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Heterogeneity of Nucleoside Transport in Mammalian Cells

Two Types of Transport Activity in L1210 and Other Cultured Neoplastic Cells

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SUMMARY

The characteristics of nucleoside transport were examined in L1210 murine leukemia cells and five other cultured neoplastic cells. The initial rates of uridine, adenosine, and thymidine transport in L1210 cells were only partially inhibited by 1 μM nitrobenzylthioinosine (NBMPR), a potent inhibitor of nucleoside transport in other cells. The IC_{50} for NBMPR inhibition of uridine transport was 5 nM, but 20% of the activity remained insensitive to concentrations as high as 3 μM. Uridine uptake in the presence of 1 μM NBMPR was saturable and was inhibited by other nucleosides, suggesting the participation of an NBMPR-insensitive transport mechanism. There appeared to be little difference in the specificity of NBMPR-sensitive and -insensitive transport for the physiological nucleosides. Uridine, adenosine, and thymidine were all substrates for both mechanisms, and the K_m values for total and NBMPR-insensitive uridine transport were the same (250 μM). Furthermore, little difference was found in the ability of several other nucleosides to inhibit total or NBMPR-insensitive uridine transport. In both cases, adenosine was the most effective inhibitor while cytidine and deoxycytidine were the least effective. The two transport processes did, however, differ from each other in their sensitivity to *p*-mercuribenzenesulfonate (pMBS). NBMPR-insensitive uridine transport was inhibited by pMBS with an $IC_{50} < 25$ μM, while the IC_{50} for NBMPR-sensitive transport was >400 μM. Cloning of the parent L1210 cell line indicated that both NBMPR-sensitive and -insensitive transport occurred in the same cell. Both types of uridine transport activity were also observed in three other cell lines (RPMI 6410, L5178Y, and P388), while two lines, S49 and Walker 256, exhibited only NBMPR-sensitive and -insensitive transport, respectively. The level of NBMPR-insensitive transport was an important determinant in the ability of NBMPR to inhibit uridine uptake over prolonged periods (10 min), with as little as 20% NBMPR-insensitive transport sufficient to render uptake over 10 min virtually insensitive to NBMPR. The existence of these two types of nucleoside transport activity in mammalian cells may have important implications in the chemotherapeutic use of transport inhibitors in combination with cytotoxic nucleosides or with inhibitors of pyrimidine and purine biosynthesis.

INTRODUCTION

The uptake of both physiological and cytotoxic nucleosides by mammalian cells is mediated by nucleoside-specific transporters which accept a wide range of nucleosides as substrates (1, 2). NBMPR[1] and other S^6-derivatives of 6-thioinosine and 6-thioguanosine (3) have proven to be specific and useful probes of the transport process in erythrocytes (4, 5) and several cultured cell lines (6–9). NBMPR binds reversibly to these cells with a K_D of about 1 nM, and occupancy of these high-affinity binding sites by NBMPR is associated with a loss of nucleoside transport activity. Furthermore, genetic impairment of nucleoside transport in sheep erythrocytes (5) and a mouse lymphoma cell line (7) is accompanied by the loss of high-affinity NBMPR-binding sites, suggesting that these sites are part of a functional nucleoside transport protein.

There is an increasing body of evidence suggesting that some cells may have, in addition to the NBMPR-sensitive transporter, a second component of nucleoside transport which is not inhibited by NBMPR. In transformed hamster fibroblasts uridine transport is inhibited by NBMPR with a K_i of 4 nM, but 20–30% of the transport

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[1] The abbreviations used are: NBMPR, 6[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine, also known as nitrobenzylmercaptopurine riboside and nitrobenzylthioinosine; pMBS, *p*-chloromercuribenzenesulfonate.

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activity remains insensitive to 100 nM NBMPR (10, 11). Similar observations have also been made in Chinese hamster ovary cells (12). In HeLa cells, the relationship between NBMPR binding and inhibition of uridine transport is complex. The dose-response curve is biphasic with ≈40% of the transport activity remaining when the high affinity NBMPR-binding sites are saturated (10 nM). Further inhibition of transport is only seen when NBMPR is increased above 1 μM (9).

In a recent study (13) we have compared nucleoside transport in Walker 256 rat carcinoma and S49 mouse lymphoma cells and have found that they differ markedly in their sensitivity to NBMPR and to sulfhydryl reagents. In Walker 256 cells, nucleoside transport is insensitive to 1 μM NBMPR, but is readily inhibited by pMBS (IC_{50} < 25 μM). Conversely, nucleoside transport in S49 cells is completely blocked by NBMPR, but is relatively insensitive to pMBS (IC_{50} ≈ 300 μM). In other aspects nucleoside transport in the two cells is quite similar. Both have the same K_m (250 μM) for uridine transport and both appear to accept a broad range of nucleosides as substrates.

In a short communication (14) we have noted that there is a component of uridine transport in L1210 and RPMI 6410 cells which is insensitive to 1 μM NBMPR but is inhibited by other nucleosides and by pMBS. In the present study, nucleoside transport in L1210 cells is characterized in greater detail and compared with that of Walker 256 and S49 cells. The presence of NBMPR-insensitive transport in other cells is also demonstrated. The physiological significance of NBMPR-insensitive transport is not known, but the existence of two types of transport activity which differ so markedly in their sensitivity to NBMPR may have important implications in the chemotherapeutic use of transport inhibitors to modulate the uptake of cytotoxic nucleosides (15, 16) or to potentiate the effects of inhibitors of pyrimidine or purine biosynthesis (17, 18).

MATERIALS AND METHODS

Cell lines and growth conditions. RPMI 6410 and S49 cells were provided by Dr. A. R. P. Paterson, University of Alberta (Edmonton, Alta., Canada); Walker 256 cells by Drs. P. M. Schwarz and R. E. Handschumacher, Yale University (New Haven, Conn.); and L1210 and L5178Y cells by Dr. A. D. Welch, St. Jude Children's Research Hospital (Memphis, Tenn.). P388 cells (CCL 46) were obtained from the American Type Culture Collection (Rockville, Md.). RPMI 6410 and L1210 cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf or horse serum, respectively. The other cell lines were grown in Fischer's medium supplemented with 10% heat-inactivated horse serum. Stock cultures were maintained in a humidified 5% CO_2/air atmosphere, and cells for transport studies were grown in roller bottles (1.5 rpm) gassed with 5% CO_2/air.

Nucleoside uptake measurements. Nucleoside uptake was determined by a modification of the method described by Harley *et al.* (19). Cells were harvested during log growth, washed twice, and resuspended in Buffer A (130 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM NaH_2PO_4, 10 mM glucose, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid adjusted to pH 7.4 at 20° with NaOH). Aliquots of the cell suspension were layered over silicone oil in microcentrifuge tubes and placed in the centrifuge. Uptake was initiated at 2-sec intervals by rapid addition of a reaction mixture (Buffer A containing twice the indicated concentration of ^{3}H-labeled nucleoside (5–10 μCi/ml), and terminated by turning on the centrifuge for 30 sec. The supernatant fractions were aspirated and the tubes washed twice with water. The oil was removed with the last wash, the pellets were dissolved in 1% Triton X-100, and total radioactivity was determined in a liquid scintillation counter.

For uptake measurements at times longer than 30 sec, incubations were carried out in a shaking water bath, and, at the times indicated, aliquots of the cell suspension were removed and layered over silicone oil. The tubes were centrifuged and the samples were processed as above.

The amount of extracellular medium carried over into the pellet was determined in parallel incubations substituting [^{14}C]sucrose (10 μCi/ml) for ^{3}H-labeled nucleoside in the reaction mixture. All uptake values were corrected for the amount of ^{3}H-labeled nucleoside in the extracellular fluid. The lag time of 2.5 sec (13) required for effective separation of the cells from the extracellular medium after activation of the centrifuge was added to the sampling time to determine the actual uptake interval. Initial rates of uptake in the absence of inhibitors were determined from the coefficients of the first-order term in parabolas fit to the uptake data as described by Harley *et al.* (19). In the presence of 1 μM NBMPR, uridine uptake in all cells except Walker 256 was linear for at least 30 sec and transport rates were determined by linear regression analysis. In some experiments, transport rates without NBMPR were estimated from uptake over 7.5-sec or 12.5-sec intervals, and those with NBMPR from 22.5-sec intervals.

Cloning of L1210 cells. Cells were diluted to ≈1,000 cells/ml in growth medium and inoculated into Cuprak tissue culture dishes (Costar, Cambridge, Mass.) containing wells varying in size from 0.05 to 2.0 μl. The plates were examined by phase-contrast microscopy, and only those wells containing a single cell were maintained. Ten clones (L1210/B1–L1210/B10) were expanded and examined for nucleoside transport activity.

Materials. RPMI 1640 and Fischer's culture media were obtained from K.C. Biologicals (Lenexa, Kan.). Horse serum, fetal calf serum, and other cell culture supplies were obtained from GIBCO Laboratories (Grand Island, N. Y.). [5-^{3}H]Uridine, [2-^{3}H]adenosine, [6-^{3}H]thymidine, and ^3H_2O were obtained from New England Nuclear Corporation (Boston, Mass.) and [^{14}C]sucrose from Schwarz/Mann, Inc. (Spring Valley, N. Y.). Unlabeled nucleosides were obtained from Calbiochem-Behring Corporation (La Jolla, Calif.) or Sigma Chemical Company (St. Louis, Mo.). NBMPR was prepared from 6-mercaptopurine riboside by Dr. Josef Nemec (St. Jude Children's Research Hospital, Memphis, Tenn.) as described by Paul *et al.* (3). 6-Mercaptopurine riboside was generously provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md.) and dipyridamole by Boehringer-Ingelheim (Ridgefield, Conn.).

RESULTS

Effect of NBMPR and dipyridamole on nucleoside transport in L1210 cells. Both NBMPR and dipyridamole are potent inhibitors of nucleoside transport, but differ in their effectiveness and specificity. NBMPR has an IC_{50} of 1–10 nM in most cells and appears to be specific for nucleoside transport (12, 20). Dipyridamole, which has no structural resemblance to a nucleoside, is at least 10-fold less effective than NBMPR as an inhibitor of nucleoside transport (9) and also inhibits the transport of nucleobases, hexoses, and phosphate (21). The effects of these two inhibitors on uridine, adenosine, and thymidine transport in L1210 cells are shown in Fig. 1. At a concentration which is sufficient to block nucleoside transport in S49 cells completely (13), NBMPR only partially inhibits nucleoside transport in L1210 cells. Approximately 20% of the initial rates of uridine and thymidine uptake and 40% of adenosine uptake remain insensitive to 1 μM NBMPR. Dipyridamole (10 μM) appears to be a little more effective, but still does not

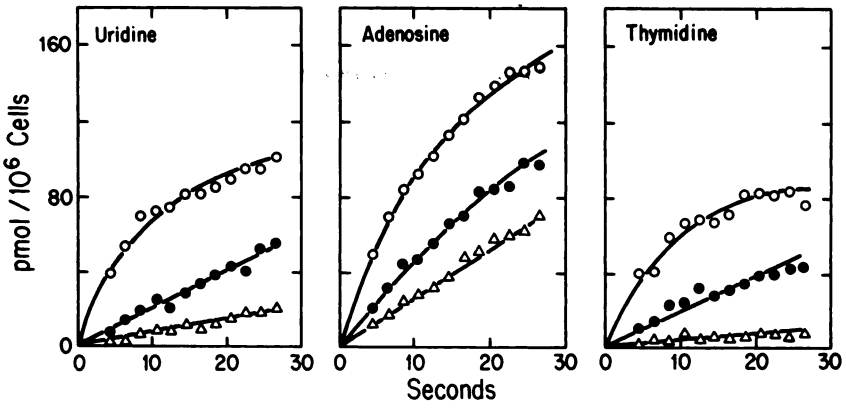

FIG. 1. *Effect of NBMPR and dipyridamole on uridine, adenosine, and thymidine transport in L1210 cells*
Cells were incubated with the indicated inhibitor for 5 min at 20° and then assayed for uridine, adenosine, or thymidine uptake as described under Materials and Methods. The nucleoside concentration was 100 μM. ○, No additions; ●, 1 μM NBMPR; △, 10 μM dipyridamole.

completely block the uptake of these nucleosides. The effect of NBMPR on uridine transport is further examined in Fig. 2. Cells were incubated with increasing concentrations of NBMPR for 20 min and then assayed for uridine transport. About 80% of the transport activity is inhibited with an IC_{50} of 5 nM, but a plateau is reached in the inhibition curve at 10 nM NBMPR. Further inhibition is seen only when the concentration is increased to 10 μM. The wide plateau in the inhibition curve, spanning 3 orders of magnitude, suggests that the inhibition observed at 10 μM NBMPR occurs by a mechanism different from that involved at lower concentrations of NBMPR. We have therefore used uptake in the presence of 1 μM NBMPR to define the component of uptake which is relatively insensitive to NBMPR.

Is NBMPR-insensitive nucleoside uptake due to a second transport mechanism or diffusion? The uptake of nucleosides which occurs in the presence of NBMPR could be due to the presence of an NBMPR-insensitive transport mechanism such as that observed in Walker 256 cells (13), or it could be the result of simple diffusion of nucleosides across the plasma membrane. Two criteria may be used to distinguish between these possibilities: (a) saturability and (b) inhibition by other substrates for the same transport mechanism. As shown in Fig. 3, the initial rate of uridine uptake in either the presence or absence of 1 μM NBMPR obeys Michaelis-Menten kinetics with similar K_m values (247 ± 47 and 252 ± 34 μM, respectively) but a 4-fold difference in the V_{max} values (8.3 ± 0.4 and 32 ± 1 pmoles/10^6 cells-sec, respectively). The similarity of the K_m values is in keeping with our observations that the K_m for uridine transport in the NBMPR-insensitive cell line Walker 256 does not differ significantly from that of the NBMPR-sensitive line S49 (13).

The effect of other nucleosides on the uptake of uridine in the presence and absence of NBMPR is examined in Fig. 4. Both total and NBMPR-insensitive uptake are inhibited by a variety of nucleosides, including both purine and pyrimidine, and ribo- and deoxyribonucleosides. In both cases, adenosine is the most effective inhibitor and cytidine and deoxycytidine are the least effective. These experiments indicate that NBMPR-insensitive nucleoside uptake in L1210 cells occurs via a carrier-mediated process. Furthermore, it appears that there is little difference in the specificity of NBMPR-sensitive and -insensitive transport for the physiological

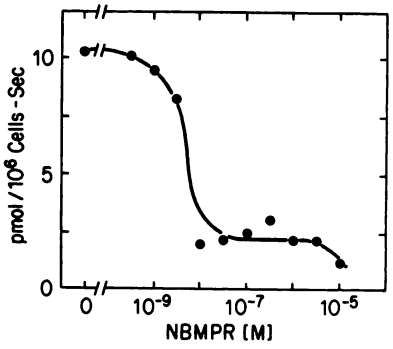

FIG. 2. *Dose-response curve for NBMPR inhibition of uridine transport in L1210 cells*
Cells were incubated with the indicated concentration of NBMPR for 20 min at 20°. Uridine (100 μM) uptake was determined over 24-sec time courses as described under Materials and Methods. Initial rates of transport were calculated from parabolas fit to the first 12 sec of the uptake curves at NBMPR concentrations <10 nM and by linear regression analysis of the entire time course at NBMPR concentrations ≥10 nM.

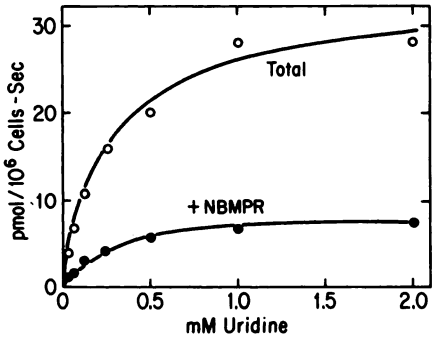

FIG. 3. *Kinetics of uridine transport ± NBMPR in L1210 cells*
Cells were incubated with (●) or without (○) 1 μM NBMPR for 5 min at 20°. Total uridine uptake was determined over 7.5-sec intervals, and NBMPR-insensitive uptake over 22.5-sec intervals at the concentrations indicated. The kinetic constants were determined by direct computer fit of the nonlinear rate equation as described by Cleland (22).

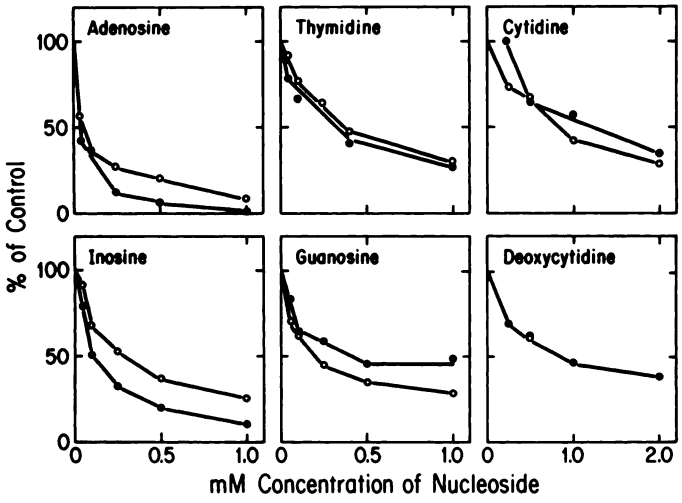

FIG. 4. *Effect of other nucleosides on uridine transport ± NBMPR in L1210 cells in the presence and absence of NBMPR*

Cells were incubated with (●) or without (○) 1 μM NBMPR for 5 min at 20° and then assayed for uridine uptake over 12.5-sec intervals. The indicated concentrations of unlabeled nucleosides were added simultaneously with [^{3}H]uridine (100 μM).

nucleosides—a conclusion also reached in the comparison of these two types of transport activity in Walker 256 and S49 cells (13). Whether there are any differences in their specificity for cytotoxic nucleoside analogues has yet to be determined.

Effect of pMBS on uridine transport. The nucleoside transporters of S49 and Walker 256 cells differ in their response to pMBS as well as to NBMPR, with Walker 256 (IC_{50} < 25 μM) cells at least 10-fold more sensitive than S49 cells (IC_{50} ≈ 300 μM) (13). To determine whether the two transport processes in L1210 cells also differ in their sensitivity to pMBS, cells were incubated with increasing concentrations of pMBS and then assayed for total and NBMPR-insensitive uridine transport (Fig. 5). The effect of low concentrations of pMBS (<100 μM) on total uridine transport is quite variable, with inhibition ranging from 0 to 20% in different experiments, but the

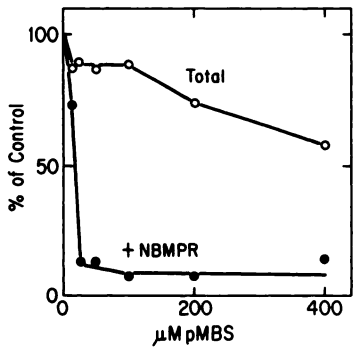

FIG. 5. *Effect of pMBS on uridine transport ± NBMPR in L1210 cells*

Cells were incubated with the indicated concentration of pMBS for 20 min at 20°. Where indicated, 1 μM NBMPR was added during the last 5 min of the incubation. Uridine (100 μM) uptake was determined over 12.5-sec intervals for total (○) uptake and 22.5-sec intervals (●) for NBMPR-insensitive uptake. Control values were 88 pmoles/10^6 cells-12.5 sec for total uptake and 47.6 pmoles/10^6 cells-22.5 sec for NBMPR-insensitive uptake.

NBMPR-insensitive component is consistently inhibited with an IC_{50} less than 25 μM. At higher pMBS concentrations, inhibition of total transport is more reproducible, but the IC_{50} is greater than 400 μM. Inhibition of the NBMPR-insensitive component of transport by pMBS is readily reversed by β-mercaptoethanol (Table 1), indicating the presence of an active sulfhydryl group in this transporter. It was not possible to assess the reversibility of pMBS inhibition of total uridine transport because cells treated with higher concentrations of pMBS (>200 μM) were very fragile, and the washing and incubation conditions employed resulted in nonspecific membrane damage as evidenced by an increase in the sucrose-accessible space.

The susceptibility of the NBMPR-insensitive component of transport in L1210 cells to pMBS and the reversal of inhibition by β-mercaptoethanol is similar to that of Walker 256 cells. Likewise, the relative resistance (IC_{50} > 400 μM) of NBMPR-sensitive transport to this reagent is similar to that of S49 cells (13). In some respects the effect of pMBS on uridine transport in L1210 cells is also similar to that reported in hamster fibroblasts (11) and HeLa cells (23). In both of these cell lines it was observed that pMBS (≤100 μM) alone does not inhibit transport but appears to act synergistically with NBMPR to block uridine transport completely. Although we cannot rule out the possibility that pMBS acts to increase the sensitivity of a nucleoside transport-mechanism to NBMPR, as suggested by these authors (11, 23), the simplest explanation of the results presented here is that L1210 cells possess two distinct types of nucleoside transport which differ in their sensitivity to NBMPR and pMBS.

Two types of transport in the same cell or different cell populations? The presence of both NBMPR-sensitive and -insensitive transport activity in L1210 cells could be due to a heterogeneous cell population or two types of transport in the same cell. To address this question, uridine transport was examined in 10 sublines cloned from the parent line. All of these clones exhibit NBMPR-insensitive transport, and the relative level of NBMPR-insensitive to total activity (range = 16%–32%) does not differ significantly from that of the parent line (data not shown). It can therefore be concluded that both types of transport activity are present in the same cell.

NBMPR-insensitive nucleoside transport in other

TABLE 1

Reversal of pMBS inhibition of NBMPR-insensitive transport by β-mercaptoethanol

L1210 cells were incubated with or without 100 μM pMBS for 10 min at 20° and then washed with cold buffer and incubated with 1 mM β-mercaptoethanol at 37°. At the times indicated, aliquots of the cell suspension were removed and cooled to 20°, and 1 μM NBMPR was added to the cell suspension. Uridine uptake was then determined over a 22.5-sec interval. The data presented are the means of duplicate assays.

Time with β-mercaptoethanol	Uridine transport	
	Control cells	pMBS-Treated cells
min	*pmoles/10^6 cells-22.5 sec*	
0	40.8	7.0
10	44.0	22.0
30	40.6	37.6

TABLE 2

Effect of NBMPR on the initial rate of uridine transport and on uptake at 10 min

Cells were incubated with or without 1 μM NBMPR for 5 min at 20° and then assayed for uridine (100 μM) transport (12- or 24-sec uptake curves) or uridine uptake over 10 min. Total initial rates of transport were calculated from parabolas fit to the uptake curves as described under Materials and Methods. For L5178Y, P388, and L1210 cells, transport rates with NBMPR were calculated by linear regression analysis from the 24-sec time courses. For S49 and RPMI 6410 cells, transport rates with NBMPR were determined from 30-min and 5-min time courses, respectively. Data are the means ± standard deviation of three to seven experiments.

Cell line	Transport			Uptake at 10 min
	−NBMPR	+NBMPR	% Insensitive to NBMPR	% Inhibition by NBMPR
	pmoles/10⁶ cells-sec			
S49	5.9 ± 2.5	<0.05[a]	<1[a]	>95[a]
RPMI 6410	10.0 ± 1.3[b]	0.2 ± 0.04	2	72 ± 4
L5178Y	8.5 ± 1.3	0.9 ± 0.2	10	42 ± 10
P388	12.4 ± 2.9	1.3 ± 0.4	10	23 ± 11
L1210	10.9 ± 1.1	2.2 ± 0.2	20	8 ± 8
Walker 256	6.9 ± 0.8	6.9 ± 0.6	100	< 5[a]

[a] Limit of detection.

[b] From Belt and Welch (24).

cells. We have now examined the effect of NBMPR on uridine transport in six cultured neoplastic cell lines, and the results of these studies are summarized in Table 2. Two of the lines, S49 and Walker 256, each exhibit only a single type of transport activity and have been described elsewhere (13) as models for NBMPR-sensitive and -insensitive transport, respectively. All of the other four lines (RPMI 6410, L5178Y, P388, and L1210) exhibit both types of transport activity, with the NBMPR-insensitive component varying from 2% to 20% of the total activity.

All of the results discussed to this point have involved measurements made over 30 sec or less. This is because nucleoside transport is very rapid, and in less than 1 min intracellular and extracellular nucleoside pools equilibrate and phosphorylation of the nucleoside becomes rate-limiting for uptake (25). However, measurements over longer periods of time may be more relevant to the uptake of nucleosides and their cytotoxic analogues under physiological conditions. In Table 2 the effect of NBMPR on uridine uptake over 10 min is also examined. In S49 cells, which have no detectable NBMPR-insensitive transport, 1 μM NBMPR completely blocks uptake over 10 min. Interestingly, in RPMI 6410 cells, where the NBMPR-insensitive component is only 2% of the initial rate of transport, uptake over 10 min is inhibited only 70% by 1 μM NBMPR. L5178Y and P388 cells have higher levels of NBMPR-insensitive transport (10%), and uptake over 10 min in these cells is inhibited less than 50% by NBMPR. In L1210 cells, the NBMPR-insensitive component is still a minor part (20%) of the total transport rate, but NBMPR has little or no effect on uptake over 10 min. These results suggest that the level of NBMPR-insensitive transport is an important determinant in the ability of NBMPR to inhibit nucleoside uptake over prolonged periods and that even 20% NBMPR-insensitive transport can prevent NBMPR inhibition of uptake over longer times.

DISCUSSION

The results presented here suggest that L1210 cells have two distinct components of nucleoside transport which differ in their sensitivity to NBMPR and to pMBS. The first of these, which we shall define as Type 1, is inhibited by NBMPR with an IC_{50} of 5 nM, but is relatively insensitive to pMBS (IC_{50} > 400 μM). Type 2 transport, on the other hand, is not affected by NBMPR at concentrations below 1 μM, but is sensitive to pMBS with an IC_{50} less than 25 μM. In other respects the two transport activities are quite similar. Both mechanisms accept uridine, adenosine, and thymidine as substrates, and the K_m values for uridine are the same (250 μM). Inhibition experiments also suggest that there are no significant differences in the specificity of these mechanisms for several other physiological nucleosides. In all aspects thus far examined, Type 1 transport of L1210 cells resembles that of S49 cells, which exhibit only NBMPR-sensitive transport (13). Conversely, Type 2 transport in L1210 cells is similar to that of Walker 256 cells, which lack high-affinity NBMPR-binding sites[2] and are inhibited by NBMPR only at concentrations greater than 1 μM (13). In L1210 cells, both types of transport appear to reside in the same cell, although Type 2 activity is a minor component (20%).

Chello *et al.* (26) have previously reported a duality of transport mechanisms for adenosine in L1210 cells; however, the second transport system reported by these authors and the Type 2 transporter reported here are not the same. The second transporter reported by Chello *et al.* (26) has a marked specificity for adenosine and its analogues and is inhibited by 6-(2-hydroxy-5-nitrobenzyl)thioguanosine, a congener of NBMPR which competes with NBMPR for its high-affinity binding sites (7). The Type 2 transporter reported here, however, does bear some resemblance to a second transport system proposed by Bibi *et al.* (27) for hamster cells.

In addition to L1210, three cell lines examined in the present study (RPMI 6410, L5178Y, and P388) also appear to have both Type 1 and Type 2 nucleoside transport, with Type 2 activity contributing from 2% to 20% of the total rate of uridine transport. The role of Type 2 transport in the uptake of the nucleosides in these cells under physiological conditions is not clear. Transport is very rapid, and within 1 min the intracellular pool of free

[2] J. A. Belt, E. S. Jakobs, and A. R. P. Paterson, unpublished data.

nucleoside reaches a steady state and phosphorylation of the nucleoside becomes rate-limiting for uptake (25). It is unlikely that even the 20% contribution of the Type 2 mechanism to the transport rate in L1210 cells has a signficant effect on uptake after the 1st min. However, the rate of Type 2 transport may be an important determinant in the ability of NBMPR to protect a cell or tissue from cytotoxic nucleosides (2, 15, 16, 28), or to potentiate the action of inhibitors of pyrimidine or purine biosynthesis through blockade of nucleoside salvage (17). We have demonstrated that as little as 20% Type 2 activity in a cell (L1210) is sufficient to render the uptake of uridine at 10 min virtually insensitive to 1 μM NBMPR. Paterson and his colleagues (15, 16, 28) have found that NBMPR can protect mice from lethal doses of certain cytotoxic nucleosides. Furthermore, they have demonstrated that coadministration of NBMPR with an otherwise lethal dose of tubercidin (7-deazaadenosine) (15) or nebularine (purine riboside) (16) to tumor-bearing mice results in increased life-span and some long-term survivors. The mechanism by which NBMPR protects critical normal tissues of mice without also blocking uptake of the toxic nucleoside by the tumor is not known. It is possible that this apparent selectivity of NBMPR resides in differing levels of Type 1 and Type 2 transport in various tissues and tumors.

ACKNOWLEDGMENTS

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