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Nitrobenzylthioinosine-sensitive and -resistant nucleoside transport in normal and transformed rat cells

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Cultured Novikoff rat hepatoma and Walker 256 carcinoma cells have previously been reported to express only nitrobenzylthioinosine (NBTI)-resistant uridine transport and to lack high affinity NBTI-binding sites, whereas the latter are common on all other types of cultured mammalian cells from different species ($(1-7)\cdot 10^5$ sites/cell) which have been investigated with the exception of a transport-deficient cell variant which lacks high-affinity NBTI-binding sites. The present study shows that lack of NBTI sensitivity of transport and of NBTI-binding sites in Novikoff and Walker 256 cells are not related to the species or tissue origin of these cells. Uridine transport in a variant (NRM) of Novikoff hepatoma cells, in HTC rat hepatoma cells, normal rat kidney (NRK) cells, rat erythrocytes and rat hepatocytes was inhibited 15–60% by 10–500 nM NBTI and the cells expressed high-affinity NBTI-binding sites ($K_d = 0.1-0.6$ nM). The apparent turnover numbers for the NBTI-sensitive nucleoside carriers fell into two classes, with those for transformed cells about 10-times higher than those for the normal rat cells.

Introduction

On the basis of sensitivity to inhibition by 6([4-nitrobenzyl]thio)-9-β-D-ribofuranosylpurine (nitrobenzylthioinosine, NBTI), two types of nucleoside transport have been recognized in mammalian cells [1,2]. One type (NBTI-sensitive type) is inhibited by very low concentrations of NBTI ($IC_{50} = 1-10$ nM). With this type, inhibition of transport correlates with the binding of NBTI to high-affinity binding sites on the cells ($K_d =$ 0.2–1 nM). In contrast, the IC_{50} of the second type (NBTI-resistant type) is about 5–10 μM. As far as has been determined, the two types do not differ substantially in their broad substrate specificity or kinetically [1–3], and both types are completely inhibitable by the less specific inhibitors dipyridamole and papaverine [2,4]. Although it has not been excluded yet that the two types of transport are mediated by independent gene products, we favor the view that they represent two conformational states of a single protein in the membrane which differ in their expression of high-affinity NBTI-binding sites [2]. Human erythrocytes, S49 mouse lymphoma cells and RPMI 6410 mouse cells are the only types of cells recognized so far that possess practically only NBTI-sensitive transport [1,2,5–7], and a variant of S49 cells that is deficient in nucleoside uptake [8] lacks high-affinity NBTI-binding sites [9]. In most cell types investigated, both NBTI-sensitive and NBTI-resistant transport co-exist, most likely in single cells. For example, NBTI-sensitive nucleoside transport represents 80–90% of total transport in mouse P388, L1210 and L5178 leukemia and mouse

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Abbreviation: NBTI, nitrobenzylthioinosine.

L929 cells, 60–70% in Chinese hamster ovary (CHO) cells and 40–60% in human HeLa cells [1,2,4,10,11]. At the other extreme are Novikoff rat hepatoma and Walker 256 rat mammary gland carcinoma cells [1–3] whose nucleoside transport capacities are as high as those of other cell types, but whose transport is little, if at all, affected by 1 μM NBTI. This resistance to inhibition in Novikoff cells correlates with a lack of high-affinity NBTI-binding sites [2]. Novikoff and Walker 256 cells differ in both species and tissue origin from all other types of cells investigated. This finding raised the question of whether the unique properties of the nucleoside transporter of these cells might be related to the species or tissue origin of these cells. These considerations led to the present study in which we have investigated nucleoside transport in various normal and transformed rat cells and its sensitivity to NBTI.

Experimental procedures

Materials. [5-^{3}H]uridine and [G-^{3}H]NBTI were obtained from Moravek Biochemicals (Brea, CA, U.S.A.) and diluted to the desired specific radioactivity with unlabeled uridine or NBTI. Unlabeled nucleosides were obtained from Sigma (St. Louis, MO, U.S.A.) and unlabeled NBTI from Calbiochem (San Diego, CA, U.S.A.). Dipyridamole (Persantin) was a gift from Geigy Pharmaceuticals (Yonkers, NY, U.S.A.).

Cell culture. Wild-type Novikoff rat hepatoma cells used in the present study (NRM) represent a subline of our strain [12] which has been propagated in the laboratory of Dr. A.R.P. Paterson for about 1 year and has been received from him. The cells were propagated in suspension culture in Swim's medium 67 as described previously [13]. The cells were cloned both by limiting dilution in 96-well tissue culture plates and in semisolid agarose medium [14] in 60-mm culture dishes. With both methods, the plating efficiency was about 100%. Rat hepatoma HTC cells (obtained from Dr. Gordon Ringold, Stanford University) were propagated as described for Novikoff cells. Normal rat kidney cells were obtained from the American Type Culture Collection and propagated in plastic T flasks or in 24-well culture plates with Eagle's minimum essential medium supplemented with serum, D-glucose and non-essential amino acids as described previously [13].

Isolation of hepatocytes. Hepatocytes were isolated by perfusing the livers of fed, male rats (250–330 g) with collagenase (Sigma type IV, 0.5 mg/ml) according to the protocol of Seglen [15]. The cell preparations were centrifuged through a Percoll gradient [16], a procedure which removes Kupffer cells and injured hepatocytes, and the hepatocytes were suspended to $(1–4) \cdot 10^6$ cells/ml of balanced salt solution containing 1% (w/v) of lipid-free bovine serum albumin. Final hepatocyte preparations were greater than 95% impermeable to Trypan blue.

Isolation of erythrocytes, unsealed erythrocyte ghosts and lymphocytes. Rats were bled by cardiac puncture. The red cells were washed three times in saline containing 5 mM Tris-HCl (pH 7.4; Tris-saline) whereby the buffy coat containing lymphocytes was removed; alternatively, the red cells were collected and freed of lymphocytes by centrifugation through Ficoll-Hypaque [17] and then washed in Tris-saline. The Ficoll-Hypaque layer was diluted with 2 vol. of Tris-saline containing 5% (v/v) fetal calf serum, and the lymphocytes were collected by centrifugation and washed twice in Tris-saline containing fetal calf serum. Human red cells were obtained as a byproduct of lymphocyte isolations from Dr. J. Kersey (Department of Pathology) and washed thrice in Tris-saline. Unsealed white erythrocyte ghosts were prepared as described by Steck [18].

Nucleoside transport measurements. Zero-*trans* influx of uridine was measured in cell suspensions at 25°C as we have described previously [19,20]. Novikoff (NRM) and HTC cells were harvested by centrifugation from mid to late exponential phase cultures and suspended to $6 \cdot 10^6$ to $2 \cdot 10^7$ cells/ml of basal medium 42 (BM42). Washed rat and human red cells were suspended to about $6 \cdot 10^8$ cells/ml of Tris-saline. Time-courses (comprising 15 time-points) of transmembrane equilibration of 500 μM radiolabeled uridine were determined by rapid kinetic techniques. Data for NRM and HTC cells, hepatocytes and rat erythrocytes were evaluated by fitting an appropriate integrated rate equation, based on a simple carrier with directional symmetry and equal mobility of empty and nucleoside-loaded carrier [19,21] whereby K, the

Michaelis-Menten constant, was fixed at 250 μM [19,21]. The nucleoside carrier of human erythrocytes exhibits differential mobility of empty and loaded carrier [7,22,23]. Thus, data for these cells were evaluated by integrated rate analysis as described previously [7,22] with K fixed at 100 μM [22] and R_{ee}, the resistance factor for equilibrium exchange, fixed at a value experimentally determined for the cell population under investigation (5.3 s/mM). The slopes of these fits at $t = 0$ were taken as initial velocity (v_{12}^{zt}) and are expressed, if not indicated otherwise, as pmol of labeled permeant transported per second and μl of cellular H_2O [19,21,22]. For measuring the effects of NBTI and dipyridamole on uridine transport, these were added to cell suspensions (at 25°C) at least 2 min prior to transport assay.

For nucleoside transport measurements in NRK cells, confluent cultures in 24-well culture plates were rinsed with balanced salt solution. Then, 500 μM [^{3}H]uridine was added at timed intervals, 0.2 ml/well, at 25°C. After all wells had received substrate (12 time-points in duplicate per time-course), the fluid was rapidly decanted, and the plate was immersed in ice-cold balanced salts solution containing 20 μM dipyridamole as a stopper. The fluid was again decanted, and the rinse was twice repeated in this manner (the procedure took less than 10 s). The wells were drained, the cells of each well were scraped into 0.5 ml of an aqueous solution of 1% Triton X-100 and the lyate was analyzed for radioactivity.

Equilibrium binding of NBTI. Equilibrium binding of [^{3}H]NBTI was measured as described previously [2,24]. The final cell densities per ml were $5 \cdot 10^6$ to $2 \cdot 10^7$ for Novikoff (NRM) and HTC cells, $(2–3) \cdot 10^6$ for NRK cells, $5 \cdot 10^5$ to $2 \cdot 10^6$ for hepatocytes, $4 \cdot 10^8$ for rat red cells and $2 \cdot 10^7$ for rat lymphocytes. The following equation was fitted to concentrations of bound ligand (L_b; measured as total ligand minus free ligand) and free ligand (L_f):

$$L_b = \frac{NL_f}{K_d + L_f} + k'L_f \qquad (1)$$

where N = number of binding sites per liter, K_d = dissociation constant and k' = a coefficient of non-saturable binding.

Data analysis. The theoretical equations were fitted to data by a generalized least-squares regression program based on the algorithm of Dietrich and Rothmann [25] and implemented on a Hewlett-Packard 9825 computer. Parameter values are reported ± standard error of the least-squares estimate [26], unless indicated otherwise.

Results and Discussion

In previous studies we have shown that uridine transport in wild-type Novikoff cells is not significantly inhibited by NBTI up to a concentration of 1 μM and that the cells lack a measurable number of high-affinity NBTI-binding sites [2,3]. We found, however, that a uridine kinase-deficient variant of Novikoff cells (1-14-7) possessed a low number of high-affinity binding sites and that 10–15% of the total uridine transport by this variant was NBTI-sensitive. Paterson (personal communication) similarly found that the wild-type Novikoff cells he had received from us and had propagated in his laboratory (designated NRM) also exhibited a low number of high-affinity NBTI-binding sites. We have confirmed this finding. A similar number of high-affinity binding sites were detected in Novikoff (NRM) cells in four independent experiments (Table I) and their presence correlated with an inhibition of uridine transport in these cells. Fig. 1A shows representative time-courses of uptake of 500 μM uridine by the NRM Novikoff cells in the presence of the indicated concentrations of NBTI and illustrates the general method of measuring zero-*trans* influx in all experiments presented. Chromatographic analysis showed that after 1 min of incubation less than 10% of the intracellular radioactivity was associated with nucleotides (data not shown). Thus, uptake reflected transmembrane equilibration of unmodified uridine. Initial zero-*trans* entry velocity (v_{12}^{zt}) was estimated by integrated rate analysis as the zero-time slope of the uptake curve [19,20].

In Fig. 2, the v_{12}^{zt} values are plotted as a function of the NBTI concentration. On the average, about 20% of total uridine transport of Novikoff (NRM) cells was found to be inhibited by concentrations of NBTI between 10 and 500 nM. Although these experiments confirm clearly a correlation between the presence of high-affinity binding sites and sensitivity of a portion of uridine

TABLE I

PARAMETERS FOR EQUILIBRIUM BINDING OF NBTI TO VARIOUS TYPES OF RAT CELLS

The equilibrium binding of 0.15, 0.3, 0.6, 1, 2, 4, 7, 10, 15, 20 and 40 nM NBTI was measured and Eqn. 1 was fitted to the data. k' as defined in Eqn. 1 is dimensionless; the values presented are normalized to μl cell water, since nonspecific binding is presumably proportional to cell density and volume.

Cell type	Expt.	K_d (nM)	Binding sites per cell ($\times 10^{-3}$)	k' (μl suspension/ μl cell H_2O)
Novikoff (NRM)	1	0.18 ± 0.05	18 ± 2	7
	2	0.10 ± 0.05	24 ± 5	6
	3	0.57 ± 0.80	31 ± 8	11
	4	0.20 ± 0.21	32 ± 8	18
HTC	1	0.57 ± 0.16	31 ± 30	32
	2	0.54 ± 0.10	69 ± 11	22
	3	0.20 ± 0.08	26 ± 3	10
NRK	1	0.21 ± 0.20	380 ± 80	26
	2	0.12 ± 0.08	170 ± 30	23
	3	0.13 ± 0.05	330 ± 50	34
Hepatocytes	1	0.43 ± 0.18	800 ± 90	47
	2	0.19 ± 0.13	940 ± 110	10
	3	0.22 ± 0.09	560 ± 60	23
Erythrocytes [a]	1	– [b]		27
	2	0.48 ± 0.21	0.56 ± 0.2	25
	3	0.89 ± 0.97	0.70 ± 0.3	16
Erythrocyte white ghosts	2	0.26 ± 0.16	0.094 ± 0.02 [c]	[d]
	3	0.21 ± 0.10	0.108 ± 0.02 [c]	[d]

[a] Each experiment represents a pool of red cells from 2–3 rats.
[b] No specific binding was apparent.
[c] Expressed per ghost, assuming a recovery of 1 ghost/cell.
[d] Nonspecific binding was less than 5% of that of intact erythrocytes

transport to inhibition by low concentrations of NBTI, they do not address the question of whether the correlation extends to single cells in the population and whether all cells in a population express NBTI-binding sites. The possibility that NBTI binding and sensitivity of uridine transport were attributable to contaminating cells also needed to be considered. We have, therefore, isolated and analyzed six independent clones of Novikoff (NRM) cells. All exhibited similar kinetics of NBTI binding, number of binding sites and proportion of NBTI-sensitive uridine transport (data not shown). These results, combined with those presented previously [2], indicate that the lack of expression of high-affinity binding sites in Novikoff cells is a relatively stable property but that populations that express a low number of NBTI-binding sites may arise. We have not been able to ascertain yet whether the observed variations in the expression of NBTI-binding sites is under genetic or environmental control.

Other results showed that all other types of rat cells, whether normal or transformed, exhibited combinations of NBTI-sensitive and resistant uridine transport (Figs. 1B and 2 and Table II) and that in all instances NBTI-sensitive uridine transport was associated with the presence of high-affinity NBTI-binding sites (Table I). Thus, a lack of high-affinity NBTI-binding is not a characteristic of rat cells. Furthermore, it is not a general property of hepatoma cells or related to the liver origin of these tumors. In fact, about 60% of uridine transport in rat hepatocytes was NBTI-sensitive (Fig. 2) and the cells possessed a rela-

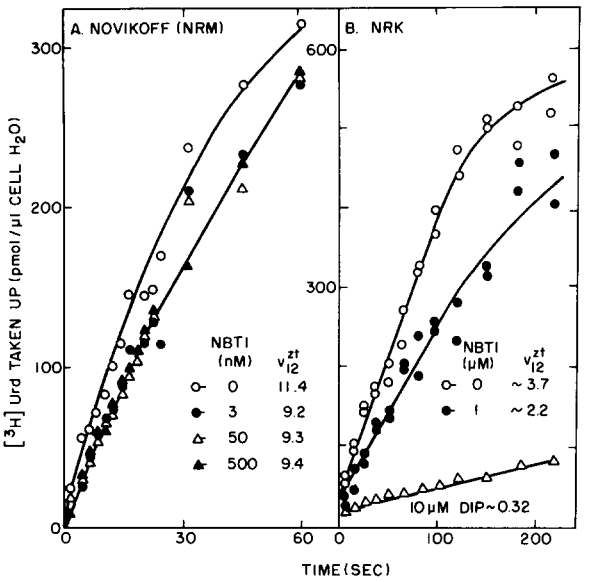

Fig. 1. Effect of NBTI and dipyridamole (DIP) on zero-*trans* influx of uridine in Novikoff (NRM) (A) and NRK (B) cells. (A) Samples of a suspension of $1.5 \cdot 10^7$ NRM cells/ml of BM42B were supplemented with 0, 0.3, 1, 3, 10, 50, 150 and 500 nM NBTI. Then, the time-course of uptake of 500 µM [^{3}H]uridine (1 cpm/pmol) was measured at 25°C and the initial velocities (v_{12}^{zt} in pmol/µl cell water per s) were computed by integrated rate analysis of the data as described under Experimental procedures. Only representative time-courses of uptake are shown. The v_{12}^{zt} values for the treated cells are summarized in Fig. 2 as percent of control. (B) The uptake of 500 µM [^{3}H]uridine (11 cpm/pmol) was measured in confluent cultures of NRK cells at 25°C as described under Experimental procedures. NBTI and dipyridamole were added to the indicated final concentrations simultaneously with substrate. v_{12}^{zt} was estimated graphically from the initial linear phase of uptake.

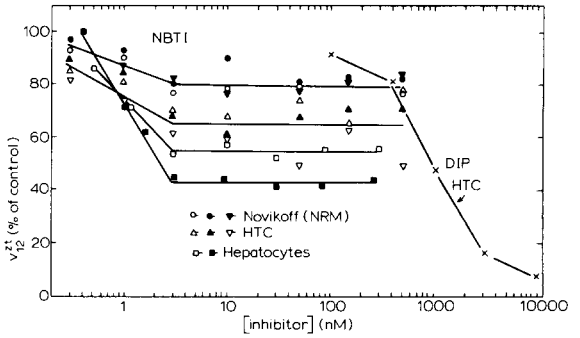

Fig. 2. Zero-*trans* uridine influx in various types of rat cells as a function of NBTI and dipyridamole (DIP) concentrations. The experiments were conducted as described in the legend to Fig. 1A and under Experimental procedures. The means of the v_{12}^{zt} values for the untreated controls of the different types of cells ± S.E. are listed in Table III (column 3).

tively high number of NBTI-binding sites (Table I). Between 30–50% of uridine transport in HTC hepatoma cells was NBTI-sensitive, and the same was true for NRK cells. Uridine transport in NRK cells was measured in most experiments in monolayer cultures (Fig. 1B), but in one experiment the cells were removed from the culture vessels by treatment with trypsin and EDTA and the suspended cells were assayed for uridine transport. The transport velocity and its reduction by NBTI were comparable to those obtained with monolayer cultures (data not shown).

As reported by Jarvis et al. [23], uridine transport in rat erythrocytes was relatively slow (Table II). In one batch of cells it was not significantly inhibited by NBTI up to concentrations of 500 nM (expt. 1), but it was inhibited 10–15% in two other batches of cells (expts. 2 and 3). The reasons for this difference between these batches of red cells, each of which was obtained from 2–3 rats, are unclear, but the absence and presence of NBTI-sensitive uridine transport correlated with the absence and presence of a low number (about 600/cell) of high-affinity NBTI-binding sites on the cells (Table I). The number of binding sites is similar to that reported by Jarvis et al. [23] for rat

TABLE II

EFFECT OF NBTI ON URIDINE TRANSPORT IN RAT AND HUMAN ERYTHROCYTES

The zero-*trans* influx of 500 µM uridine was measured as described under Experimental procedures. Expts. 1–3 represent the same pools of red cells which were assayed for NBTI binding (Table I). n.d., not determined.

NBTI (nM)	v_{12}^{zt} (pmol/µl H_2O per s)			
	Rat red cells			Human red cells
	Expt. 1	Expt. 2	Expt. 3	
0	0.72 ± 0.05	0.86 ± 0.04	0.81 ± 0.05	22.0
5	0.71 ± 0.04	0.76 ± 0.03	0.79 ± 0.08	14.1
50	0.81 ± 0.04	0.71 ± 0.04	n.d.	1.3
150	0.71 ± 0.07	n.d.	n.d.	1.1
500	0.88 ± 0.07	0.66 ± 0.04	0.68 ± 0.03	n.d.

red cells. To ascertain that the low levels of NBTI-sensitive uridine transport and high-affinity binding sites were not attributable to other cells contaminating the red cell suspensions, in expt. 3 the red cells were carefully freed of lymphocytes by centrifugation through Ficoll-Hypaque, but without apparent effect. Neither significant nonspecific nor high-affinity NBTI binding was detected on isolated lymphocytes at the cell density tested ($2 \cdot 10^7$ cell/ml) which was limited by the yield of these cells from three rats. This result indicates that the high-affinity NBTI binding of the rat red cells was not attributable to contaminating lymphocytes. The presence of high-affinity NBTI-binding sites on red cells was also confirmed by binding assays with unsealed white ghosts. The binding K_d was about the same as that observed for intact cells, but the number of apparent binding sites/ghost, based on a 100% recovery of ghosts, was lower than for intact cells. We deem it unlikely that this difference is due to an incomplete recovery of ghosts, since in the preparation of ghosts from human red cells using the same procedure we recover approx. 100% of the NBTI-binding capacity of intact cells (unpublished data). We consider it more likely that the number of binding sites for intact rat erythrocytes is overestimated, because nonspecific binding of NBTI limits the precision with which low levels of high-affinity NBTI binding can be measured. Ghosts bind very little NBTI nonspecifically (Table I and Ref. 23), and we therefore consider the low number of high-affinity NBTI sites for ghosts a more accurate estimate than that obtained for intact cells. In contrast to rat erythrocytes, practically all uridine transport in human erythrocytes, included for comparison, was NBTI-sensitive (Table II), and these cells possess approx. $1 \cdot 10^4$ high-affinity NBTI-binding sites/cell [5,7,23,27].

Although all rat cells exhibited some NBTI-sensitive transport, its proportion of total transport and the number of NBTI-binding sites were considerably lower than observed for various lines of mouse cells [1,2]. However, as reported for other types of cells [2], both NBTI-sensitive and resistant uridine transport in rat cells was inhibited by dipyridamole. This is illustrated for NRK and HTC cells in Figs. 1B and 2, respectively. Furthermore, all available evidence suggests that both NBTI-sensitive and resistant uridine transport exhibit similar sensitivity to inhibition by dipyridamole, although slight differences may exist [2]. Their kinetic parameters also differ only slightly [2]. The Michaelis-Menten constants for zero-*trans* entry of uridine by HTC cells (171 ± 9 and 183 ± 29 μM), NRK cells (214 ± 25 μM) and Novikoff (NRM) cells (164 ± 8 μM) were also in the same range as reported for other types of cells [2,19].

In addition, all available evidence indicates that NBTI-sensitive nucleoside transport in all types of cultured cells exhibits similar properties whether it makes up a low or high proportion of the total transport capacity of the cells [1,2]. This conclusion is further supported by the data in Fig. 3 which shows that high-affinity NBTI binding, which correlates with NBTI-sensitive transport, was inhibited in a similar dose-dependent manner by dipyridamole in Novikoff rat hepatoma (NRM) cells (about 20% NBTI-sensitive transport), Chinese hamster ovary cells (CHO; about 65% NBTI-sensitive transport) and mouse leukemia P388 cells (about 90% NBTI-sensitive transport), except that at higher concentrations of dipyridamole inhibition leveled-off to different degrees in the three cell lines. Inhibition of NBTI binding by dipyridamole, when analyzed at low dipyridamole concentrations, has been found to be of an apparent competitive nature [4,24], but the leveling-off of the inhibition at high dipyridamole concentrations is inconsistent with a competitive binding to a common substrate-binding site. These results and the finding that dipyridamole inhibits the dissociation of NBTI from high-affinity binding sites [28,29] support the view that NBTI and dipyridamole (and other substrates structurally unrelated to nucleosides) interact with the nucleoside transporter in different ways [24,28,29].

Table III summarizes our results for uridine transport and NBTI binding in rat cells and includes, for comparison, data for some mouse and hamster cell lines and human erythrocytes. The data reveal one striking difference between the normal rat cells (NRK, hepatocytes and erythrocytes) and the transformed rat cells (NRM and HTC). Although the transport capacity expressed on a per cell basis of the normal cells, except for erythrocytes, was similar (column 3) to that of the transformed rat cells, these normal cells possessed

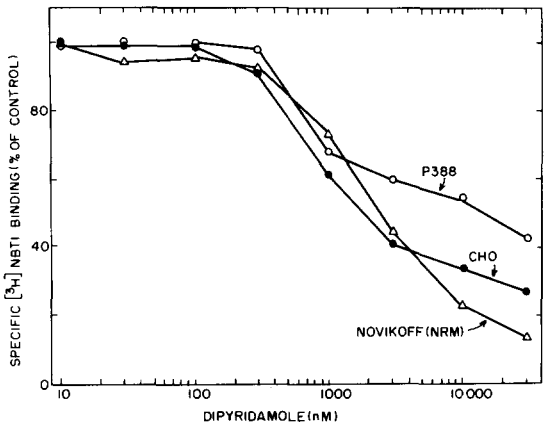

Fig. 3. Concentration-dependent inhibition by dipyridamole of NBTI binding by various cell lines. Equilibrium binding of 0.8 nM [^{3}H]NBTI in the presence of the indicated concentrations of dipyridamole or 1 μM unlabeled NBTI (to estimate nonspecific binding) was measured as described in Experimental procedures. The final cell densities per ml were $1.45 \cdot 10^7$ P388 cells, $7.5 \cdot 10^6$ CHO cells and $1.7 \cdot 10^7$ Novikoff (NRM) cells. At equilibrium, control cells of these lines had bound, respectively, 57, 70 and 22% of the total NBTI to high-affinity binding sites and 35, 18 and 53% nonspecifically.

a much higher number of high-affinity NBTI-binding sites per cell than did the transformed cells (column 5). On the basis of these values, the volume of the cells (column 2) and assuming that each high-affinity NBTI-binding site represents a single nucleoside carrier, we calculated that the turnover number for the NBTI-sensitive carriers of the transformed cells was 5–10-times higher than that of the carriers of the normal cells (column 6). The turnover numbers are a function of three quantities, each measured independently, and each containing measurable uncertainties as quantitated by the standard errors of the means. The value calculated from the pooled data for nontransformed rat cells (NRK, hepatocytes and erythrocytes) is significantly different, in a statistical sense, from that for transformed rat cells (NRM and HTC). However, additional work is required to determine whether this difference pertains to other cell systems. The carrier turnover numbers for transformed rat cells are comparable to those of the mouse leukemia L1210 and P388 lines and even less than that for the human red cell carrier, but the turnover numbers for the NBTI-sensitive carriers of L929 and CHO cells fell between these extremes.

We have no explanation for the apparent differences in catalytic efficiency of the carriers from different cells. One possibility is that in some types of cells, including rat hepatocytes and NRK cells, many of the carriers quantitated by high-affinity NBTI binding may be nonfunctional, perhaps because they are associated with internal membranes rather than the plasma membrane. Because of its high lipid solubility, NBTI equilibrates across membranes within a few seconds and thus internal carriers, though nonfunctional in transport, should be readily accessible to NBTI in equilibrium binding. The relatively low carrier turnover calculated for rat red cells seems to contradict this view, since these cells lack internal membranes. However, there is some uncertainty as to the exact number of high-affinity binding sites on these cells. If carrier turnover for these cells is calculated on the basis of the number of binding sites estimated for ghosts (Table I), it will fall in the same range as estimated for some of the cell lines (43 molecules/carrier per s). We have no information at present on the distribution of the nucleoside carrier between plasma membrane and internal membranes of the cultured cells or hepatocytes. However, substantial proportions of the sugar transporters of rat adipocytes and diaphragm have been reported to be associated with internal membranes [31–33].

We also have no direct information on the molecular structure of high-affinity NBTI-binding sites, but all evidence indicates that binding of NBTI to these sites results in transport inhibition. Other remaining questions concern the factors that regulate the expression of the high-affinity binding sites, and thus the proportion of NBTI-sensitive to resistant nucleoside transport in cells. As shown in this paper, the majority of the nucleoside carriers of rat cells are present in the NBTI-resistant form, and some rat cells only express this form. This property, however, seems not to be invariable as indicated by the isolation of Novikoff cell variants with a significant number of high-affinity NBTI-binding sites, but whether these changes are regulated by genetic, epigenetic or environmental factors is not known. Whenever cells express NBTI-resistant and sensitive carriers both seem to be present on single cells, and they are indistinguishable on the basis of several other criteria [2].

It has been shown that co-administration of

TABLE III

SUMMARY OF PARAMETERS OF NBTI BINDING AND TURNOVER OF NBTI-SENSITIVE NUCLEOSIDE CARRIER OF VARIOUS TYPES OF RAT CELLS, OF VARIOUS MOUSE AND HAMSTER CELL LINES AND OF HUMAN ERYTHROCYTES

Values for cell volume, v_{12}^{zt} and NBTI-binding sites/cell for the rat cells are for the data presented in Table I and Fig. 2. In three experiments with rat erythrocytes, three experiments with hepatocytes and two experiments with NRM cells, uridine influx and NBTI binding were measured in the same population of cells. All other values are for separate cell populations. Values for human red cells have been calculated from data presented in Ref. 7 and from unpublished work. Those for P388, L1210, L929-2 and CHO cells have been calculated from data presented in Refs. 2 and 30. All values for cell volume, v_{12}^{zt} and NBTI-binding sites/cell are means ± S.E. calculated from the number of experiments indicated in parentheses. The standard errors associated with turnover number were computed according to Ref. 26:

$$\frac{S_v}{\bar{v}} = \sqrt{\left(\frac{S_x}{\bar{x}}\right)^2 + \left(\frac{S_y}{\bar{y}}\right)^2 + \left(\frac{S_z}{\bar{z}}\right)^2}$$

where x, y and z correspond to values in columns 2, 3 and 5 of the table.

Cell type	Cell volume ($\mu l/10^6$ cells)	v_{12}^{zt} (pmol/10^6 cells per s) [a]	NBTI-sensitive transport (% of total)	NBTI-binding sites/cell ($\times 10^{-3}$)	Carrier turnover (molecules/carrier per s) [a]
Novikoff (NRM)	1.6 ±0.03 (3)	17.7 ±0.6 (12)	≈ 20	26 ± 1.8 (4)	82 ± 16
HTC	3.4 ±0.2 (3)	17.8 ±3.3 (3)	≈ 40	42 ± 2.9 (4)	102 ± 22
NRK	3.0 (1)	13.1 ±0.8 (5)	≈ 35	293 ± 63 (3)	9.4 ± 2.1
Hepatocytes	5.1 ±0.1 (3)	29.9 ±2.2 (3)	≈ 60	767 ±111 (3)	14.1 ± 2.3
Rat erythrocytes	0.066 ± 0.03 (3)	0.053 ± 0.003 (3)	≈ 15	0.66 ± 0.10(2)	7.3 ± 3.5
P388	0.99 ±0.05(17)	36 ±3 (8)	≈ 90	240 ±100 (2)	81 ± 35
L1210	1.32 ±0.12(10)	47 ±2 (7)	≈ 85	240 ± 25 (2)	100 ± 14
L929-2	1.96 ±0.20(11)	68 ±6 (6)	≈ 85	690 ± 40 (2)	50 ± 7.4
CHO	2.13 ±0.10(30)	36 ±1 (14)	≈ 65	500 ± 75 (2)	28 ± 4.5
Human erythrocytes	0.171 ± 0.06(18)	8.0 ±0.9 (11) [b]	≈ 100	15 ± 1 (7)	320 ±120

[a] At 500 μM uridine, which approaches maximum velocity. Turnover is calculated for NBTI-sensitive carriers assuming that each high-affinity binding site represents a single carrier and is for 25°C.

[b] v^{ec} at 500 μM uridine which is higher than v_{12}^{zt} because of the differential mobility of loaded and empty carrier in human red cells [7,19].

NBTI and its monophosphate (NBTI-P) lowers the toxicity of various nucleoside analogs to mice [34]. Thus, by allowing the administration of potentially lethal doses of these drugs, it increased the efficacy of chemotherapy against several transplantable mouse leukemias, perhaps by selectively inhibiting the uptake of these drugs by normal host cells [35,36]. It has therefore been proposed that such co-administration of NBTI or NBTI-P with toxic nucleosides might be of general usefulness in cancer chemotherapy. However, the finding that nucleoside transporters of both transformed and normal cells of species other than the mouse, including humans, exist to a large extent in the NBTI-resistant form raises questions as to the applicability of this approach to other species.

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References

1 Belt, J.A. (1983) Mol. Pharmacol. 24, 479–484
2 Plagemann, P.G.W. and Wohlhueter, R.M. (1984) Biochim. Biophys. Acta 773, 39–52
3 Wohlhueter, R.M., Marz, R. and Plagemann, P.G.W. (1979) J. Membrane Biol. 42, 247–264
4 Paterson, A.R.P., Lau, F.Y., Dahlig, E. and Cass, C.E. (1980) Mol. Pharmacol. 18, 40–44

5 Cass, C.E., Gaudette, L.A. and Paterson, A.R.P. (1974) Biochim. Biophys. Acta 345, 1–10
6 Cass, C.E. and Paterson, A.R.P. (1976) Biochim. Biophys. Acta 419, 285–294
7 Plagemann, P.G.W. and Wohlhueter, R.M. (1984) Biochim. Biophys. Acta 778, 176–184
8 Cohen, A., Ullman, B. and Martin, D.W., Jr. (1979) J. Biol. Chem. 254, 112–118
9 Cass, C.E., Kolassa, N., Uehara, Y., Dahlig-Harley, E., Harley, E.R. and Paterson, A.R.P. (1981) Biochim. Biophys. Acta 649, 769–777
10 Lauzon, G.J. and Paterson, A.R.P. (1977) Mol. Pharmacol. 13, 883–891
11 Dahlig-Harley, E., Eilam, Y., Paterson, A.R.P. and Cass, C.E. (1981) Biochem. J. 200, 295–305
12 Plagemann, P.G.W. and Swim, H.E. (1966) J. Bacteriol. 91, 2317–2326
13 Plagemann, P.G.W. and Wohlhueter, R.M. (1982) Biochim. Biophys. Acta 688, 505–514
14 Coffino, P., Baumal, R., Laskov, R. and Scharff, M.D. (1972) J. Cell. Physiol. 79, 429–440
15 Seglen, P.O. (1976) Methods Cell. Biol. 13, 29–83
16 Dalet, C., Fehlman, M. and Debey, P. (1982) Anal. Biochem. 122, 119–123
17 Mishell, B.B. and Shiigi, S.M. (1980) Selected Methods in Cellular Immunology, pp. 24–25, W.H. Freeman and Co., San Francisco
18 Steck, T.L. (1974) Methods Membrane Biol. 2, 245–281
19 Plagemann, P.G.W. and Wohlhueter, R.M. (1980) Curr. Top. Membrane Trans. 14, 225–330
20 Wohlhueter, R.M., Marz, R., Graff, J.C. and Plagemann, P.G.W. (1978) Methods Cell. Biol. 20, 2111–236
21 Wohlhueter, R.M. and Plagemann, P.G.W. (1982) Biochim. Biophys. Acta 689, 249–260
22 Plagemann, P.G.W., Wohlhueter, R.M. and Erbe, J. (1982) J. Biol. Chem. 257, 12069–12074
23 Jarvis, S.M., Hammond, J.R., Paterson, A.R.P. and Clanachan, A.S. (1983) Biochem. J. 208, 83–88
24 Wohlhueter, R.M., Brown, W.E. and Plagemann, P.G.W. (1983) Biochim. Biophys. Acta 731, 168–176
25 Dietrich, O.W. and Rothmann, O.S. (1975) Keyboard (Hewlett-Packard) 7, 4–7
26 Bevington, P.R. (1969) Data Reduction and Error Analysis for the Physical Sciences, McGraw-Hill, New York
27 Jarvis, S.M., Hammond, J.R., Paterson, A.R.P. and Clanachan, A.S. (1983) Biochem. J. 210, 457–461
28 Jarvis, S.M., Janmohamed, S.N. and Young, J.D. (1983) Biochem. J. 216, 661–667
29 Koren, R., Cass, C.E. and Paterson, A.R.P. (1983) Biochem. J. 216, 299–308
30 Plagemann, P.G.W. and Wohlhueter, R.M. (1984) Arch. Biochem. Biophys. 233, 489–500
31 Kono, T., Suzuki, K., Dansey, L.E., Robinson, F.W. and Belvins, T.L. (1981) J. Biol. Chem. 256, 6400–6407
32 Karnieli, E., Zarnowski, M.J., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981) J. Biol. Chem. 256, 4772–4777
33 Wardzala, L.J. and Jeanrenaud, B. (1981) J. Biol. Chem. 256, 7090–7093
34 Paterson, A.R.P., Paran, J.H., Yang, S.-E. and Lynch, T.P. (1981) Cancer Res. 39, 3607–3611
35 Lynch, T.P., Jakobs, E.S., Paran, J.H. and Paterson, A.R.P. (1981) Cancer Res. 41, 3200–3204
36 Lynch, T.P., Paran, J.H. and Paterson, A.R.P. (1981) Cancer Res. 41, 560–565