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Sodium-dependent and inhibitor-insensitive uptake of adenosine by mouse peritoneal exudate cells

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[8-^{3}H]Adenosine uptake in mouse peritoneal exudate cells, harvested following i.p. challenge with Complete Freund's Adjuvant from BALB/c mice, was found to be insensitive to common nucleoside transport inhibitors such as dilazep or 6-[(4-nitrobenzyl)mercapto]purine ribonucleoside and to require sodium ion, being inactive when sodium was replaced by lithium or potassium. These findings also applied to the adherent (macrophages) and nonadherent (polymorphonuclear cells) cell fractions prepared from the peritoneal cell mixture. Uptake was inhibited by several nucleosides including deoxyadenosine, inosine, uridine, thymidine and, to a lesser extent, by the adenosine analog tubercidin, while adenine, fructose, glucose and ribose were without effect. Uptake [8-^{3}H]adenosine was fully matched by rapid intracellular phosphorylation to AMP, ADP and ATP. Inosine was a substrate for the transporter, but tubercidin was not. The system clearly is distinct from carrier-mediated, nonconcentrative transport and has similarities to concentrative, sodium-dependent nucleoside transporters described in other cell types.

Introduction

The most widely studied mechanism of nucleoside transport across cellular membranes is that of carrier-mediated, facilitated diffusion. A specific transporter system appears to exist in membranes which exhibits structure-activity selectivity for nucleoside permeants. The process is non-concentrative and bidirectional, depending on the direction of the permeant concentration gradient, without any evidence of energy requirements on part of cells (cf. Refs. 1 and 2). This mediated, facilitated transport of nucleosides can be effectively inhibited by a number of drugs including nucleoside derivatives such as NBMPR and others including dipyridamole, dilazep or mioflazine derivatives, all of which are structurally dissimilar from nucleosides and exhibit prominent vasodilator activity [1–5]. A mediated but NBMPR-insensitive transport system also has been described but its physiological significance and distribution in normal tissues is not clear, having been observed largely in several cultured and transformed cell lines [6] but also in rat erythrocytes (cf. Refs. 2 and 6).

More recently it has been observed that in some cell types nucleosides also can be transported by a sodium-dependent, concentrative mechanism operating against a transmembrane concentration gradient, including in hepatocytes [7], choroid plexus [8,9], intestinal epithelial cells [10,11] and a derived cell line [12] as well as in functionally undefined splenocytes [13,14]. Contrasting with facilitated diffusion, this sodium-dependent transport of nucleosides is not inhibitable by the above drugs. Structure-activity studies with different nucleosides as inhibitors also have demonstrated that such sodium dependent transport systems are diverse [13] and that in one cell type more then one transporter with differing specificity for permeants may exist [11], although it remains to be established which the physiologically significant permeants actually are.

Our interest in the use of cytotoxic nucleosides as antiparasitic drugs, including against leishmaniasis, led us to investigate the nucleoside transport characteristics of macrophages, the resident cells of *Leishmania* amastigotes. Using mouse peritoneal exudate cells and cellular fractions thereof as a model, we made the unexpected observation that the apparent transport of adenosine in these cells was insensitive to the common transport inhibitors NBMPR and dilazep and that it

Abbreviation: NBMPR, 6-[(4-nitrobenzyl)mercapto]purine riboside.

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required sodium ion. This finding suggests that Na^+-dependent, probably active nucleoside transport is operative in different immune cell types and thus raises interesting questions regarding its physiological significance.

Methods and Materials

Cell isolation

Twenty BALB/c mice were injected i.p. with 0.2 ml Complete Freund's Adjuvant and killed by cervical dislocation after three days. Then 10 ml of transport medium (see below) was injected into the peritoneal cavity and withdrawn. The medium was centrifuged at $350 \times g$ for 10 min and the supernatant discarded. Erythrocyte lysing medium (0.83% NH_4Cl, 0.75 ml) was added to the cell pellet and after 3–5 min the cells were suspended in 10 ml transport medium, centrifuged and washed twice with the same medium. The final pellet was suspended in 2–4 ml medium to obtain a count of $4 \cdot 10^7$ cells/ml (determined by hemocytometer).

Cells isolated and prepared in this manner are referred to throughout this manuscript as 'peritoneal (exudate) cells' and contained about 50% monocytes (macrophages) besides polymorphonuclear cells based on microscopic inspection. These were separated for some studies into adherent and nonadherent cells as follows.

10-ml portions of peritoneal cell suspensions were added to Petri dishes (Corning 100×20 mm, polystyrene) and incubated for 2.5 h at 37 °C in an atmosphere of 5% CO_2 and 97% relative humidity. Then nonadherent cells were collected by removing the medium from dishes and centrifugation. After rinsing dishes with 10 ml medium, the adherent cells were gently detached by a rubber scraper, suspended in medium and concentrated by centrifugation. Based on the original cell number, about 20% of cells were recovered in the adherent and 40% in the nonadherent cell fraction. Cell composition in the fractions was assessed by microscopic examination after Giemsa staining. The adherent cells contained 88% monocytes, and the nonadherent cells, representing mostly polymorphonuclear cells, 6% monocytes.

In some experiments, erythrocytes were removed from the peritoneal cell mixture by centrifugation through a layer of Ficoll-Paque. About 10^8 cells in 5 ml medium were layered gently on 4 ml of Ficoll-Paque and centrifuged at $700 \times g$ for 25 min. Cells accumulating at the interface were collected and washed twice with medium before use in transport assays.

Mouse blood was drawn by cardiac puncture and erythrocytes were obtained by repeated washing in transport medium and removal of buffy coats [15].

Transport assays

Adenosine transport measurements were conducted essentially as described by Paterson et al. [16] with modifications. Assays were carried out in a transport medium (Basal Medium Eagle, buffered with 10 mM Na-Hepes, pH 7.4), and all component stock solutions were made up in this medium unless otherwise indicated. Before dispensing component stock solutions, 50 µl dibutyl phthalate was added to the assay tubes (1.5 ml polypropylene Eppendorf tubes). [8-^{3}H]Adenosine (approx. 100000 dpm) was 1 µM and the number of cells per assay was approx. $2 \cdot 10^6$ (peritoneal cells) or $2 \cdot 10^7$ (erythrocytes). Assay volumes were 200 µl, and incubations were at 22 °C. Reactions were stopped by the addition of 200 µl of 5 mM unlabeled adenosine followed by rapid centrifugation at $14000 \times g$ in an Eppendorf 5141 microcentrifuge for 20 s. Subsequently, further dibutyl phthalate (400 µl) was added to tubes without disturbing the already present oil layer separating cell pellet from medium. The following steps of medium removal, washing and determining the radioactivity content of the cell pellet were performed as previously detailed [15]. Assays with mouse erythrocytes were conducted under essentially identical conditions. Unless otherwise stated assays were limited to duplicates, considering their excellent reproducibility and the preciousness of the preparations. Transport of inosine was measured by first treating an [8-^{3}H]adenosine stock solution with a small quantity of adenosine deaminase, fully transforming adenosine to inosine. Tubercidin transport was measured in the same manner using [G-^{3}H]tubercidin as permeant.

Transport rates were calculated and expressed on a cellular basis, i.e., as 'mol permeant/cell per s [15]. The average intracellular water space was determined for the mixed cell preparation using [^{3}H]H_2O as a marker, with correction for extracellular H_2O in the cell pellet using [^{3}H]inulin as a marker, and found to be 0.3 pl. Thus a rate of $1 \cdot 10^{-20}$ mol/cell per s corresponds to approximately 0.03 pmol/µl intracellular water per s.

Adenosine metabolism

Incubation conditions were identical to those used in transport assays, except for using a three times higher level of specific radioactivity. After incubation and stopping reactions by addition of unlabeled adenosine, the entire volumes were rapidly transferred to vials containing 400 µl dibutyl phthalate underlayed with 35 µl 3 M perchloric acid and immediately centrifuged. The overlaying medium was aspirated and a portion of it saved for metabolite analysis, followed by two washes with 1 ml water and aspiration of the oil. To the acid underlay a titrated volume of 3 M KOH was added to neutralize the perchloric acid. The tubes were left on ice for 0.5 h, and following centrifugation the supernatants were collected for metabolite analysis by HPLC. A volume of 20 µl of each sample supernatant as well as of overlaying medium was diluted with 10 µl of carrier solution (containing about 0.17 mM each of ATP, ADP,

AMP, inosine and adenosine and 0.08 mM hypoxanthine). 20 μl of these mixtures was subjected to reverse phase HPLC on a Lichrosorb Hibar stainless steel column (250 × 4 mm, 5 μm particle size, RP-Select B type, E. Merck, Darmstadt, F.R.G.) and an LKB HPLC system equipped with a photodiode array Rapid Spectral Detector. The mobile phase was 0.3 mM $NH_4H_2PO_4$ with 2% methanol and 0.4% tetrahydrofuran (pH 4.0) at a flow rate of 0.7 ml/min. UV peaks identified by markers were collected and counted by liquid scintillation counting with quench correction.

Ion-defined saline media

Taking the measured osmolality of the above transport medium (240 mosmolal) as a reference, solutions of NaCl, KCl and LiCl, each with 10 mM Hepes buffer (pH 7.4), were prepared and adjusted to the same osmolality. All component stock solutions were made up in the respective saline solution and peritoneal cells or erythrocytes were suspended and washed in these solutions twice before using in respective transport assays.

Materials

Dilazep (*N*,*N*′-bis[3-(3,4,5-trimethoxybenzoyloxy)-propyl]homopiperazine) was gift from F. Hoffmann-LaRoche Co., Basel, Switzerland. NBMPR was a gift from Dr. A.R.P. Paterson, Cancer Research Unit, University of Alberta, Edmonton, Canada. [8-^{3}H]Adenosine (31 Ci/mmol) and [G-^{3}H]tubercidin (20 Ci/mmol) were purchased from Moravek Biochemicals, Brea, CA, U.S.A., and [^{3}H]inulin (1.7 Ci/mmol) and [^{3}H]H_2O from Amersham International, Buckinghamshire, U.K. Nucleosides were obtained from Boehringer Mannheim Corp., Mannheim, F.R.G., or from Sigma Chemical Co., St. Louis, MO, U.S.A. Complete Freund's Adjuvant was purchased from Calbiochem Behring Diagnostics, LaJolla, CA, U.S.A., and Ficoll-Paque from Pharmacia-LKB Biotechnology Inc., Pisataway, NJ, U.S.A.

Results

When attempting to measure [8-^{3}H]adenosine transport in peritoneal exudate cells, we noted that the process of intracellular accumulation of label continued despite the addition of dilazep (Fig. 1, Panel A) commonly used to rapidly terminate the process of facilitated diffusion of nucleosides into mammalian cells. Similar results were obtained with the transport inhibitor NBMPR (data not shown), indicating that these compounds were ineffective as stopping agents in these cells and suggesting the presence of an inhibitor insensitive transport or uptake process. Accordingly, an alternative method to terminate transport, such as addition of a high concentration of unlabeled permeant, was

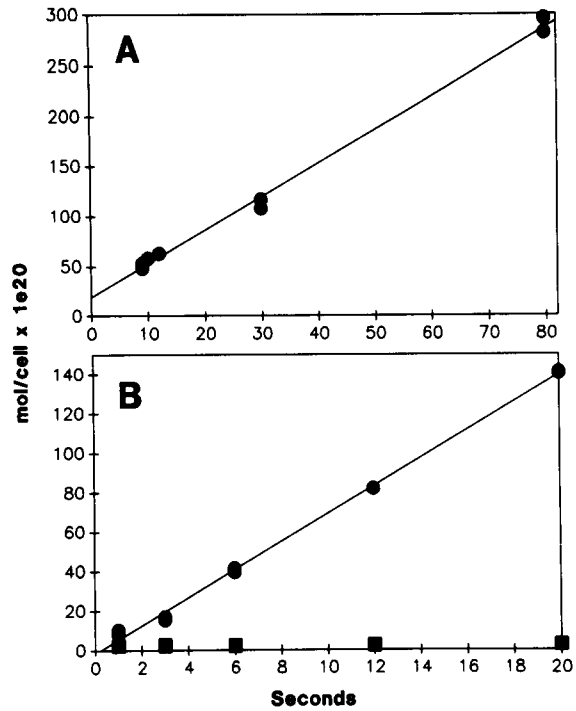

Fig. 1. Time dependence of [8-^{3}H]adenosine uptake by peritoneal cells. (Panel A) Continued transport or uptake in the presence of dilazep: 200 μl 'stop' solution containing 0.3 mM dilazep was added simultaneously with cells to assay tubes, and centrifugation through oil layer was carried out at the times indicated on the abscissa. (Panel B) Using 5 mM unlabeled adenosine stop solution to terminate transport of [8-^{3}H]adenosine. Stop solution was added at time intervals (●) to terminate the uptake process, as well as at zero time (■) with centrifugation being carried out at time intervals as in the experiment shown in panel A.

explored and subsequently used in all studies reported below. As evident in panel B of Fig. 1, the addition of 200 μl 5 mM unlabeled adenosine in place of dilazep or NBMPR was effective in preventing any significant cellular accumulation of radioactive material and thus could be used to stop the apparent uptake or transport process and follow it with precise timing.

Fig. 1 (Panel B) indicated that [8-^{3}H]adenosine uptake in peritoneal cells was linear over the 20 s time period, but repetitions of this type of experiment showed that the time period of linearity extended to at least 60 s, the slope of time curves then being reduced gradually by about 20% during the subsequent period of 1–5 min (data not shown). From the initial slopes of various time course experiments as well as fixed time studies, apparent rates of [8-^{3}H]adenosine (1 μM) uptake or transport could be calculated, yielding values ranging between $5 \cdot 10^{-20}$ and $9 \cdot 10^{-20}$, corresponding to about 0.15–0.27 pmol/μl intracellular water per s.

In none of the time course studies, some of which also included earlier sampling points of 2–5 s of incubation, was there evidence of any initial curvature with an initial, more rapid phase of transport or uptake reported for several other cell types (cf. Refs. 2–4) and

TABLE I

Metabolism of [8-^{3}H]adenosine during transport assays

The distribution of [8-^{3}H]adenosine-derived radioactivity in metabolites in supernatant medium and cell pellet after different incubation times of peritoneal exudate cells under conditions of transport assays was measured.

Sample and time point	Percent radioactivity [a]					
	ADO	INO	HX	AMP	ADP	ATP
Supernatant medium						
Zero time	96	1	3	<1	<1	<1
10 s	96	1	2	<1	<1	<1
20 s	93	2	3	<1	1	1
30 s	93	2	2	<1	2	1
60 s	93	2	3	<1	2	1
Cell pellet						
10 s	3	3	2	4	28	60
20 s	4	1	2	4	28	62
30 s	1	3	2	6	40	51
60 s	1	2	3	6	42	46

[a] Adenosine (ADO); inosine (INO); hypoxanthine (HX).

interpreted to indicate a decrease of the transmembrane concentration gradient due to intracellular accumulation of free permeant. Straight line fits to data points always were highly significant and extrapolations to zero time yielded intercepts on the ordinate which were identical for time courses of uptake and control (the latter with unlabeled adenosine present throughout).

Metabolism of [8-^{3}H]adenosine

The metabolism of [8-^{3}H]adenosine was assessed by HPLC analysis of radioactive materials accumulating in cells as well as remaining in the supernatant medium following different periods of incubation. As shown in Table I, essentially all radioactivity within cells was associated with the nucleotides ATP, ADP and AMP beginning with the earliest sampling point of 10 s of incubation. Radioactivity present in the supernatant was fully associated with adenosine, with no indication of increased formation or accumulation of metabolites such as inosine or hypoxanthine at any time point. It should be noted that analysis of extracellular radioactivity distribution, i.e., in the medium, did not allow any assessment of intracellular metabolism of [8-^{3}H]adenosine but only served to determine any possible gross metabolism of the permeant due, for example, to enzymes leaking from cells. We calculated that if the equivalent of 10% of intracellularly accumulated nucleotides were in the form of metabolites (inosine, hypoxanthine) and fully leaked from cells, that after about 20 s of incubation this would represent less then 0.25% of the label present in the medium under the experimental conditions used.

Effect of inhibitors or competing nucleosides

To assess whether the inhibitor insensitive uptake of [8-^{3}H]adenosine represented a specific process of nucleoside transport, the effect of various nucleosides, sugars and the nucleobase adenine were determined. As evident from Fig. 2, substantial, concentration dependent inhibition of [8-^{3}H]adenosine uptake was seen only with nucleosides (adenosine, deoxyadenosine, inosine and tubercidin), while the nucleobase adenine and sugars (glucose, ribose, fructose) essentially remained without any effect. In additional experiments (data not shown), uridine and thymidine also were found to be highly effective as inhibitors of [8-^{3}H]adenosine uptake in peritoneal cells. For comparison, Fig. 2 shows the effects of these compounds on the uptake of [8-^{3}H]adenosine in mouse erythrocytes. Except for some quantitative differences, the results were similar to those with peritoneal cells. Thus the free sugars were inactive, but adenine showed a small inhibitory effect in erythrocytes. The nucleosides appeared to be more potent inhibitors in peritoneal cells, with the exception of the

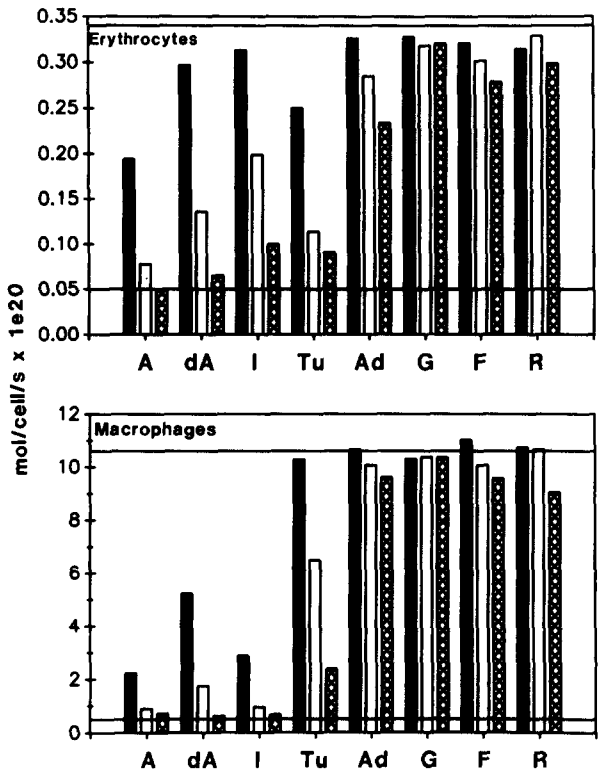

Fig. 2. Effect of various compounds on adenosine uptake (over 10 s) in erythrocytes and peritoneal cells, including unlabeled adenosine (A), deoxyadenosine (dA), inosine (I), tubercidin (Tu), adenine (Ad), glucose (G), fructose (F), and ribose (R). Concentrations were 25 μM (closed bars), 250 μM (open bars) and 2550 μM (hatched bars). The solid lines across bar graphs represent: total transport rates in the absence of drug additions (upper lines) and maximal inhibition of uptake by 0.25 mM dilazep (erythrocytes) and 1.25 mM unlabeled adenosine (peritoneal cells) (lower lines). Means of duplicate assays are shown, and individual values differed by less than 12% of each other.

analog tubercidin which was more potent in erythrocytes.

The lack of any significant effects of inhibitors of facilitated diffusion such as dilazep and NBMPR (10–25 μM) on [8-^{3}H]adenosine uptake was confirmed repeatedly in peritoneal cells, while control experiments confirmed that these drugs completely inhibited transport in mouse erythrocytes.

Transport or uptake of other nucleosides

The question of whether some of the inhibitory nucleosides served as substrates of the transporter in peritoneal cells was investigated with inosine and tubercidin as permeants. Thus [8-^{3}H]inosine was found to be taken up at about the same rate as [8-^{3}H]adenosine and without any inhibitory effect of NBMPR (data not shown). However, labeled tubercidin was not significantly taken up (cf. Fig. 3) by peritoneal cells although being transported by mouse erythrocytes at a rate comparable to that of [8-^{3}H]adenosine (data not shown). The uptake or transport of inosine (in peritoneal cells) and tubercidin (in erythrocytes) was linear throughout the experimental time period of 20 s.

Ion requirements

In order to investigate the ion requirement of the inhibitor insensitive [8-^{3}H]adenosine uptake in peritoneal cells, it was studied in the presence of saline media where sodium was replaced fully either by potassium or lithium, and in the presence and absence of 10 μM NBMPR in each case. Again, the effects of using different saline media on [8-^{3}H]adenosine uptake were compared in peritoneal cells and erythrocytes. Results of this experiment are shown in Fig. 3. It is evident that after replacing sodium with lithium or potassium ion the uptake of [8-^{3}H]adenosine in peritoneal cells was almost fully eliminated, contrasting with erythrocytes where ion replacement had no effect. The lower panel of Fig. 3 also shows that [G-^{3}H]tubercidin was essentially not transported by the macrophage system in the presence of either sodium, potassium or lithium ion. There was no evidence of any NBMPR effect on the residual, apparent transport processes with [8-^{3}H]-adenosine or [G-^{3}H]tubercidin as permeants in lithium or potassium saline media.

Effect of cell preparation on transport studies

Time course studies and single time point assays in the absence and presence of NBMPR, as well as studies on the ion dependence of [8-^{3}H]adenosine uptake in peritoneal cells were conducted also with cells freed of contaminating erythrocytes by layering on Ficoll-Paque. In all cases the experimental results were identical to those obtained with preparations in which erythrocytes were lysed by NH_4Cl treatment (data not shown). Thus the critical findings, namely absence of inhibitory effects of NBMPR and sodium dependency of [8-^{3}H]adenosine uptake in peritoneal cells, was not an artifact but reflected a true property of these cells.

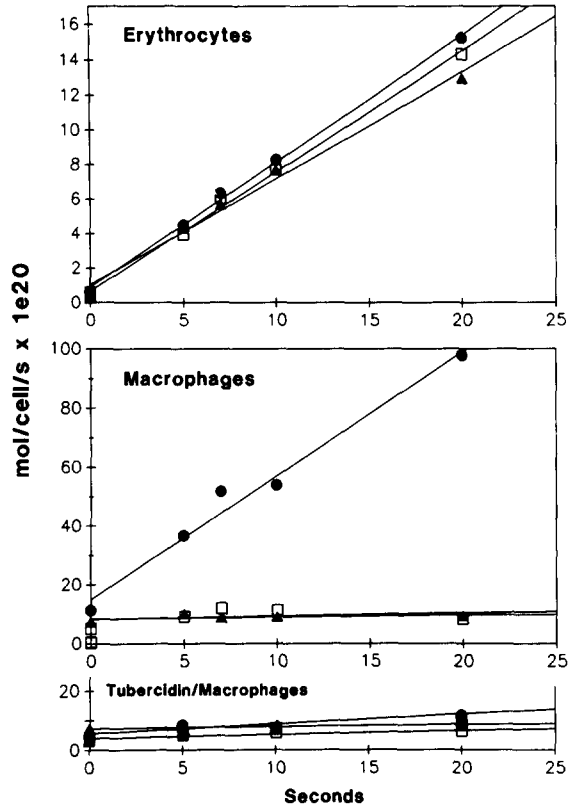

Fig. 3. Time dependence of [8-^{3}H]adenosine uptake in mouse erythrocytes (upper graph) and peritoneal cells (middle graph) as well as [G-^{3}H]tubercidin uptake in peritoneal cells (lower graph) in the presence of different cations: Na^+ (●), K^+ (▲) and Li^+ (□). Buffer and reagents were prepared as described under Methods and Materials. The lower partial plot of [G-^{3}H]tubercidin uptake in peritoneal cells is from an experiment different from that for [8-^{3}H]adenosine uptake above, but it is plotted on the same scale for direct comparison. Means of duplicate assays are shown throughout, with individual values differing from each other by less than 9%.

Adenosine uptake in different cell fractions

Since the peritoneal cell preparation was not pure and contained different cell types, notably macrophages as well as polymorphonuclear cells, we assessed the presence and nature of [8-^{3}H]adenosine uptake in both the adherent and nonadeherent cell fractions, the latter largely representing macrophages (monocytes). Table II lists results of transport measurements with these separated cell fractions, conducted in sodium, potassium and lithium saline media, and in the presence and absence of 10 μM NBMPR in each case.

It is evident that NBMPR had no significant inhibitory effect on [8-^{3}H]adenosine uptake in nonadherent cells and only about 20% inhibitory action in adherent cells in sodium containing medium. After replacing sodium ion with lithium or potassium ion, only about 10% of activity remained in both cell types. and this was

TABLE II

Transport activity of adherent and nonadherent peritoneal cells

Peritoneal exudate cells were separated into adherent and nonadherent cell fractions as described under Methods and Materials, and both were assayed for [8-^{3}H]adenosine uptake activity in the absence and presence of NBMPR in Na, K and Li saline buffers.

Cell type		10^{-20} mol/cell per s [a]		
		Na buffer	K buffer	Li buffer
Adherent	−NBMPR	9.07 + 0.97	0.96 + 0.07	1.65 + 0.34
	+NBMPR	7.03 + 0.41	0.37 + 0.03	1.38 + 0.14
Nonadherent	−NBMPR	6.45 + 0.71	1.07 + 0.22	0.96 + 0.24
	+NBMPR	6.65 + 0.15	0.75 + 0.27	0.41 + 0.59

[a] Means ± S.D. of triplicate determinations.

only partially sensitive to NBMPR. These results confirm in principle observations made repeatedly with the freshly prepared peritoneal exudate cell mixture, contrasting only slightly in that in the latter case always full dependence on sodium ion and complete sensitivity to NBMPR were seen (also cf. Fig. 3). Thus, findings reported above for mixed peritoneal cells appear to be representative of both macrophages and polymorphonuclear cells.

Discussion

Adenosine uptake by mouse peritoneal exudate cells clearly occurred via a mechanism insensitive to common inhibitors of facilitated diffusion of nucleosides. The concurrent presence of facilitated, inhibitor-sensitive diffusion cannot be ruled out fully, but at the concentration of 1 μM adenosine this would represent at most 10% in the cell mixture and less than 20% in the adherent and nonadherent cell fractions. The presence of an inhibitor insensitive nucleoside transport or uptake function in mixed peritoneal cells as well as partially purified polymorphs and macrophages, in combination with the fact that it was almost completely sodium dependent, suggests that the nucleoside transport system of these cells is similar to the concentrative, active transporter systems found with a number of cells [7–14] among which splenocytes [13,14] also represent an immune cell type.

The concentrative nature of the transporter in peritoneal cells could not be assessed with adenosine as a permeant because of its rapid intracellular phosphorylation to adenine nucleotides, without any evidence of accumulation of intracellular free adenosine or its nonphosphorylated metabolites during the early phases (10–20 s) of the process. The possibility that the current measurements of accumulation of label and its time course thus reflected a rate limiting enzymatic step (such as adenosine kinase) rather then the transmembrane transport of the permeant has to be considered. However, there was no indication, even during the shortest time intervals covered, of any curvature of the time curves, which has been clearly observed in studies of nucleoside uptake by animal cells [2–4]. Instead, experimental points covering 10–60 s incubation times always lay on straight lines intersecting on the ordinate at zero time with the zero-rate values or the extrapolation of control curves obtained with cold adenosine present throughout. We suggest therefore that the measurements in this study represent those of initial rates of transmembrane transport of adenosine in peritoneal cells, and that this rapid process is fully matched by rapid intracellular phosphorylation, representing a high salvage capacity of these cells. However, in the absence of any additional direct proof that our measurements were those of initial rates, the process has been described and referred to as uptake throughout this manuscript as a matter of caution.

The capacity of the nucleoside transporter in peritoneal cells to operate against a concentration gradient remains to be assessed with nonmetabolizable substrates. Work is in preparation to determine whether potential permeants such as deoxyuridine or the inosine analog formycin B which have been used by others [8,9,11,12] will be useful for this purpose in these cells.

The structure activity studies (Fig. 2) suggest that different nucleosides may serve as substrates of the inhibitor insensitive transport system in peritoneal cells, which has been demonstrated to be the case with respect to the natural nucleosides adenosine and inosine. However, the adenosine analog tubercidin, which weakly inhibited [8-^{3}H]adenosine uptake, did not serve as a permeant. This analog is readily transported by cells possessing the facilitated diffusion system for nucleosides and perhaps may serve as a tool differentiating between facilitated diffusion and sodium dependent transporters. Direct transport studies with different potential permeants in the absence and presence of competing nucleosides will be required to also assess the possibility that there are multiple transporters, as has been demonstrated in other cell systems possessing sodium dependent transporters [11,14].

We do not know of any studies demonstrating a physiological significance or need for macrophages to exhibit a high capacity to transport and salvage nucleosides. The macrophages used in this study were highly activated cells, both by exposure to Freund's Adjuvant as well as adhesion to Petri dishes. Whether this activation had any bearing on their capacity to transport and metabolize adenosine remains an open question. Other types of immune cells, of course, undergo rapid and extensive proliferation in response to various stimuli, and a high capacity salvage pathway for nucleosides may be of significance under this condition.

Apart from the question of physiological role of sodium dependent nucleoside transporters in immune

cells, there may be concern over the possible selective risk to macrophage and immune cell function during the use of cytotoxic nucleosides in anticancer or antiparasitic therapy (cf. Ref. 17), since the concomitant use of host protecting nucleoside transport inhibitors would not protect these particular cells. On the other hand, the existence of a special transporter in macrophages with differing structural requirements for permeants also may provide a window for a selective, therapeutic approach with suitable nucleoside derivatives toxic to intracellular leishmanial amastigotes.

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