Dipyridamole-insensitive Nucleoside Transport in Mutant Murine T Lymphoma Cells*

(Received for publication, May 9, 1986)

Bruce Aronow†, Darwin Toll‡, Joseph Patrick+, Kathleen McCarta§, and Buddy Ulman††

From the †Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536 and the ‡Department of Biochemistry, The Oregon Health Sciences University, Portland, Oregon 97201

From a mutagenized population of S49 murine T lymphoma cells, a mutant cell line, JPA4, was selected that expressed an altered nucleoside transport capability. JPA4 cells transported low concentrations of pyrimidine nucleosides and uridine more readily than the parental S49 cell line. The transport of these nucleosides by mutant cells was insensitive to inhibition by either dipyridamole (DPA) or 4-nitrobenzylthioinosine (NBMPR), two potent inhibitors of nucleoside transport in mammalian cells. Kinetic analyses revealed that the apparent $K_m$ values for the transport of uridine, adenosine, and inosine were 3-4-fold lower in JPA4 cells compared to wild type cells. In contrast, the transport of both thymidine and cytidine by JPA4 cells was similar to that of parental cells, and transport of these pyrimidine nucleosides remained sensitive to inhibition by both NBMPR and DPA. Furthermore, thymidine was a 10-12-fold weaker inhibitor of inosine transport in JPA4 cells than in wild type cells. Thus, JPA4 cells appeared to express two types of nucleoside transport activities; a novel (mutant) type that was insensitive to inhibition by DPA and NBMPR and transported pyrimidine nucleosides and uridine, and a parental type that retained sensitivity to inhibitors and transported cytidine and thymidine. The phenotype of the JPA4 cell line suggests that the sensitivity of mammalian nucleoside transporters to both NBMPR and DPA can be genetically uncoupled from its ability to transport certain nucleoside substrates and that the determinants on the nucleoside transporter that interact with each nucleoside are not necessarily identical.

Many of the physiological and cytotoxic actions of nucleosides and nucleoside analogs require permeation of the plasma membrane. The transport of nucleosides into mammalian cells occurs by a facilitated diffusion mechanism which appears to be mediated by a single carrier of broad specificity (1-5). This single carrier model is based on kinetic evidence indicating competitive inhibition among nucleosides for transport (1-4) and on the existence of mutant cells genetically deficient in nucleoside transport capability (5). Mutant S49 murine T cell lines have been characterized which are unable to transport virtually all purine and pyrimidine nucleosides tested and consequently have lost their high affinity binding sites for p-nitrobenzylthioinosine (NBMPR) (5-11), a highly specific inhibitor of mammalian nucleoside transport (12-15).

There are, however, discrepancies with the premise that only a single type of carrier is responsible for all nucleoside transport in all mammalian cells. For example, sodium-dependent (16, 17) and purine nucleoside phosphorylase-dependent (18-20) nucleoside carriers have been described in mammalian cells and tissues. In addition, various mammalian cells possess a component of their nucleoside transport activity (2-100%) which is insensitive to inhibition by NBMPR (9, 15, 21, 23-27), implying the existence of at least two classes of nucleoside transport differing in NBMPR sensitivity. Efforts to distinguish between the biochemical properties of NBMPR-sensitive and NBMPR-insensitive nucleoside transport activities have not revealed differences in permeant specificity. However, different susceptibilities to inactivation by non-permeating sulfhydryl reactive reagents suggest possible structural differences between the NBMPR-sensitive and NBMPR-insensitive transporter components (9, 21, 25).

Mutant mouse S49 cells with alterations in their sensitivity to the effects of NBMPR have been generated. Unlike wild type cells, these mutant cells are capable of proliferating in medium containing hypoxanthine, methotrexate, thymidine, and NBMPR and possess nucleoside transport components that are insensitive to inhibition by NBMPR (9). The NBMPR-insensitive nucleoside transporter component of mutant S49 cells, however, is insensitive to inhibition by dipyridamole (DPA), another inhibitor of mammalian nucleoside transport (28). DPA is structurally unrelated to NBMPR, but like nucleosides, is capable of displacing NBMPR from its specific binding sites (9, 29). The extent to which DPA inhibits nucleoside transport (26, 30) and interferes with the binding of NBMPR (31) differs among mammalian cell lines. Thus, genetic variation of mammalian nucleoside transporters could explain phenotypic variation of their sensitivities to inhibitors.

In the present study we have extended this genetic dissection of inhibitor interactions with mammalian nucleoside transport systems by selecting and characterizing mutant S49 cells expressing a nucleoside transport component that was insensitive to DPA-mediated inhibition.

MATERIALS AND METHODS

Chemicals and Reagents—[3H]Adenosine (20 Ci/mmol), [3H]Cytidine (22 Ci/mmol), [3H]Guanosine (11 Ci/mmol), [3H]Deoxyguanosine (72 Ci/mmol), [3H]Deoxyadenosine (28 Ci/mmol), [3H]Hypoxanthine (17 Ci/mmol), [3H]Thymidine (75 Ci/mmol), [3H]Juridine (40 Ci/mmol), [3H]Thymidine (25 Ci/mmol), and [3H]NBMPR (28 Ci/mmol) were purchased from American Radiolabeled Chemicals and Reagents—[3H]Adenosine (20 Ci/mmol), [3H]Cytidine (22 Ci/mmol), [3H]Guanosine (11 Ci/mmol), [3H]Deoxyguanosine (72 Ci/mmol), [3H]Deoxyadenosine (28 Ci/mmol), [3H]Hypoxanthine (17 Ci/mmol), [3H]Thymidine (75 Ci/mmol), [3H]Juridine (40 Ci/mmol), [3H]Thymidine (25 Ci/mmol), and [3H]NBMPR (28 Ci/mmol) were purchased from American Radiolabeled Chemicals and Reagents.

*The abbreviations used are: NBMPR, p-nitrobenzylthioinosine; DPA, dipyridamole; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

**Recipient of a Research Career Development Award from the National Institutes of Health.

††This work was funded by Grants CA32580 and CA42539 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

14467
Genetically Altered Nucleoside Transport

purchased from Moravek Biochemicals. [3H]H2O (1.0 mCi/ml) and [methoxy-3H]methoxyaminulin (470 mCi/g) were obtained from New England Nuclear. Methylxanthate was obtained from Lederle Parenterals Inc. in a 25 mg/ml solution. N-methyl-N-nitro-N-nitrosoguanidine was provided by the K and K Laboratories Inc. of ICN Chemical Corp. Ethylo-9-(2-hydroxy-5-ronyl)adenine was bought from the Burroughs-Wellcome Corp. NBMPR, azaserine, and N-methyl-N-nitro-N'-nitroguanidine were obtained from Sigma.

Cell Culture—The SA9 cell line is a T cell lymphoma originally derived from a BALB/c mouse by Horibata and Harris (32). Its growth characteristics and lymphocytic properties have been described previously in detail (29, 33). The origin and description of the KAB5 cell line, which possesses a nucleoside transport component completely refractory to inhibition by NBMPR, has been described in detail (9, 34). All S49 cells grew in suspension culture in a humidified 10% CO2 atmosphere at 37°C in Dulbecco's modified Eagle medium containing 3.7 g/liter sodium bicarbonate and 4.5 g/liter d-glucose supplemented with 10% horse serum which had been heat-inactivated at 56°C for 30 min.

Mutagenesis and Selection of the JPA4 Cell Line—KAB5 cells were grown to a density of 10^6 cells/ml in a volume of 200 ml and mutagenized by incubation with 3 mg/ml N-methyl-N-nitro-N-nitrosoguanidine for 3 h at 37°C as previously described (5, 6, 8). Cells were separated from mutagen by centrifugation and resuspended in fresh growth medium. Approximately 75% of the cells were killed by N-methyl-N-nitro-N-nitrosoguanidine exposure. Surviving cells were then expanded in non-selective growth medium for 7-10 generations in order to allow expression of mutant phenotypes and cloned in 0.3% agarose (Sea Kem-ME) overlaying mouse embryo fibroblast feeder layers, as originally described by Coffino et al. (35). JPA4 cells were selected in semi-solid medium containing 100 μM hypoxanthine, 11 μM azaserine, a potent inhibitor of the purine biosynthetic pathway (36), and 10 μM DPA. This concentration of DPA blocks the salvage of hypoxanthine by azaserine-treated wild type S49 cells (11). Azaserine rather than methotrexate was used to render cells auxotrophic for purines, since the toxicity of the former could be circumvented by all three cell lines (Fig. 1). In contrast, DPA substantially protected JPA4 cells to salvage hypoxanthine in the absence and presence of DPA.

Growth Rate Determinations—The ability of wild type, KAB5, and JPA4 cells to salvage hypoxanthine were assayed in Costar multwell (24-well) tissue culture plates. Azaserine was added to experimental wells to block de novo purine biosynthesis (9, 36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine. Two wells lacking experimental wells to block de novo purine biosynthesis (36), along with varying concentrations of hypoxanthine.
Genetically Altered Nucleoside Transport

14469

5-FLUOROURIDINE CONC (nM)

DEOXYGUANOSINE CONC (μM)

ARABINOSYLCYTOSINE

FIG. 2. Sensitivity of wild type and mutant cells to growth inhibition by three cytotoxic nucleosides in the absence and presence of DPA. The abilities of wild type (○, ○), KAB5 (□, ■), and JPA4 (△, △) cells to grow in the presence of increasing concentrations of 5-fluorouridine (panel A), deoxyguanosine (panel B), and arabinosylcytosine (panel C) in the absence (open symbols) or presence (closed symbols) of 10 μM DPA were ascertained according to the procedures described under “Materials and Methods.” The results are those of single typical experiment versions which have been repeated 2–5 times with the same results.

able to interfere with the salvage of hypoxanthine or the toxicity of arabinosylcytosine.

Transport Measurements—Since 5-fluorouridine, deoxyguanosine, and deoxyadenosine are metabolized by genetically and biochemically distinct enzymes in S49 cells (37–39), the results of the growth determinations suggested that the peculiar growth phenotype of JPA4 cells might be attributable to their expression of an altered nucleoside transport capability. In order to determine whether the failure of DPA to protect JPA4 cells from the cytotoxic effects of some nucleosides was due to the presence of a DPA-insensitive transport function, nucleoside transport activities were measured in the three cell lines in the absence and presence of DPA. The data in Fig. 3 demonstrated that JPA4 cells transported low concentrations of inosine more rapidly than either KAB5 or wild type cells. Moreover, inosine transport into JPA4 cells, unlike that in wild type and KAB5 cells, was not inhibited by 10 μM DPA. To ascertain the substrate specificity of this DPA-insensitive transport capability, the abilities of all three cell lines to transport a variety of nucleosides was determined. As shown in Table I, JPA4 cells transported 5 μM concentrations of all purine nucleosides tested more rapidly than parental or wild type cells, and this purine nucleoside influx was insensitive to inhibition by either DPA or NBMPR. The transport of pyrimidine nucleosides was not, however, uniformly elevated or insensitive to inhibition by DPA or NBMPR. The transport of both cytidine and thymidine by JPA4 cells was neither augmented nor sensitive to inhibition by DPA and NBMPR, while uridine transport was 3-fold elevated and

FIG. 3. Inosine transport by wild type and mutant S49 cells. The conditions for the measurement of inosine transport are described under “Materials and Methods.” The abilities of wild type (○, ○), KAB5 (□, ■), and JPA4 (△, △) cells to transport [3H]inosine (10 μCi/ml) in the absence (open symbols) or presence (closed symbols) of 10 μM DPA are depicted. The inhibitory effects of 10 μM DPA were determined by preincubation of the cells in their growth medium with DPA at 37°C for 15 min. Cells were then harvested by centrifugation, and resuspended in transport media which also contained 10 μM DPA.

TABLE I

The rates at which various nucleosides and hypoxanthine (Hyp) are transported by wild type, KAB5, and JPA4 cells in the absence and presence of either 10 μM DPA or 10 μM NBMPR

Rates reported are those obtained with 5 μM concentrations of nucleoside or hypoxanthine. The indicated rates were obtained in spot assays based upon the exposure of cells to radiolabeled compounds for intervals between 2.8 and 61.8 s at room temperature.

| Cell line | Addition | Ado | dGuo | dThd | Cyd | Urd | Hyp |
|-----------|----------|-----|------|------|-----|-----|-----|
| Wild type | Control  | 0.5 | 0.15 | 0.1  | 0.16| 0.06| 0.7 |
|           | DPA      | 1.0 | 0.8  | 0.1  | 0.2 | 0.03|     |
| KAB5      | Control  | 1.0 | 0.8  | 0.1  | 1.0 | 1.3 | 1.7 |
|           | DPA      | 1.1 | 0.3  | 0.5  | 0.3 | 0.45| 0.45|
| JPA4      | Control  | 118 | 58   | 11   | 54  | 1.62| 1.62|
|           | DPA      | 122 | 60   | 1.0  | 0.4 | 0.54| 0.54|
|           | Both     | 124 | 60   | 1.1  | 0.5 | 0.46| ND  |

* ND, not determined.
Insensitive to both inhibitors (Table I). These results implied that JPA4 cells possessed two types of nucleoside transport activity, a DPA-sensitive and a DPA-insensitive form, the latter more capable of transporting low concentrations of purine nucleosides and uridine, but not thymidine, cytidine, or hypoxanthine.

Kinetic Characterization of Purine Nucleoside Transport in JPA4 Cells—Since results from the transport assays performed at low ligand concentration were consistent with the hypothesis that JPA4 cells possessed a novel transport capability, the kinetic parameters associated with nucleoside transport in JPA4 cells were compared to those of wild type cells. Previous detailed kinetic analyses have demonstrated that these kinetic parameters are identical in wild type and KAB5 cells (9, 34). The augmented transport of low adenosine concentrations by JPA4 cells could be attributed to a 3-fold reduced apparent $K_m$ value for adenosine transport by JPA4 cells compared to both KAB5 (9) and wild type cells (Fig. 4A).

The maximal transport capacities were equivalent in all three cell lines. Since the transport of thymidine by JPA4 cells was not elevated and remained sensitive to the inhibitory effects of DPA and NBMPR, we postulated that thymidine might not be an effective substrate for the DPA-insensitive transporter. Thus, the extent of competition between thymidine and adenosine was measured for JPA4 cells. Thymidine was a considerably weaker competitive inhibitor of adenosine transport into JPA4 cells than into wild type cells, Fig. 4A.

Apparent $K_m$ values for adenosine transport were 0.15 mM thymidine in wild type cells and 1.6 mM thymidine in JPA4 cells. Pretreatment of JPA4 cells with NBMPR or DPA did not affect this $K_m$ value (data not shown). Thus, thymidine is both a poor permeant for transport and an ineffective inhibitor of adenosine transport into JPA4 cells.

The kinetic parameters for inosine transport into wild type and JPA4 cells were also evaluated. The apparent $K_m$ values for inosine (Fig. 4B) and uridine (data not shown) transport into JPA4 cells were also reduced severalfold over those of wild type cells. Moreover, the $K_v$ value of adenosine for inosine transport reflected the apparent $K_m$ values obtained with adenosine (Fig. 4). Thus, JPA4 cells apparently possess a transport capability that has a greater affinity for adenosine, inosine, and uridine, but a weaker affinity for thymidine than the wild type nucleoside transporter.

That purine nucleoside transport was augmented and essentially unaffected by thymidine in JPA4 cells could be explained by a dissociation of the purine recognition portion of the nucleoside transporter from the cytidine-thymidine recognition site. To test this premise, the ability of purine nucleosides to inhibit cytidine transport was determined. As demonstrated in Fig. 5, inosine inhibited cytidine transport into wild type and JPA4 cells in a similar manner. Thus, the transport system for cytidine in JPA4 cells appeared identical to the wild type cytidine transport activity. This suggested that the normal nucleoside transport function was intact in JPA4 cells but distinct from the altered purine nucleoside-uridine transport capability.

To substantiate the substrate specificity of the DPA-insensitive nucleoside transport activity in JPA4 cells, the ability of various nucleosides to inhibit deoxyguanosine influx in JPA4 and parental cells was compared. The results in Fig. 6 indicated that uridine and inosine were more effective inhibitors of deoxyguanosine transport into JPA4 cells than cytidine or thymidine. In parental KAB5 cells, the differences in effectiveness among uridine, inosine, thymidine, and cytidine were greater than in JPA4 cells.

![FIG. 5. Inosine inhibition of cytidine transport by KAB5 and JPA4. The ability of various concentrations of inosine to inhibit the transport of 5 μM cytidine was determined for KAB5 (□) and JPA4 cells (△). This experiment was performed once with adenosine rather than inosine and gave equivalent results.](image)
p-Chloromercuribenzene sulfonate, a sulphydryl reactive
dine in inhibiting deoxyguanosine transport were insignifi-
cant.

**NBMPR Binding Measurements**—NBMPR binding to the
surface of S49 cells has served as a useful indicator for
nucleoside transport activity, since cells genetically deficient
in nucleoside transport lack NBMPR binding sites (7, 9).
Therefore, the number and affinity of the cell surface binding
sites were determined for wild type, KAB5, and JPA4 cells.
Previously, we have shown that KAB5 cells possess only 30–
50% of the number of NBMPR binding sites as wild type cells
(9). As demonstrated in Fig. 7A, the number of NBMPR
binding sites on JPA4 and KAB5 cells was about 27,000/cell,
about half the number found on the surface of wild type cells.
The affinity of NBMPR for the binding site was approxi-
mately 0.3 nM for all three cell lines. The ability of DPA to
interfere with the binding of [3H]NBMPR to the cell surface
sites was also similar in all three cell lines (Fig. 7B). Therefore,
the presence of the mutant transport function in JPA4 cells
did not appear to affect the binding of the inhibitors to the
cell surface.

**Sensitivity of the DPA-insensitive Transporter to Sulphy-
dryl Reagents**—In order to detect structural differences in
transport, the sensitivities of the nucleoside transport func-
tions toward sulphydryl reagents were determined (9, 21, 25).
p-Chloromercuribenzenesulfonate, a sulphydryl reactive
agent which has been exploited previously to demonstrate structural alterations in the KAB5 nucleoside transporter (9),
inhibited the transport of inosine into JPA4 cells to the same
extent as it did into wild type or parental cells (Fig. 8). The
extent of inhibition was much less than that observed with
the NBMPR-insensitive component of KAB5 cells. Thus, by
this criterion the structure of the DPA-insensitive transporter
appears to be more related to the wild type nucleoside trans-
porter than to the NBMPR-insensitive transporter of KAB5
cells.

**DISCUSSION**

Studies into the mechanism by which nucleosides permeate
mammalian plasma membranes have been greatly enhanced
by the existence of two potent inhibitors of mammalian
nucleoside transport, DPA and NBMPR. While NBMPR
binding abolishes nucleoside transport in S49 cells (7, 9) and
in human erythrocytes (1–4), the extent to which NBMPR
inhibits nucleoside transport into other cells varies consider-
ably. Many cultures cell lines possess both NBMPR-sensitive
and NBMPR-insensitive nucleoside transport components
(15, 24–26, 40). Uridine influx into RPMI 1640, P388, L1210,
Chinese hamster ovary, and L929 cells is biphasic with respect
to NBMPR inhibition, each cell line possessing both
NBMPR-sensitive and NBMPR-insensitive components (24–
26). The NBMPR-sensitive and NBMPR-insensitive nucleo-
side transport systems are indistinguishable with respect to
substrate specificity, affinity for nucleosides and DPA, and
temperature dependence. Considerable variation has also
been observed among mammalian cells with respect to the
concentration of DPA required to inhibit nucleoside transport
(26) and to displace [3H]NBMPR from its high affinity bind-
ing sites (31). For instance, Shi et al. (31) showed that DPA
interferes with the binding of [3H]NBMPR to guinea pig lung
membranes, but not to rat lung membranes. This difference
is specific for DPA, since adenosine and uridine inhibit the
[3H]NBMPR binding to rat and guinea pig lung membranes
equivalently (31).

Three mutant S49 cell lines have been isolated in this
laboratory that express an NBMPR-insensitive nucleoside
transport component which retains its sensitivity to inhibi-
tion by DPA (9, 34, 41). This suggested that the structural
elements that allow for the interaction of NBMPR and DPA
with the S49 nucleoside transporter are distinct. The reten-
tion of DPA sensitivity by the NBMPR-insensitive nucleoside
transport components does not necessarily imply that nucleoside trans-
porters have specific binding domains for DPA. Moreover,
the differences in DPA sensitivity among mammalian cells and
tissues do not prove that the nucleoside transporters are
the plasma membrane components that vary genetically, and
therefore account for the altered DPA responsiveness. In fact,
DPA has inhibitory effects on the passive diffusion of L-
glucose and cytosine (42), the influx of phosphate and fucose
(43), and the efflux of 3-O-methylglucose, uracil, and cyclo-
leucine (43). Thus, to study the molecular basis of the inter-
action of nucleoside transport with DPA, mutant cell lines
were generated in which the nucleoside transport activity was

---

**Fig. 7.** NBMPR binding sites in wild type, KAB5, and JPA4 cells. The number and affinity of NBMPR
binding sites were determined at varying concentrations of [3H]NBMPR for wild type (○), KAB5 (□), and JPA4
(△) cells, as demonstrated in panel A. The ability of DPA to displace NBMPR from these binding sites is compared
for all three cell lines in panel B. Wild type cells possessed about 51,000 NBMPR binding sites/cell with an
apparent $K_d$ value of 0.32 nM. JPA4 and KAB5 cells had 28,000 binding sites/cell with an apparent $K_d$ value of
0.30 nM.
Phenotype in JPA4 cells is the activation of a distinct but DPA-insensitive nucleoside transport of 5-β adenosine was determined by the rapid kinetic sampling assay in wild type and mutant cells treated for 5 min with sulphydryl reagent, with 0.5 mM N-ethylmaleimide (NEM).

The transport of purine nucleosides and uridine by JPA4 cells was insensitive to DPA, while thymidine, cytidine, and hypoxanthine transport remained DPA-sensitive. That the transport of some nucleosides could be refractory to DPA inhibition in the same S49 mutant cell in which the transport of others retained DPA sensitivity suggests that the action of DPA on nucleoside transport must be an intrinsic property of DPA-sensitive transporters.

There are several plausible hypotheses that can account for the genetic basis of expression of the DPA-insensitive nucleoside transport system of JPA4 cells. The first is that the unique DPA-insensitive transporter is derived by mutation. Such a mutation could either be in an allele coding for the NBMPR-sensitive nucleoside transport component of KAB5 cells or in one coding for the NBMPR-insensitive nucleoside transport component. If the phenotype of the JPA4 transporter could be attributed to mutation, its unique substrate specificity suggests that the nucleoside recognition site may not promiscuously interact with all purine and pyrimidine nucleosides. Instead, it is possible that the structural determinants required for the recognition of thymidine, cytidine, and hypoxanthine can be distinguished from the determinants that recognize purine nucleosides and uridine. An alternative model for the genetic origin of the DPA-insensitive transport phenotype in JPA4 cells is the activation of a distinct but previously "silent" genetic locus coding for a DPA-insensitive transport function. This second model, however, cannot account for the lack of DPA-sensitive transport in JPA4 cells nor can it explain the $K_m$ rather than $V_{max}$ changes observed for the mutant transporter. Finally, it is possible that a genetic alteration in some secondary component affecting membrane architecture or composition is responsible for the unique phenotype of JPA4 cells. Nevertheless, whether the gene that codes for the DPA-insensitive nucleoside transport component of JPA4 cells is derived by mutation of the wild type nucleoside transport locus, mutation of the KAB5 NBMPR-insensitive locus, or by expression of a separate gene cannot be determined conclusively.

There has been a report of a mammalian nucleoside transport system that has properties similar to those of the JPA4 transport system. Cornford and Oldendorf (44) have observed rapid uptake of adenosine, guanosine, inosine, and uridine into the rat central nervous system following injection into the carotid artery. Their measurements were based upon short assay periods to minimize metabolic contributions. These four nucleosides crossed the blood brain barrier with a low apparent $K_m$ value and interfered with the uptake of the others.

Cytidine, thymidine, and arabinosylcytosine neither crossed the blood brain barrier nor interfered with the incorporation of those nucleosides that could. Sensitivity of this nucleoside uptake system to DPA was not reported.

The JPA4 cell line was selected on the basis of its ability to salvage low concentrations of hypoxanthine in the presence of azaserine and DPA. DPA inhibits the salvage of hypoxanthine by wild type S49 cells (11). Other S49 cell lines have been generated from wild type parental cells under identical selective conditions (22). These latter cell lines have a normal nucleoside transport system but express a unique high affinity nucleobase transport system (22). However, the JPA4 cell line does not possess augmented hypoxanthine transport capability, and hypoxanthine transport is completely sensitive to DPA. The growth of the JPA4 clone under the selective conditions probably resulted from their augmented ability to salvage purine nucleosides originating from lysed cells which released their contents. Whether the generation and isolation of cells possessing the JPA4 phenotype requires the presence of parental NBMPR-insensitive nucleoside transport component, as exists in KAB5 cells, is not currently known.

REFERENCES

1. Oliver, J. M., and Paterson, A. R. P. (1971) Can. J. Biochem. 49, 262-270.
2. Taube, R. A., and Berlin, R. D. (1972) Biochim. Biophys. Acta 255, 6-18.
3. Berlin, R. D., and Oliver, J. M. (1975) Int. Rev. Cytol. 42, 287-336.
4. Plagemann, P. G., and Wohlhueter, R. M. (1980) Curr. Top. Membr. Trans. 14, 225-330.
5. Cohen, A., Ullman, B., and Martin, D. W., Jr. (1979) J. Biol. Chem. 254, 122-136.
6. Ullman, B., Gudas, L. J., Clift, S. M., and Martin, D. W., Jr. (1979) Proc. Natl. Acad. Sci. U. S. A. 74, 1074-1078.
7. 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.
8. Ullman, B., Kaur, K., and Watta, T. (1983) Mol. Cell. Biol. 3, 1187-1196.
9. Aronow, B., Allen, K., Patrick, J., and Ullman, B. (1985) J. Biol. Chem. 260, 6226-6233.
10. Aronow, B., and Ullman, B. (1985) J. Biol. Chem. 260, 16274-16278.
11. Aronow, B., and Ullman, B. (1986) J. Biol. Chem. 261, 2014-2019.
12. Paterson, A. R. P., and Oliver, J. M. (1971) Can. J. Biochem. 49, 271-274.
13. Cass, C. E., and Paterson, A. R. P. (1972) J. Biol. Chem. 247, 3314-3320.
14. Pickard, M. A., Brown, R. R., Paul, B., and Paterson, A. R. P. (1973) Can. J. Biochem. 51, 566-572.
15. Wohlhueter, R. M., Marz, R., and Plagemann, P. G. W. (1978) J. Membr. Biol. 42, 247-264.
16. Le Hir, M., and Dubach, U. C. (1984) Pfugers Arch. 401, 58-63.
17. Schwenk, M., Hegazy, E., and Lopez del Pino, V. (1984) Biochim. Biophys. Acta 805, 370-374.
18. Dowd, D. J., Quinlan, D. C., and Hochstadt, J. (1977) Biochemistry 16, 4526-4532.
19. Cohen, A., and Martin, D. W., Jr. (1977) J. Biol. Chem. 252, 4428-4430.
20. Prasad, R., Shapiro, C., and Hochstadt, J. (1981) Biochim. Biophys. Acta 643, 306-318.
21. Kilam, Y., and Cabantchik, Z. I. (1977) J. Cell Physiol. 89, 831-835.
22. Plagemann, P. G. W., and Wohlhueter, R. M. (1984) Biochim. Biophys. Acta 779, 39-62.
Genetically Altered Nucleoside Transport

27. Cohen, A., Leung, C., and Thompson, E. (1985) J. Cell Physiol. 123, 431-434
28. Scholtissek, C. (1968) Biochim. Biophys. Acta 158, 435-447
29. Paterson, A. R. P., Laut, E. Y., Dahlig, E., and Cass, C. E. (1980) Mol. Pharmacol. 18, 40-44
30. Plagemann, P. G. W., and Wohlhueter, R. M. (1984) J. Membr. Biol. 81, 255-262
31. Shi, M. M., Wu, J. R., Lee, C., and Young, J. D. (1984) Biochem. Biophys. Res. Commun. 118, 594-600
32. Horiba, K., and Harris, A. W. (1970) Exp. Cell Res. 60, 61-77
33. Ullman, B., Cohen, A., and Martin, D. W., Jr. (1976) Cell 9, 205-211
34. Aronow, B., and Ullman, B. (1985) Proc. Soc. Exp. Biol. Med. 176, 463-471
35. Coffino, P., Baumal, R., Laskov, R., and Scharff, M. D. (1972) J. Cell Physiol. 79, 429-440
36. Melnick, I., and Buchanan, J. M. (1962) J. Biol. Chem. 237, 2631-2635
37. Ullman, B., Gudas, L. J., Cohen, A., and Martin, D. W., Jr. (1978) Cell 14, 205-211
38. Gudas, L. J., Ullman, B., Cohen, A., and Martin, D. W., Jr. (1978) Cell 14, 565-575
39. Ullman, B., Levinson, B., Ullman, D. H., and Martin, D. W., Jr. (1979) J. Biol. Chem. 254, 8736-8739
40. Cass, C. E., and Paterson, A. R. P. (1977) Exp. Cell Res. 105, 427-435
41. Aronow, B., Hollingsworth, P., Patrick, J., and Ullman, B. (1986) Mol. Cell. Biol. 6, 1296-1303
42. Graff, J. C., Wohlhueter, R. M., and Plagemann, P. G. W. (1977) J. Biol. Chem. 252, 4185-4190
43. Kessel, D., and Dodd, D. C. (1972) Biochem. Biophys. Acta 288, 190-194
44. Cornford, E. M., and Oldendorf, W. H. (1975) Biochem. Biophys. Acts 394, 211-219