly sonicated. Nonradioactive procainamide (15 μg) and N-acetylprocainamide (15 μg) were added to each sample. Three ml of acetonitrile were added to each sample, and the samples were mixed and centrifuged to remove precipitated protein. One hundred μl were injected onto a Bondapak phenyl column (Millipore Corp., Milford, MA), and 0.5-min fractions were collected for 17 min. The high performance liquid chromatography mobile phase was that of Lai et al. (17). At a pump speed of 0.9 ml/min, N-acetylprocainamide eluted at 5.4 min and procainamide eluted at 6.4 min. Fractions were counted in a liquid scintillation counter.

RESULTS

Time Course of [3H]Procainamide Accumulation. AB and C5 were incubated at 23°C with 50 nM [3H]procainamide, in the absence or presence of 100 μM verapamil (Fig. 1). AB progressively accumulated procainamide, reaching 4.5 fmol/μg DNA at 20 min. The rate and amount of accumulation were not influenced by verapamil (Fig. 1A). C5 accumulated procainamide but reached equilibrium earlier and at a much lower level (0.5 fmol/μg DNA) than AB. Exposure to verapamil caused C5 to accumulate procainamide with a time course similar to that of AB (Fig. 1B). To determine whether procainamide was differentially accumulated in other models of MDR, similar experiments were carried out in the human KB cell line and the human breast cell line MDA. KB-3–1 cells (sensitive to vinblastine) accumulated more procainamide than did vinblastine-resistant KB-V-1 cells (Fig. 2A), and only KB-V-1 accumulated more procainamide in the presence of verapamil (Fig. 2, B and C). The effect was not as striking in the KB line as with the CHO line. In Adriamycin-sensitive and -resistant MDA cells, we found neither a difference in procainamide accumulation nor an effect of verapamil (data not shown).

Time Course of [3H]Cimetidine Accumulation. AB and C5 were incubated at 23°C with 70 nM [3H]cimetidine, in the absence or presence of 100 μM verapamil. Both AB and C5 progressively accumulated cimetidine, with the sensitive AB line having approximately 5-fold more at 20 min than did C5 (0.49 versus 0.09 fmol/μg DNA). Verapamil did not significantly influence the accumulation of cimetidine by AB but caused a marked increase in cimetidine accumulation in C5, producing levels similar to those in the AB cell line.

Tetraethylammonium Bromide and Partition Coefficients. [3H]TEA, a quaternary amine which is virtually always charged, is secreted by the organic cation secretory mechanism in the renal tubule. AB and C5 were incubated with [3H]TEA at 23°C, but we were unable to demonstrate any accumulation in either cell with time or varying concentration, whether in the absence or in the presence of 100 μM verapamil (data not shown). Partitioning between octanol and GBSS, pH 7.4, at 23°C for these
Effect of Procainamide Concentration on Accumulation. Since procainamide was accumulated to the greatest extent in AB and C5, we used this probe for subsequent characterization. To determine whether procainamide accumulation occurred by passive diffusion or by a carrier-mediated process, AB and C5 were incubated with increasing concentrations of procainamide (14 nM to 10 μM) for 1 to 6 min at 23°C (Figs. 3 and 4). There was a linear increase in cell-associated procainamide with time (r > 0.98) at each medium procainamide concentration in both AB and C5. A plot of initial rates versus procainamide concentration was linear, without evidence of saturation (Figs. 3B and 4B). Verapamil (10 μM) was without effect in AB regardless of procainamide medium concentration but caused a large increase in procainamide accumulation in C5 at all medium procainamide concentrations (Fig. 5). These findings would be consistent with entry of procainamide by passive diffusion. Furthermore, there was no difference in procainamide accumulation in AB or C5 when excess cimetidine or TEA was in the incubation medium, as might have been expected if competition for a common carrier occurred (data not shown) (10, 11).

Effect of Other Calcium Channel Antagonists on Procainamide Accumulation. Since calcium channel antagonists other than verapamil have also been demonstrated to reverse the MDR organic cations was as follows: TEA, 0.006; procainamide, 0.275; and cimetidine, 2.101.
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Fig. 6. Effect of varying concentrations of verapamil, diltiazem, and nifedipine on procainamide accumulation in C5. Cells were incubated for 5 min with 0–100 μM verapamil (•), diltiazem (□), or nifedipine (○), with 50 nM procainamide. Each value is the mean of triplicate samples. SD was ±5%.

Table 1 Effect of organic cations on procainamide uptake by CHO cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μM)</th>
<th>Procainamide uptake (pmol/μg DNA)</th>
<th>AB</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>23.79 ± 3.01</td>
<td>5.89 ± 0.52</td>
<td></td>
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<tr>
<td>Rifampin</td>
<td>10</td>
<td>23.80 ± 1.51</td>
<td>9.90 ± 2.63*</td>
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<tr>
<td>Rifampin</td>
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<td>27.11 ± 1.76</td>
<td>12.71 ± 0.47*</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>10</td>
<td>23.21 ± 2.71</td>
<td>8.46 ± 0.21*</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
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<td>24.72 ± 2.68</td>
<td>11.84 ± 0.34*</td>
<td></td>
</tr>
<tr>
<td>Rhodamine</td>
<td>10</td>
<td>23.59 ± 0.76</td>
<td>8.15 ± 0.12*</td>
<td></td>
</tr>
<tr>
<td>Rhodamine</td>
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<tr>
<td>Ethidium</td>
<td>10</td>
<td>23.30 ± 1.8</td>
<td>8.37 ± 0.97*</td>
<td></td>
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<tr>
<td>Ethidium</td>
<td>100</td>
<td>25.18 ± 1.6</td>
<td>11.3 ± 0.13*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05 versus control (no added compound).

Table 1 Effect of organic cations on procainamide uptake by CHO cells

Fig. 7. Effect of temperature on verapamil effect in CHO cells. Cells were incubated with 50 nM procainamide for 5 min at either 23°C (●) or 4°C (□), in the absence or presence of 100 μM verapamil. A, AB cells; B, C5 cells. Each value is the mean ± SD of triplicate samples.

Effect of Other Organic Cations on Procainamide Accumulation. We tested some of the other organic cations which have been reported to increase accumulation of cytotoxic agents in resistant cells of several MDR models, to assess their effects on procainamide accumulation. AB and C5 were incubated with [3H]procainamide in the absence or presence of rifampin, tamoxifen, rhodamine, or ethidium. None of these compounds at the concentrations tested had an effect on procainamide accumulation in AB, but each enhanced procainamide accumulation in C5 and this appeared to be related to dose (Table 1).

Effect of Temperature on Procainamide Accumulation and Verapamil Effect. AB and C5 were incubated with [3H]procainamide for 5 min in the absence or presence of 100 μM verapamil, at either 4°C or 23°C, to determine whether the effect of verapamil on procainamide accumulation in C5 was temperature dependent. In AB cells, much more procainamide accumulated at the higher temperature but verapamil was without effect at either temperature (Fig. 7A). In C5 cells, there also was more accumulation of procainamide at the higher temperature and verapamil significantly increased procainamide accumulation only at the higher temperature (Fig. 7B). AB accumulated only 10% of the procainamide measured at the higher temperature, whereas C5 accumulated 50% of the amount measured at the higher temperature.

Preincubation with Verapamil and Subsequent Effect. Since verapamil did not increase procainamide accumulation at 4°C, C5 cells were incubated with 10 μM verapamil at 4°C for 10 min and then washed repeatedly before incubation with [3H]procainamide at 23°C. Similarly treated cells had no verapamil present or verapamil only present during the 23°C incubation. Preliminary incubations at 4°C with [3H]verapamil demonstrated that the washing technique removed >99% of the verapamil, to a level which did not increase procainamide accumulation in our hands (data not shown). Procainamide accumulation in C5 increased significantly (approximately 4-fold) with both verapamil treatments (Fig. 8). These results demonstrate that preincubation with verapamil at 4°C is sufficient for the
Fig. 9. Effect of verapamil on procainamide efflux from CHO cells. Cells were incubated with 50 nM procainamide, with or without 10 μM verapamil, for 90 min at 23°C. Procainamide was removed, cells were rapidly washed, and cells were placed in procainamide-free medium with or without 10 μM verapamil. Cells were harvested following an additional wash at the times indicated. A, efflux from AB and C5 without exposure to verapamil; B, efflux from AB with or without exposure to verapamil; C, efflux from C5 with or without exposure to verapamil. Each point is the mean of triplicate samples. SD was <5%.

subsequent increase in procainamide accumulation in C5 and that verapamil need not be present in the incubation medium containing procainamide.

Effect of Verapamil on Procainamide Efflux from CHO Cells. The difference in levels of cytotoxic drug accumulation between sensitive cell lines and resistant cell lines expressing the mdr gene is due to gp-170 acting as an efflux pump. To determine whether the differences in procainamide accumulation found between AB and C5 could be explained by differences in rates of efflux, AB and C5 were incubated with [3H]procainamide for 90 min (a time sufficient to reach equilibrium; data not shown) at 23°C. Cell-associated procainamide remaining at various times after medium procainamide removal and cell washing was quantitated. Since removal of medium radioactivity and cell washing take approximately 30 s/well, it is possible to miss very rapid efflux. Therefore, some cells were brought to 4°C following the 90-min incubation at 23°C and cells were washed at 4°C. The equilibrium value (0 min of efflux) was identical whether obtained at 4°C or 23°C, making a missed early rapid efflux unlikely. Procainamide efflux from AB and C5 was biexponential (Fig. 9A). The efflux rate from 0 to 5 min for AB was 0.283 ± 0.058 fmol/μg DNA/min (t1/2 = 2.45 min), and the efflux rate for C5 was 0.272 ± 0.017 fmol/μg DNA/min (t1/2 = 2.55 min). The presence of verapamil in the incubation, whether during the uptake period or during the efflux period, had no significant effect on procainamide efflux from either AB (Fig. 9B) or C5 (Fig. 9C). Efflux half-times for AB (in min) (where the first plus or minus refers to the presence or absence of verapamil in the 90-min uptake incubation and the second plus or minus refers to the presence or absence of verapamil in the efflux medium) were: −/+; 2.56; +/−; 2.24; and ++/−; 2.56. Efflux half-times for C5 (in min) were: −/+; 2.14; +/−; 2.02; and ++/−; 1.75. Verapamil did increase procainamide accumulation in C5 when present during the uptake period, as expected. Verapamil (20 μM) had no effect on procainamide efflux from KB-V-1 (data not shown).

Effect of Verapamil on Rate of Procainamide Uptake. Initial rates of procainamide accumulation were measured in AB and C5 following incubation with 50 nM [3H]procainamide, in the absence or presence of 10 μM verapamil. The rate of procainamide accumulation was 2.6-fold greater in AB than in C5 at early time points (Fig. 10A). Verapamil was without effect on the rate of procainamide accumulation in AB (Fig. 10B) but increased the rate of procainamide accumulation 2.8-fold in C5 cells (Fig. 10C).
**Organelle Cation Uptake by CHO Cells**

**Effect of Azide in Glucose-free Medium on Procainamide Accumulation.** One of the central features suggesting an efflux pump in other models of MDR studied with cytotoxic drugs has been the finding that azide in glucose-free medium increases accumulation in the resistant cell line, suggesting that energy is required to maintain the pump (18, 19). AB and C5 cells were incubated with procainamide, in glucose-free GBSS, alone or in the presence of 1 mg/ml added glucose, 0.1% sodium azide, or both. Procainamide accumulation was essentially the same in AB cells regardless of the addition of glucose, azide, or both (Fig. 11). However, azide increased the amount of procainamide accumulated in C5 cells ($P < 0.05$ versus control) and this was reversed when glucose was added with the azide (Fig. 11), suggesting that net procainamide accumulation might be energy dependent. A similar experiment was also performed with 3-O-methylglucose, a substrate for the glucose transporter but not an energy source. Unlike glucose, 3-O-methylglucose did not reverse the increase in procainamide accumulation in C5 (Fig. 11). The influence of azide on procainamide efflux from C5 cells was also studied, to determine whether the azide effect was due to a decrease in efflux rate. C5 cells were incubated for 30 min with procainamide in GBSS, glucose-free GBSS, or glucose-free GBSS containing 0.1% azide. Radioactive medium was removed, cells were washed, and appropriate medium was replaced. Azide-containing medium resulted in a higher zero-time (equilibrium) procainamide accumulation than did glucose-free medium without azide (Fig. 12A). However, the rates of procainamide efflux were similar, with a half-life for glucose-free medium of 14.5 min and a half-life for azide-containing medium of 11.5 min. Rates of procainamide accumulation were also studied, and azide was found to increase procainamide accumulation 2.4-fold (Fig. 12B). Taken together, these data suggest that the effect of azide in glucose-free medium is on procainamide uptake rather than procainamide efflux.

**Lack of Procainamide Metabolism in CHO Cells.** Confluent T25 flasks of AB and C5 cells and a flask without cells were incubated with $[^3]H$procainamide for 3 h at 37°C. There was no evidence for metabolism (no new peaks or any decrease in recovery of procainamide) in either AB or C5.

**DISCUSSION**

Energy-dependent active efflux appears to be the mechanism by which gp-170 renders a cell multiply drug resistant, as shown by many in vitro observations (1–3) and occasionally by direct visualization (20–22). Other explanations have also been suggested (1). Since gp-170 is a constituent of organs which normally engage in organic cation transport (8, 9), well characterized noncytotoxic transport probes (10, 11) were used in models of MDR, to determine their handling. These agents have the advantage of being very poorly bound to proteins and cellular constituents or plastic culture dishes and are, thus, easily used in kinetic studies.

This study demonstrates that procainamide and cimetidine, but not TEA, accumulate in the colchicine-sensitive AB line to a greater extent than in the resistant C5 line. Procainamide uptake was passive in both AB and C5, and there was no difference in procainamide efflux between the two cell lines. Influx was greater in the sensitive cell line than in the resistant cell line. Influx was increased by verapamil in the resistant cell line, but verapamil had no effect on procainamide efflux. Influx was also dependent on energy state, in that influx increased in C5 in the presence of azide in glucose-free medium and could be reversed by addition of glucose but not 3-O-methylglucose. Procainamide efflux was not different between AB and C5 in
the presence of azide in glucose-free medium.

There was no evidence for an active organic cation transport system, such as that found in the kidney, as an explanation for the difference in procainamide accumulation in AB and C5. Procainamide accumulation was by passive diffusion. If accumulation is totally passive for each of the three probes tested, TEA would be excluded from the cells in short term experiments, since TEA is always charged and very poorly lipid soluble. Indeed, this was what was found. Horio et al. (23) also reported that TEA had no effect on vinblastine transport by the Madin-Darby canine kidney cell line. Cimetidine, however, would be expected to accumulate to a greater extent than procainamide, since at pH 7.4 there is approximately 100-fold more uncharged cimetidine and it is 10-fold more lipid soluble. This was not the case, suggesting that cimetidine accumulation may be determined by factors besides lipid solubility and charge. Efflux rates for procainamide were not different in AB and C5 and, thus, would not explain the difference in accumulation in the two cell lines. This finding does not indicate that mediated efflux does not exist, although we were unable to demonstrate trans-stimulation in either cell line (data not shown). We were also unable to inhibit overall renal secretion of the MDR substrate colchicine with the organic cations TEA or N-methyl nicotinamide in vivo (24).

How, then, can the difference in procainamide and cimetidine accumulation be explained? One possibility is that the membranes of the two cell lines are intrinsically different, such that permeability, even if passive, is not the same as was originally suggested by Ling and Thompson (13). While this possibility cannot be excluded, it seems inconsistent with the observations that procainamide accumulation is dependent on energy state (actually increased by energy depletion in the resistant cell line) and increased in the resistant cell line by verapamil and other agents known to interact with gp-170.

Another possibility is that procainamide and cimetidine are substrates for gp-170. There are other examples in the literature in which differences in drug accumulation in the MDR cell have been reported to be due to less drug uptake than in its sensitive counterpart and not due to a significant difference in drug efflux. For example, Fojo et al. (6) found slower drug influx in multiply drug-resistant KB cells, compared to sensitive KB cells, without a difference in rates of drug efflux. Verapamil increased drug influx in the resistant KB cells. Sirotnak et al. (25) reported a 24-fold difference in vincristine influx in resistant Chinese hamster lung cells, compared to sensitive cells, but only a 2-fold difference in efflux rates between the two cell lines. Danks et al. (26) reported a 10-fold higher rate of influx of VP-16 in sensitive human leukemia cells, compared to resistant cells, whereas VP-16 efflux was actually 2.5-fold slower in the resistant cells. Ramu et al. (27) reported that doxorubicin influx was more rapid in sensitive P388 cells than in resistant P388 cells, whereas there was no difference in doxorubicin efflux between the two cell lines. If procainamide and cimetidine are substrates for gp-170, the mechanism by which drug accumulation is accomplished would not seem to be due to active efflux, since we found no difference between the sensitive and resistant cells. Verapamil had no measurable effect on procainamide efflux from either AB or C5 cells, whether present during procainamide uptake or efflux or both. An early, very rapid, efflux component was sought but not found, and verapamil had the expected enhancing effect on procainamide accumulation in C5.

Another possibility for the difference in procainamide and cimetidine accumulation is that it occurs secondary to some action of gp-170. MDR cells have an increased cytosolic pH, which is thought to be a consequence of the multidrug transporter (28-30). In one report, C5 intracellular pH was 0.17 units higher than that in AB (29), whereas the difference has been reported to be as much as 0.44 pH units in other cells (28). Addition of verapamil results in intracellular pH returning to levels found in sensitive cells. Thus, gp-170 may act as a proton pump. Drugs such as procainamide and cimetidine might be expected to accumulate to a greater degree in cells with a lower intracellular pH, due to trapping as the cation. This has been demonstrated for cimetidine and procainamide in rabbit renal brush-border membrane vesicles with an outwardly directed proton gradient (31). The procainamide observations in CHO cells might be explained as a result of the effect of gp-170 on intracellular pH. Cimetidine would accumulate to a lesser extent, in part due to its lower pKa. TEA could not accumulate because it is always charged and water soluble and cannot passively enter the cell to a significant degree. The variable degree to which intracellular pH is affected in MDR cells might explain why we found no difference in procainamide accumulation in sensitive and resistant MDA cells and a smaller difference in KB cells than in CHO cells. To explain a 2.5-fold difference in initial rates of uptake between AB and C5 or in C5 in the presence and absence of verapamil, a cytosolic pH difference of 0.4 units would be necessary, whereas explanation of a 5-fold difference in procainamide accumulation would require a cytosolic pH difference of 0.7 units (32), which is greater than has been previously reported. We do not know the cytosolic pH values in the CHO cells in our experiments, nor do we know whether procainamide stays in the cytosol or moves into other, more acidic, compartments, such as lysosomes (33).


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