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High-affinity, equilibrative nucleoside transporter of pig kidney cell line (PK-15)

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Nucleoside transport was determined in the cloned porcine kidney cell line PK-15 which exhibits properties of tubular cells. The cells did not express any Na^+-dependent, concentrative nucleoside transport. They exhibited only nitrobenzylthioinosine-sensitive equilibrative nucleoside transport. Their transport activity, however, was only about 10% of that observed in many other mammalian cell lines. This low transport activity correlated with a relatively low number of high-affinity nitrobenzylthioinosine binding sites ($5 \cdot 10^3$/cell). Furthermore, although the equilibrative transporter of PK-15 cells exhibited a similar broad substrate specificity as the equilibrative nucleoside transporters of other mammalian cells, it exhibited a much higher affinity for certain nucleosides, especially cytidine and uridine, than the latter. The Michaelis-Menten constants for zero-*trans* transport and equilibrium exchange of uridine in ATP-depleted cells were about the same (about 40 μM), indicating equal mobility of the nucleoside-loaded and empty carrier. Concentrative nucleoside transport was detected in one set of PK-15 cultures, but was found to be due to mycoplasma contamination.

Introduction

The primary nucleoside transport system of mammalian cells is an equilibrative system with broad substrate specificity [1,2]. Two forms of equilibrative nucleoside transport have been distinguished on the basis of their sensitivity to inhibition by nitrobenzylthioinosine (NBTI) [1,2]. One form is strongly inhibited by nanomolar concentrations of NBTI (designated NBTI-sensitive) which results from the binding of NBTI to a nucleoside transporter-associated high-affinity binding site on the plasma membrane ($K_d \leq 1$ nM). The other form is not associated with such binding site and is inhibited only by μmolar concentrations of NBTI (designated NBTI-resistant). Both forms, however, are inhibited by other inhibitors such as dipyridamole and dilazep. Some cells express only one or the other of these two equilibrative nucleoside transporters, but many cells express both in different proportions [2].

A second type of nucleoside transport, Na^+-dependent, concentrative nucleoside transport has been demonstrated in epithelial cells of the kidney and intestine of various mammalian species and brush border membranes thereof where it seems to be involved in absorption of nucleosides into the blood [2–13]. This function is suggested by its localization in the brush border membrane and the presence of an equilibrative nucleoside transporter in the basolateral membranes [7,12]. Two forms of the concentrative transporter have been distinguished on the basis of substrate specificity. The more common form transports uridine and purine nucleosides. It is also present in mouse and rat macrophages, mouse lymphocytes and all mouse cell lines that have been investigated [14–21]. However, in the mouse cell lines Na^+-dependent concentrative transport represents only a minor nucleoside transport component; equilibrative transport is the main component. No Na^+-dependent, concentrative nucleoside transport has been detected in mouse and rat erythrocytes, rat and pig lymphocytes, Novikoff rat hepatoma cells, Chinese hamster ovary cells, and three lines of human T cells [16,17].

A second form of concentrative transporter that is specific for pyrimidine nucleosides, adenosine and adenosine analogs has been detected in mouse intestinal epithelial cells and rat and bovine renal brush border membrane vesicles [5,11,13] but not in other types of cells [16,17]. Both concentrative nucleoside transporters are highly resistant to inhibition by NBTI and other inhibitors of equilibrative nucleoside trans-

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port, such as dipyridamole and dilazep [6–17]. The present study shows that a cloned porcine kidney cell line, PK-15, does not express either of the two Na^+-dependent, concentrative nucleoside transporters. This is surprising since PK-15 cells are believed to represent a single type of distal convoluted tubular cell expressing calcitonin-responsive cyclic AMP synthesis [22]. Lack of a concentrative nucleoside transporter is probably not simply a consequence of immortalization since lines of mouse, rat and rabbit kidney and of rat intestinal epithelial cells express considerable Na^+-dependent, concentrative nucleoside transport. Only equilibrative nucleoside transport is expressed by PK-15 cells, but at a much lower level than observed in many other cell lines. On the other hand, its affinity for cytidine and uridine, but not some other nucleosides, is higher than that of the equilibrative nucleoside transporter of other cell lines.

Experimental procedures

Cells. PK-15 cells have been derived from a clone of a porcine kidney cell line (PK 2a) that was originally established in 1955 [23]. They were obtained from the American Type Culture Collection (ATCC; CCL-33) and propagated in monolayer culture in T-flasks in RPMI supplemented with 4% (v/v) fetal bovine serum and 4% (v/v) bovine serum. The population doubling time was about 28 h. cAMP synthesis in the ATCC line has been shown to be greatly stimulated by treatment with calcitonin, but not by parathyroid hormone or vasopressin [22]. These cells express low alkaline phosphatase activity [22]. Their model chromosome number is 37, that is one less than the diploid number for pigs [23]. PK-15 cultures were routinely screened for mycoplasma contamination using a highly sensitive assay for adenosine phosphorylase activity [24,25]. Mammalian cells do not possess this enzyme, whereas it has been found in all mycoplasma species that have been analyzed. Except where indicated, all results are for mycoplasma-free cultures.

Transport measurements. Transport studies were conducted with monolayer cultures of PK-15 cells (see Fig. 1) or suspensions of these cells (see Fig. 2), which were prepared from monolayer cultures. In the former case, the cells were propagated in 24-well cell culture plates. When slightly subconfluent or confluent, the cultures were rinsed with balanced salt solution (BSS) or, where indicated, with isotonic choline chloride containing 5 mM Tris-HCl, pH 7.4 (Tris-choline chloride). Then the cultures were overlaid with 0.5 ml of RPMI or, where indicated, with Tris-choline chloride or isotonic NaCl containing 5 mM Tris-HCl, pH 7.4 (Tris-saline). Where indicated, the cells were preincubated in glucose-free basal medium containing 5 mM KCN, 5 mM iodoacetate and 100 μg gramicidin/ml to deplete the cells of ATP [26,27] and to abolish the Na^+-transmembrane gradient [15,16], respectively. [^{3}H]Formycin B and [^{3}H]thymidine uptake was measured by adding them, at timed intervals, to 4–6 wells of a plate. At the completion of the incubation, the medium was dumped out and the wells rapidly rinsed thrice (within 15 s) with ice-cold balanced salt solution (BSS) as described previously [16–18]. Where indicated the medium was supplemented with dipyridamole or NBTI at least 2 min before nucleoside uptake was measured. For determining the effects of other nucleosides on [^{3}H]formycin B or [^{3}H]thymidine uptake these were added along with the radiolabeled nucleosides [16–18].

For assessing the metabolism of the radiolabeled nucleosides, BSS-washed cell layers were extracted with 0.5 M trichloroacetic acid and the acid-insoluble material analyzed for radioactivity [26]. The acid extracts were further processed and analyzed by ascending paper chromatography using a solvent composed of 30 ml 1 M ammonium acetate (pH 5.0) and 70 ml of 95% ethanol (solvent 28) as described previously [14,26]. This procedure separates nucleoside tri-, di-, and mono-phosphates and nucleosides from each other.

For measuring the kinetics of cytidine, uridine, thymidine and formycin B transport, the PK-15 cells were propagated in large T-flasks, removed from the culture flasks by trypsinization and ATP-depleted by incubation in glucose-free medium containing 5 mM KCN and 5 mM iodoacetate [26,27]. For zero-trans influx measurements, samples of the suspension were incubated with KCN and iodoacetate at 37°C for 20 min, equilibrated at 25°C and then supplemented with six different concentrations of radiolabeled substrate. At various times of incubation at 25°C, the cells from 0.5 ml of each suspension were collected by centrifugation through an oil layer and analyzed for radioactivity as described previously [1,27,28]. For measurements of uridine equilibrium exchange, samples of the suspension were supplemented with six concentrations of unlabeled uridine along with KCN and iodoacetate and incubated for 50 min at 37°C and equilibrated at 25°C. Then the suspensions received [^{3}H] uridine at the same concentration as used for pre-equilibration and the uptake of radioactivity was measured as described already. Radioactivity in cell pellets was corrected for that attributable to extracellular space as estimated with [^{14}C]inulin [27,28]. Intracellular water space was measured with ^3H_2O [27,28]. The cells were enumerated with a Coulter counter. For estimating the kinetic parameters of zero-*trans* influx and equilibrium exchange appropriate integrated rate equations based on the simple carrier model were fitted to the time courses of uptake of the six nucleoside concentrations assuming directional symmetry of the carrier as described previously [1,2,28]. The Michaelis-Menten parameters were estimated by least-squares regression [1,2,28].

Equilibrium binding of NBTI. The equilibrium binding of [^{3}H] NBTI was measured with cells propagated in 24-well culture plates [17]. Cultures of cells in duplicate wells were overlaid with RPMI (0.5 ml/well) containing [^{3}H]NBTI to final concentrations of 0.05 to 10 nM. After 20 min of incubation at room temperature the culture fluid was analyzed for radioactivity. The wells were rinsed three times with ice-cold BSS and the rinsed cells analyzed for radioactivity. Measured radioactivity was converted to concentrations of free ligand (L_f) and bound ligand (L_b), respectively, and the data were analyzed by the method of Scatchard [29].

Materials. ^{3}H-labeled nucleosides were purchased from Moravek Biochemicals (Brea, CA), α-amino[1-^{14}C]isobutyrate from ICN (Irvine, CA) and unlabeled nucleosides and gramicidin from Sigma (St. Louis, MO).

Results and Discussion

First we compared the uptake of formycin B by PK-15 cells in isotonic NaCl and choline chloride media (Fig. 1A). Formycin B is a C-analog of inosine which is an efficient substrate for both equilibrative nucleoside transporters as well as the uridine-purine nucleoside-specific Na^+-dependent, concentrative transporter [2,6,13,15,16,30]. It is only poorly metabolized by mammalian cells so that its accumulation by cells can be measured unimpeded by significant metabolism [6,30]. Uptake of formycin B by the PK-15 cells was about the same in both media and strongly inhibited in both media by 10 μM dipyridamole. Furthermore, 1 μM NBTI inhibited formycin B uptake to about the same extent as 10 μM dipyridamole. The results indicate that the cells do not express Na^+-dependent concentrative transport of formycin B. This conclusion is supported by the finding that the cells did not accumulate formycin B in a concentrative manner during long term incubation in the presence of dipyridamole (Fig. 1C). Such concentrative accumulation of formycin B in the presence of dipyridamole has been observed in all cell lines that express the concentrative transporter even when it represents only a minor transport component [16,17]. It results from the inhibition of the efflux via equilibrative transport of the formycin B that is transported into the cells by the concentrative transporter which is resistant to dipyridamole [15–17]. Furthermore, formycin B uptake was inhibited by all

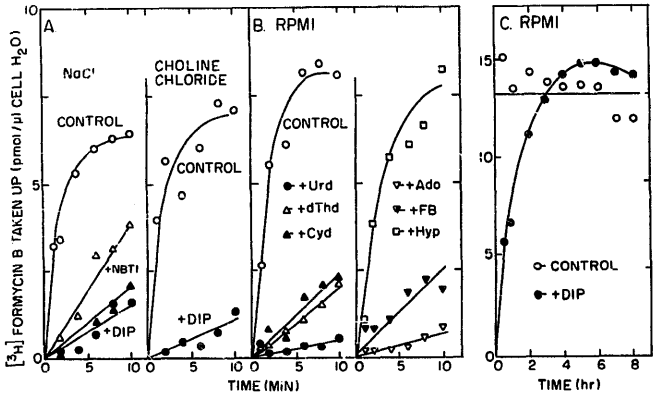

Fig. 1. Effects of Na^+-depletion, NBTI, dipyridamole and other nucleosides on the uptake of [^{3}H]formycin B by PK-15 cells. (A) Confluent cultures in 24-well plates ($4 \cdot 10^5$ cells/well) were rinsed with Tris-choline chloride and the cells overlayed with 0.5 ml of the same or Tris-saline and supplemented where indicated with 10 μM dipyridamole (DIP; ●——●) or 100 nM (△——△) or 1 μM (▲- - - -▲) NBTI. Then the uptake of 5 μM [^{3}H]formycin B (130 cpm/pmol) was measured at 25°C as described under Experimental procedures. (B) Similar plate cultures were rinsed with BSS, overlayed with 0.5 ml RPMI and then the uptake of 5 μM [^{3}H]formycin B was measured in the absence and presence of 200 μM uridine (Urd), thymidine (dThd), cytidine (Cyd), adenosine (Ado), formycin B (FB) or hypoxanthine (Hyp). (C) Two samples of a suspension of $4 \cdot 10^6$ PK-15 cells/ml of RPMI (harvested by trypsinization from monolayer cultures) were supplemented with 10 μM [^{3}H]formycin B (13 cpm/pmol) and one sample also with 10 μM dipyridamole. At various times of incubation at 37°C, the cells from 0.5-ml samples of each suspension were collected by centrifugation through an oil layer and analyzed for radioactivity.

nucleosides tested, which included thymidine and cytidine (Fig. 1B), which are not substrates for concentrative formycin B transporter [13,15,17]. Uridine and adenosine inhibited formycin B uptake most strongly (~ 97%), whereas hypoxanthine had no effect.

Formycin B has been reported not to be a substrate for the other, less common, Na^+-dependent concentrative nucleoside transporter of certain epithelial cells of the kidney and intestine whose substrates are pyrimidine nucleosides, adenosine and adenosine analogs [5,11,13]. The potential presence of this concentrative transporter in PK-15 cells was examined by measuring the uptake of 10 μM [^{3}H]thymidine by the cells (Figs. 2A–C). Thymidine is not a substrate for the uridine-purine nucleoside specific concentrative transporter [18]. At 10 μM, thymidine first accumulates in cells primarily in unmodified form because the K_m for the intracellular phosphorylation of thymidine in various types of cells is about 0.5 μM and the K_m and V_{max} for thymidine transport are at least 100-times higher than those for in situ thymidine phosphorylation [1,31]. This conclusion held for PK-15 cells. Chromatographic analysis of acid extracts showed that after 10 min of incubation with 10 μM [^{3}H]thymidine (see Fig. 2A), only 15–20% of the intracellular radioactivity was associated with nucleotides (data not shown); less than 5% was associated with acid-insoluble material. Thus, at concentrations $\geq$ 10 μM, thymidine transmembrane equilibration can be measured without significant interference by intracellular metabolism.

Thymidine uptake by the PK-15 cells was only little affected by combined ATP depletion and gramicidin treatment (Fig. 2A). The slight reduction in uptake effected by the treatment reflected the inhibition of intracellular thymidine phosphorylation, rather than of transport. The data in Figs. 2A–C also show that

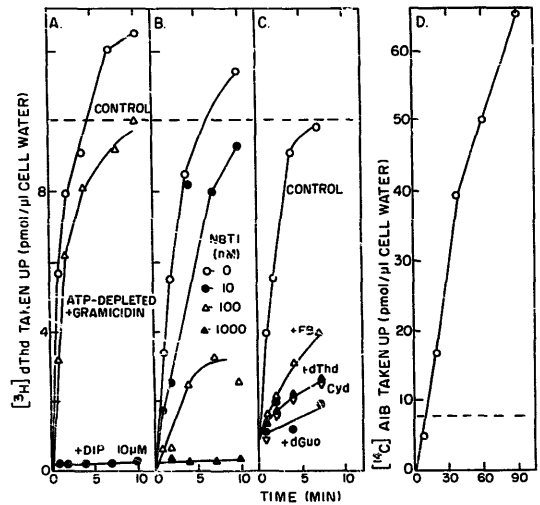

Fig. 2. Effects of ATP depletion, gramicidin treatment, dipyridamole, NBTI and other nucleosides on [^{3}H]thymidine uptake by PK-15 cells (A–C) and uptake of α-amino[^{14}C]isobutyrate by the cells (D). Confluent cultures in 24-well plates ($5.5 \cdot 10^5$ cells/well) were rinsed with BSS and overlayed with 0.5 ml RPMI, except for one set of cultures in (A) which received 0.5 ml of glucose-free basal medium containing 5 mM KCN, 5 mM iodoacetate and 100 μg gramicidin/ml and were preincubated at 37°C for 10 min (Δ —— Δ). Where indicated in (A) the medium was supplemented with 10 μM dipyridamole (DIP) or in (B) with various concentrations of NBTI. In (A) and (B), the uptake of 10 μM [^{3}H]thymidine (27 cpm/pmol) was then measured as described under Experimental procedures. Where indicated in (C), cytidine (Cyd), formycin B (FB), deoxyguanosine (dGuo) or unlabeled thymidine (dThd) were added to 300 μM simultaneously with the [^{3}H]thymidine. In (D), the uptake of 8 μM α-amino[^{14}C]isobutyrate (AIB; 92 cpm/pmol) was measured as described for nucleosides. The broken lines indicate the intracellular concentration of substrate equivalent to that in medium.

[^{3}H]thymidine uptake was almost completely inhibited by 10 μM dipyridamole and 1 μM NBTI and also inhibited by unlabeled formycin B, thymidine, cytidine and deoxyguanosine. Combined, the results indicate that PK-15 lack both of the two recognized Na^+-dependent, concentrative nucleoside transporters.

We also assessed whether PK-15 cells fail to express Na^+-dependent concentrative transport in general, perhaps because of a defect in the maintenance of a Na^+ transmembrane gradient. This is not the case since the cells concentratively accumulated 8 μM α-amino[^{14}C]isobutyrate (Fig. 2D).

The finding that NBTI inhibited formycin B and thymidine transport to about the same extent as dipyridamole (Figs. 1A and 2A and B) indicates that NBTI-sensitive, equilibrative transport is the primary nucleoside transporter of the PK-15 cells. However, the rate of formycin B and thymidine transport in these cells was significantly lower than observed with many other mammalian cell lines. This was also indicated by a kinetic analysis of zero-*trans* influx of various nucleosides in PK-15 cells that had been ATP-depleted by preincubation with KCN and iodoacetate in glucose-free medium to prevent nucleoside phosphorylation. The V_{max} values for uridine and thymidine zero-*trans* influx (V_{12}^{zt}; Figs. 3A and C) were only about 10% of those observed for many other cell lines [2]. This was also the case for the zero-*trans* influx of formycin B ($V_{12}^{zt} = 1.08 \pm 0.23$ μM/s) and of cytidine ($V_{12}^{zt} = 2.1 \pm 0.2$ μM/s, data not shown).

The Michaelis-Menten constants for the zero-*trans* influx of some, but not all, nucleosides by PK-15 cells were also much lower than those observed for other cell lines. For example, K_{12}^{zt} for uridine influx in PK-15 cells (44 μM; Fig. 3A) was only about 1/8 the value observed for many other mammalian cell lines [1,2]. Even more striking was the difference for cytidine influx ($K_{12}^{zt} = 63 \pm 19$ μM) which was about 1/20 of those observed in other cell lines (2–4 mM) [1,2]. On the other had, the K_{12}^{zt} for thymidine influx in PK-15 cells (108 μM, Fig. 3C) was only slightly lower than that for thymidine transport in other types of cells (150–250 μM) [1,2] and the K_{12}^{zt} for formycin B influx in PK-15 cells (317 ± 150 μM, data not shown) fell in the range observed for other cell lines (180–350 μM) [16].

The relatively low K_{12}^{zt} values for uridine and cytidine transport explain the unexpectedly strong inhibition of formycin B (Fig. 1B) and thymidine (Fig. 2C) influx by these nucleosides in PK-15 cells. For example, uridine inhibited [^{3}H]formycin B influx more strongly than thymidine or unlabeled formycin B (Fig. 1B). Cytidine was also strongly inhibitory at concentrations (200–300 μM) that have only a minor effect on the

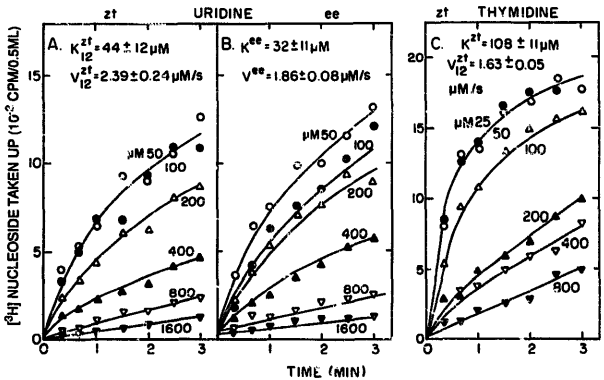

Fig. 3. Kinetic analyses of the transport of uridine and thymidine by ATP-depleted PK-15 cells. The cells were harvested from monolayer cultures by trypsinization and ATP depleted by incubation in glucose-free medium containing KCN and iodoacetate. Then the zero-*trans* influx of the indicated concentrations of (A) [^{3}H]uridine (400 cpm/μl, irrespective of concentration) or (C) [^{3}H]thymidine (270 cpm/μl, irrespective of concentration) was measured at 25°C as described under Experimental procedures. In (B) the samples of the suspension were incubated with 50–1600 μM unlabeled uridine along with the KCN and iodoacetate for 50 min at 37°C and then the equilibration of [^{3}H]uridine at the preincubation concentrations (400 cpm/μl, irrespective of concentration) was measured at 25°C. The kinetic parameters listed in each frame were estimated by integrated rate analysis of each set of uptake time courses.

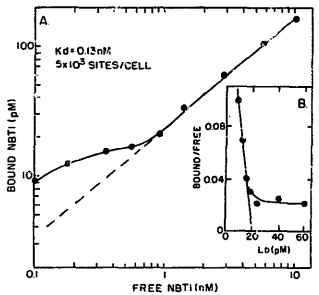

Fig. 4. Amounts of [^{3}H]NBTI bound at equilibrium to PK-15 cells as a function of the concentration of free NBTI (A) and Scatchard analysis of the data (B). The equilibrium binding of [^{3}H]NBTI at concentrations ranging from 0.1 to 21 nM was measured in confluent cultures of PK-15 cells in 24-well plates ($7 \cdot 10^5$ cells/well) as described under Experimental procedures. The kinetic parameters for binding listed in (A) were calculated by the method of Scatchard (B).

transport of other nucleosides in other types of cells because of the low affinity of the carriers of these cells for cytidine [1,2,32,33].

The data in Figs. 3A and B are of additional interest in that they indicate that $K^{zt} \approx K^{cc}$. This finding in combination with the fact that the first-order rate constant (V/K) was about the same for equilibrium exchange and zero-*trans* transport (see Fig. 3) indicate that the mobility of the equilibrative nucleoside transporter of PK-15 cells is the same whether or not it is loaded with substrate [1,2]. This is also the case for the equilibrative nucleoside transporters with lower substrate affinity found in all other mammalian cells that have been investigated, except for human, pig, and guinea pig erythrocytes. The transporters of the latter move several times more rapidly when substrate-loaded than when empty, at least when loaded with pyrimidine nucleosides [2,32,34,35]. This fact is most readily detected by higher V^{cc} than V^{zt} [2].

Consistent with a primary expression of NBTI-sensitive equilibrative nucleoside transport in PK-15 cells was the presence of high-affinity NBTI binding sites on these cells (Fig. 4). K_d values for NBTI binding observed in four independent experiments ranged from 0.13 nM (see Fig. 4) to 1 nM. However, the number of NBTI binding sites/PK-15 cell, which varied between $5 \cdot 10^3$ and $2 \cdot 10^4$ in these experiments, were much lower than those reported for many other cell lines [2,36], in spite of the much larger size of the PK-15 cells. The intracellular water volume of the PK-15 cells was 4–5 μl/10^6 cells, which compares to 0.7–1 μl/10^6 P388 or L1210 mouse leukemia cells and 1–3 μl/10^6 cells for various other cell lines [25,36]. The relatively low number of NBTI binding sites on PK-15 cells, however, is consistent with the low nucleoside transport activity of these cells. In fact, the carrier turnover number for the NBTI-sensitive transporter of these cells (about 100 molecules/carrier per s) falls within the range reported for those of other mammalian cell lines [2,34] in spite of the different substrate affinities of the carrier of the PK-15 cells.

In a previous study, we have observed that accidental contamination of cultures of S49 mouse leukemia cells with mycoplasma greatly enhanced the apparent concentrative accumulation of formycin B in the cultures because the mycoplasmas possess a highly efficient dipyridamole-resistant concentrative nucleoside transporter with broad substrate specificity and are tightly associated with the mammalian cells [25]. The mycoplasma remained associated with the S49 cells during collection of the latter by low-speed centrifugation as well as during centrifugation through an oil

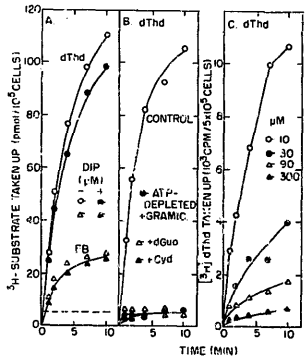

Fig. 5. [^{3}H]thymidine and [^{3}H]formycin B uptake by mycoplasma-contaminated cultures of PK-15 cells and effects of ATP-depletion/gramicidin treatment, dipyridamole and other nucleosides. (A) The uptake of 10 μM [^{3}H]thymidine (dThd; 29 cpm/pmol) and of 10 μM [^{3}H]formycin B (FB; 21 cpm/pmol) was measured in mycoplasma-contaminated PK-15 plate cultures ($3 \cdot 10^5$ cells/well) as described for uninfected cultures (see Figs. 1 and 2). Where indicated, the cultures were supplemented with 10 μM dipyridamole (DIP). (B) The uptake of 10 μM [^{3}H]thymidine was measured as in (A), except that, where indicated, the cultures were supplemented with 200 μM deoxyguanosine (dGuo) or cytidine (Cyd), or that the cultures were preincubated in glucose-free medium containing KCN, iodoacetate and gramicidin. (C) The uptake of the indicated concentrations of [^{3}H]thymidine (270 cpm/pl, irrespective of concentration) was measured in cultures of $5 \cdot 10^5$ cells/well as in (A). The broken line in (A) indicates the apparent intracellular concentration equivalent to that in the medium.

layer in the course of the nucleoside transport assay. We observed that mycoplasma contamination of monolayer cultures of PK-15 cells similarly resulted in the appearance of a concentrative nucleoside transport system, which resembled that of mammalian cells in its resistance to dipyridamole and energy dependence, but differed from the latter in its broad substrate specificity (Fig. 5). Fig. 5A shows that contaminated cultures accumulated 10 μM [^{3}H]thymidine to about 20 times and 10 μM [^{3}H]formycin B to about 6 times their concentrations in the medium during 10 min of incubation at 25°C. Chromatographic analysis of acid extracts of these cultures showed that > 95% of the cell-associated radioactivity was still located in unmodified thymidine. The uptake of thymidine and formycin B was little affected by dipyridamole (Fig. 5A), but thymidine uptake was abolished by combined ATP depletion and gramicidin treatment (Fig. 5B) and inhibited by 200 μM deoxyguanosine, cytidine (Fig. 5B), uridine and inosine (data not shown). The initial rate of uptake of thymidine by these cultures in absolute terms was about the same (2.5 pmol/5 · 10^5 PK-15 cells per s) whether the thymidine concentration in the medium was 10, 30, 90 or 300 μM (Fig. 5C), which indicates that the mycoplasma nucleoside transport system was saturated at 10 μM thymidine.

Mycoplasma contamination of the PK-15 cultures had no significant effect on the growth of the PK-15 cells or their microscopic appearance. It was readily detectable, however, by the presence of adenosine phosphorylase activity in the cultures which is a sure sign of the presence of mycoplasma [24,25]. The mycoplasmas must be tightly associated with the PK-15 cells since, after exposure to radiolabeled nucleosides, the adhering cultures were rinsed extensively with cold BSS before determination of cell-associated radioactivity. Concentrative accumulation of formycin B and thymidine was also observed in suspensions of PK-15 cells that were prepared by trypsinization of mycoplasma contaminated monolayer cultures, although at a lower level than was apparent in adhering cultures. These results indicate that the mycoplasmas became only partly dissociated from the PK-15 cells during trypsin treatment.

In summary, PK-15 cells express only NBTI-sensitive nucleoside transport in spite of possessing characteristics of renal distal tubular cells [22,23]. The carrier resembles that of other mammalian cells in its broad substrate specificity and carrier turnover number but it exhibits a much higher affinity for certain nucleosides. Especially striking is its apparent high affinity for cytidine. Why PK-15 cells lack a Na^+-dependent concentrative nucleoside transporter is unclear, since kidney epithelial cell lines from rats and mice express such system [6,13,17], just like the renal and intestinal brush border membranes of various species [3–12]. Perhaps a lack of a concentrative nucleoside transporter is a unique feature of pig kidney cells, or it could be that variants of PK-15 cells have been selected that lack a concentrative transporter during its long history of cell culture, but what selective pressure could have operated is unclear. Many mouse and rat cell lines express Na^+-dependent, concentrative nucleoside transport in spite of having been cultured for as long as the PK-15 cells [16,17]. That PK-15 cells possess only a single nucleoside transporter is also indicated by our recent single-step isolation, at a frequency of about $1.5 \cdot 10^{-6}$, of variants that lack all nucleoside transport activity (Aran and Plagemann, unpublished data). In studies of nucleoside transport in cultured mammalian cells great caution is required to avoid mycoplasma contamination because mycoplasmas possess a highly efficient concentrative nucleoside transport system, which resembles in certain properties the concentrative nucleoside transporters of mammalian cells, and because mycoplasmas are tightly associated with the mammalian cells.

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