Hypoxanthine enters human vascular endothelial cells (ECV 304) via the nitrobenzylthioinosine-insensitive equilibrative nucleoside transporter

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The transport properties of the nucleobase hypoxanthine were examined in the human umbilical vein endothelial cell line ECV 304. Initial rates of hypoxanthine influx were independent of extracellular cations: replacement of Na^+ with Li^+ , Rb^+ , *N*-methyl-D-glucamine or choline had no significant effect on hypoxanthine uptake by ECV 304 cells. Kinetic analysis demonstrated the presence of a single saturable system for the transport of hypoxanthine in ECV 304 cells with an apparent K_m of $320 \pm 10 \mu\text{M}$ and a $V_{\max}$ of $5.6 \pm 0.9 \text{ pmol}/10^6 \text{ cells per s}$. Hypoxanthine uptake was inhibited by the nucleosides adenosine, uridine and thymidine (apparent K_i 41 ± 6 , 240 ± 27 and $59 \pm 8 \mu\text{M}$ respectively) and the nucleoside transport inhibitors

nitrobenzylthioinosine (NBMPR), dilazep and dipyrindamole (apparent K_i 2.5 ± 0.3 , 11 ± 3 and $0.16 \pm 0.006 \mu\text{M}$ respectively), whereas the nucleobases adenine, guanine and thymine had little effect (50% inhibition at $> 1 \text{ mM}$). ECV 304 cells were also shown to transport adenosine via both the NBMPR-sensitive and -insensitive nucleoside carriers. Hypoxanthine specifically inhibited adenosine transport via the NBMPR-insensitive system in a competitive manner (apparent K_i $290 \pm 14 \mu\text{M}$). These results indicate that hypoxanthine entry into ECV 304 endothelial cells is mediated by the NBMPR-insensitive nucleoside carrier present in these cells.

INTRODUCTION

The synthesis of nucleotides in mammalian cells can occur by two separate routes: either the *de novo* pathway or salvage from exogenous nucleosides and nucleobases [1]. The first step in the salvage pathway is transport across the plasma membrane. Conversely, the catabolism of adenine nucleotides and adenosine gives rise to hypoxanthine, a process that is accelerated during ischaemia [2–5]. Subsequent post-ischaemic reperfusion results in re-oxygenation of the affected tissue and a burst in production of reactive oxygen species due to the action of xanthine oxidase on its substrate, hypoxanthine, and the transfer of electrons directly to molecular oxygen rather than NAD^+ [6]. The contribution of endothelial xanthine oxidase to the release of reactive oxygen species is still under debate, but the enzyme is distributed widely in capillary and small vessel endothelium in human tissues [7]. It has also been shown to be the primary source of reactive oxygen species when cultured human aortic endothelial cells are exposed to hypoxic conditions and re-oxygenated [8]. Thus, endothelial cells are likely to be an important source of the oxygen free radicals that mediate post-ischaemia damage. Despite the important role of hypoxanthine in both purine salvage and post-ischaemic injury, little is known about the transporters responsible for the influx and efflux of hypoxanthine in endothelial cells.

Studies on nucleobase transport have been restricted to a few cell types. Human erythrocytes possess a single saturable facilitated diffusion transport system that exhibits a medium affinity for purine bases (apparent K_m $13\text{--}180 \mu\text{M}$) but a low affinity for pyrimidine bases (apparent K_m ~ 4 and 5.8 mM for 5'-fluorouracil and uracil, respectively) [9–11]. This purine transporter is distinct from the nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter present in human erythrocytes and has

also been demonstrated to be present in certain lymphoma cells, human T-lymphoblastoid CCRF-CEM cells and pig renal epithelial LLC-PK₁ cells [12–15]. In other mammalian cell lines, such as Chinese hamster ovary cells, Chinese hamster lung cells, Ehrlich ascites cells and Novikoff rat hepatoma cells, hypoxanthine influx proceeds via the NBMPR-insensitive nucleoside carrier [16–18]. [The current established convention describes a facilitative diffusion nucleoside carrier as being NBMPR-insensitive (*ei*) when nucleoside fluxes via the transporter are resistant to inhibition by concentrations of NBMPR less than $1 \mu\text{M}$. However, in many cases micromolar concentrations of NBMPR will inhibit nucleoside transport catalysed by the NBMPR-insensitive transporter. In contrast, transport via the NBMPR-sensitive nucleoside transporter (*es*) is inhibited by NBMPR with an apparent IC_{50} value of $0.1\text{--}3 \text{ nM}$. Thus relative to the *es* transporter, the *ei* carrier is insensitive to inhibition by NBMPR.] However, the affinity for hypoxanthine influx via the NBMPR-insensitive nucleoside transporter in all cells investigated to date is low (apparent $K_m \sim 2\text{--}3 \text{ mM}$) with the notable exception of Novikoff rat hepatoma cells (apparent K_m $300\text{--}400 \mu\text{M}$) [18]. Evidence for the involvement of the NBMPR-sensitive nucleoside transporter in the translocation of nucleobases comes from studies with a single cell type, mouse S49 lymphoma cells [19]. However, the low efficiency of hypoxanthine transport via the NBMPR-sensitive nucleoside carrier in S49 cells raises doubts as to the physiological role of the transporter in mediating hypoxanthine flux. Further evidence for heterogeneity in the transport of nucleobases is the recent demonstration of sodium-dependent nucleobase transport in cultured renal epithelial cells and guinea-pig renal brush-border membrane vesicles and ventricular myocytes [14,20,21]. Of particular note is that the affinity of both purine and pyrimidine bases for this co-transporter is high (apparent K_m values in the

Abbreviations used: NBMPR, 6-[(4-nitrobenzyl)thio]-9- β -D-ribofuranosylpurine (nitrobenzylthioinosine); ECV 304, endothelial cell vein line; NMG⁺, *N*-methyl-D-glucamine.

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range 1–10 μM) and that adenine is not a permeant. Recently, a Na^+ -dependent nucleobase transporter in rabbit choroid plexus has been described with properties distinct from those nucleobase co-transporters in kidney and heart [22].

Given the above evidence for the presence of multiple transporters for nucleobases, the possible clinical interest in hypoxanthine being used as an indicator of tissue hypoxia [5], and our lack of knowledge of how hypoxanthine is removed from the vascular bed, we have investigated the properties of hypoxanthine transport in human endothelial cells. For this study we have used ECV 304 cells, a spontaneously transformed human umbilical vein endothelial cell line [23]. We demonstrate that hypoxanthine entry into ECV 304 cells proceeds via an NBMPR-insensitive nucleoside transporter with a medium affinity. A preliminary report of some of these results has been published in abstract form [24].

EXPERIMENTAL

Cell culture

ECV 304 cells were grown at 37 °C in a humidified air and 5 % CO_2 atmosphere in M199 medium supplemented with 10 % (v/v) fetal bovine serum, 3.2 mM glutamine, 100 I.U. penicillin/streptomycin and subcultured for serial passages [23]. Confluent cells were resuspended (2×10^5 cells/ml) and plated into 24-well tissue-culture plates and used for experiments 24–48 h later when the cells had reached confluency. The experiments were performed using cells between passages 110 and 126. Cells used in the present study were free of mycoplasma as determined using Hoechst 33258 stain.

Transport measurements

Zero-*trans* uptake of 1 μM [^3H]hypoxanthine (10 $\mu\text{Ci/ml}$) and [^3H]adenosine (1 $\mu\text{Ci/ml}$) was determined at 22 °C in phosphate-free medium as described previously [14]. In inhibition studies, test compounds and radioactive substrate (1 μM) were added to triplicate monolayer wells simultaneously, except for NBMPR, dipyrindamole or dilazep which were preincubated for 30 min before addition of radiolabel. In these inhibition studies, mediated transport was defined as that not inhibitable by a 1000-fold excess of unlabelled permeant. Transport was terminated by aspirating the transport medium and adding 1 ml of ice-cold buffer. In the case of adenosine transport measurements, the ice-cold buffer also contained 1 mM dilazep. The cell layer was rinsed with a further two washes of ice-cold medium. Radioactivity associated with monolayers at time zero was determined by exposing the cell layer simultaneously to radiolabelled medium and ice-cold solution. Cells were lysed by the addition of 0.2 ml of 0.5 M NaOH and incorporated cellular radioactivity determined by liquid scintillation counting. Cells in replicate wells were enumerated after trypsin/EDTA detachment. Control experiments using [^{14}C]mannitol (0.05–0.4 μCi) as an extracellular marker confirmed that less than 3 % of the radioactivity incorporated into cells was due to trapping of the radiolabel.

[^3H]NBMPR binding

Steady-state specific [^3H]NBMPR binding to triplicate monolayers of ECV 304 cells at 22 °C was determined as described previously for early-passage human umbilical vein cell monolayers [25].

Hypoxanthine metabolism

Intracellular [^3H]hypoxanthine in ECV 304 monolayers was analysed by TLC as described previously for adenosine [25].

Acid-soluble extracts (100 μl) were mixed with 20 μl of solution containing hypoxanthine, xanthine, inosine, uric acid and IMP. Samples (20–40 μl) were then chromatographed on silica-gel-coated plates containing a fluorescent indicator using butan-1-ol/ethyl acetate/methanol/ammonia (7:4:3:4, by vol.) [14]. The percentage recovery of radioactivity loaded on to the TLC plate was $101 \pm 6\%$ (mean $\pm$ S.E.M., $n = 4$).

Data analysis

All transport and binding experiments were carried out in triplicate. The errors given in Table 1 are $\pm$ S.E.M. and those in the Figures are $\pm$ S.D. The kinetic parameters of hypoxanthine influx were determined by non-linear regression analysis of self-inhibition as discussed recently for the uptake of D-glucose [26]. Least-square analysis of [^3H]NBMPR binding data was performed using the computer program Enzfitter (Elsevier, Biosoft) with the points weighted according to their S.D. The data from inhibition experiments were fitted to the mathematical equation describing either a single-site model or a two-site model.

Materials

Fetal calf serum was purchased from GIBCO. Medium 199, penicillin, streptomycin, NBMPR, dipyrindamole and nucleosides were obtained from Sigma. [$\text{G-}^3\text{H}$]Hypoxanthine (6.6 Ci/mmol), [2,8,5'- ^3H]adenosine (60 Ci/mmol) and D-[1- ^{14}C]mannitol (56 Ci/mmol) were obtained from Amersham International (Amersham, Bucks., U.K.). [$\text{G-}^3\text{H}$]NBMPR (23 Ci/mmol) was purchased from Moravsek Biochemicals (Brea, CA, U.S.A.). Dilazep was a generous gift from Hoffman-La Roche (Basel, Switzerland). All other reagents were of analytical grade.

RESULTS

Hypoxanthine uptake and metabolism by ECV 304 cells

The time course of 1 μM [^3H]hypoxanthine uptake in phosphate-free medium in the presence and absence of sodium by ECV 304 monolayers at 22 °C is shown in Figure 1. Uptake of hypo-

Figure 1 Time course of hypoxanthine uptake by confluent ECV 304 monolayers

Culture medium was aspirated and cell monolayers washed with three 1 ml aliquots of phosphate-free medium. Uptake of 1 μM hypoxanthine at 22 °C was initiated by the addition of 0.2 ml of transport medium in the presence of 140 mM NaCl (●) or 140 mM NMG⁺ (○). After the appropriate time interval, transport was terminated by washing the monolayer with three 1 ml aliquots of ice-cold medium. Cells were lysed with 0.5 M NaOH, and the incorporated radioactivity was determined.

Figure 2 Kinetic parameter determination of hypoxanthine transport in ECV 304 cells

ECV 304 cells were incubated with $1 \mu\text{M}$ [^3H]hypoxanthine and increasing amounts of unlabelled hypoxanthine (0–4.5 mM) and initial rates of total hypoxanthine transport determined. Non-mediated hypoxanthine influx was estimated from the residual flux in the presence of 4.5 mM hypoxanthine and subtracted from the total hypoxanthine uptake values. Non-linear regression analysis of the displacement curve (A) of the mediated transport gave K_m and $V_{\max}$ values of $308 \pm 25 \mu\text{M}$ and $3.8 \pm 0.2 \text{ pmol}/10^6 \text{ cells per s}$ with a Hill slope of 0.9 ± 0.12 . The concentration dependence plot of the data is shown in (B).

xanthine was linear for approx. 1 min and reached a steady-state plateau by 5 min. The initial rate of hypoxanthine influx was estimated by fitting a monoexponential curve to the data as described previously for adenosine uptake [27]. The average rate of $1 \mu\text{M}$ [^3H]hypoxanthine influx from five separate experiments was $2.8 \pm 0.2 \text{ pmol}/10^6 \text{ cells per min}$ (mean $\pm$ S.E.M.). This value was not significantly different from that estimated using the 60 s uptake rate ($2.4 \pm 0.2 \text{ pmol}/10^6 \text{ cells per min}$). Thus, a time interval of 60 s was used in further studies to approximate initial rates of hypoxanthine transport.

Figure 1 also shows the effect of the presence or absence of extracellular Na^+ on hypoxanthine influx in ECV 304 cells. An apparent inwardly directed Na^+ electrochemical gradient had no effect on the initial rate of hypoxanthine influx as compared with the flux in the presence of *N*-methyl-D-glucamine (NMG $^+$). Similarly, no differences were found in the initial rate of $1 \mu\text{M}$ [^3H]hypoxanthine influx when Na^+ was replaced by the monovalent cations Li^+ , Rb^+ or choline (chloride salts at 140 mM) (results not shown). Analysis by TLC of intracellular ^3H in ECV 304 cells following an incubation period of 1 min with $1 \mu\text{M}$ [^3H]hypoxanthine revealed that $79.8 \pm 1.6 \%$ ($n = 4$) of the intracellular radioactivity was recovered as unmetabolized hypo-

Table 1 Effect of nucleobases, nucleosides and transport inhibitors on [^3H]hypoxanthine uptake in ECV 304 cells

Zero-trans uptake (60 s) of $1 \mu\text{M}$ [^3H]hypoxanthine was determined at 22°C in phosphate-free medium containing various concentrations of test compound. The mediated component of hypoxanthine influx was calculated as the difference in the rate of influx in the presence and absence of 1 mM hypoxanthine. The uptake rates were plotted as a percentage of the mediated control flux as a function of the concentration of test compounds (see Figure 3) and the IC_{50} values determined. Inhibition constant (K_i) values were calculated from the equation $K_i = \text{IC}_{50}/(1 + L/K_m)$, where the K_m value was taken as $320 \mu\text{M}$ and $L = 1 \mu\text{M}$ hypoxanthine. The values are the means $\pm$ S.E.M. of three separate experiments.

Inhibitor	Apparent K_i value (μM)
Adenine	2700 ± 820
Thymine	1400 ± 500
Uracil	1700 ± 220
Adenosine	41 ± 6
Uridine	240 ± 27
Thymidine	59 ± 8
NBMPR	2.5 ± 0.3
Dilazep	11 ± 3
Dipyridamole	0.16 ± 0.01

xanthine, with the remaining ^3H associated with metabolic products. Phosphoribosylation of hypoxanthine by ECV 304 cells in the absence of phosphate is thus low, similar to that reported previously with LLC-PK $_1$ cells [14].

Concentration dependence of hypoxanthine transport

The initial rate of hypoxanthine influx by ECV 304 cells was measured at extracellular hypoxanthine concentrations ranging from $10 \mu\text{M}$ to 1.5 mM (Figure 2). Hypoxanthine influx was composed of a saturable component and conformed to a single transporter system with simple Michaelis–Menten kinetics. The mean kinetic hypoxanthine influx parameters, determined by non-linear regression analysis, were an apparent K_m value of $320 \pm 10 \mu\text{M}$ and a $V_{\max}$ value of $5.6 \pm 0.9 \text{ pmol}/10^6 \text{ cells per s}$ (mean $\pm$ S.E.M. from three separate experiments). The goodness of fit for a one-site model was confirmed by the Hill coefficients not being statistically different from 1 (0.95 ± 0.07).

Inhibition of hypoxanthine transport

The specificity of hypoxanthine uptake was studied by investigating the ability of selected nucleosides, nucleobases and transport inhibitors to inhibit $1 \mu\text{M}$ [^3H]hypoxanthine influx by ECV 304 cells. The results are summarized in Table 1 and Figure 3. High concentrations of purine and pyrimidine nucleobases had little effect on hypoxanthine uptake, with K_i values $> 1 \text{ mM}$. For example, no significant inhibition of hypoxanthine influx was observed by adenine at concentrations less than 1 mM (Figure 3). In contrast, nucleosides and the inhibitors NBMPR, dipyridamole and dilazep were potent inhibitors of hypoxanthine influx by ECV 304 cells. Adenosine was more potent (K_i value $41 \pm 6 \mu\text{M}$) than the pyrimidine nucleosides uridine and thymidine [K_i values 240 ± 27 and $59 \pm 8 \mu\text{M}$ (see Table 1) respectively]. The classical facilitated diffusion nucleoside transport inhibitor, NBMPR, a potent inhibitor ($K_i = 0.11 \text{ nM}$) of adenosine influx in early-passage human umbilical vein endothelial cells [25], inhibited hypoxanthine influx with an apparent K_i value of $2.5 \pm 0.3 \mu\text{M}$. The nucleoside transport inhibitor dipyridamole was approx. 15-fold more potent than NBMPR at inhibiting hypoxanthine influx, with an apparent K_i value of $0.16 \pm 0.01 \mu\text{M}$, whereas dilazep was 4-fold less potent (apparent K_i $11 \pm 3 \mu\text{M}$).

Figure 3 Effect of adenosine, uridine and adenine on hypoxanthine influx by ECV 304 monolayers

The initial rate of $1 \mu\text{M}$ [^3H]hypoxanthine influx was determined in the presence of adenosine (●), uridine (▲) and adenine (▼). The results are from a single experiment and expressed as a percentage of the control influx rate ($0.64 \pm 0.03 \text{ pmol}/10^6 \text{ cells per min}$) (see Table 1 for apparent K_i values).

Figure 4 Dose-response curve for NBMPR inhibition of adenosine uptake in ECV 304 monolayers

Confluent ECV 304 monolayers were incubated with 1 ml aliquots of graded concentrations of NBMPR for 30 min. The medium was then removed and the initial rate of [^3H]adenosine influx ($1 \mu\text{M}$, 30 s) determined at 22°C in the presence of a similar concentration of NBMPR to that used in the preincubation medium.

Adenosine transport and NBMPR binding by ECV 304 cells

The results of Figure 3 and Table 1 raise the possibility that hypoxanthine influx into ECV 304 cells is being mediated by a nucleoside carrier. To explore further the relationship between hypoxanthine and nucleoside transport, we investigated the nucleoside transport properties of ECV 304 cells. In preliminary experiments, we demonstrated that initial rates of adenosine influx by ECV 304 cells could be determined using an incubation interval of 30 s. Figure 4 shows that the initial rate of zero-*trans* adenosine influx ($1 \mu\text{M}$) by ECV 304 cells is inhibited by NBMPR in a biphasic manner, with 70–80% inhibition achieved at $< 100 \text{ nM}$ (mean IC_{50} value $0.011 \pm 0.002 \text{ nM}$, $n = 3$). The residual transport activity was blocked at NBMPR concentrations above $1 \mu\text{M}$ with an IC_{50} value of $390 \pm 110 \text{ nM}$ ($n = 3$). Thus, ECV 304 cells are similar to many other cultured cell lines in expressing both NBMPR-sensitive and -insensitive nucleoside

Figure 5 Effect of hypoxanthine on adenosine influx by ECV 304 monolayers

ECV 304 cells were preincubated in the presence (○) and absence (●) of 100 nM NBMPR for 15 min. The initial rate of $1 \mu\text{M}$ [^3H]adenosine influx was then determined at 22°C in the presence of various concentrations of hypoxanthine. The results are given as a percentage of the control flux (20.6 ± 1.3 and $2.4 \pm 0.12 \text{ pmol}/10^6 \text{ cells per min}$, in the absence and presence of NBMPR, respectively).

Figure 6 Dixon plot of hypoxanthine influx inhibition by adenosine

The reciprocals of mediated hypoxanthine influx at 30 (●), 60 (▲) and 120 (▼) μM are plotted against the respective concentrations of adenosine. Apparent $K_i = 47 \mu\text{M}$.

transporters [16]. A functional separation between the two transporters in ECV 304 cells was achieved at 100 nM NBMPR (see Figure 4).

Confirmation of the presence of an NBMPR-sensitive nucleoside transporter in ECV 304 cells was obtained with the observation that these cells possess specific [^3H]NBMPR binding sites. Steady-state values for [^3H]NBMPR binding to monolayers of ECV 304 cells were: apparent K_d $0.44 \pm 0.06 \text{ nM}$ ($n = 3$) and B_{max} $0.33 \pm 0.04 \text{ pmol}/10^6 \text{ cells}$ ($n = 3$).

Inhibition of adenosine transport by hypoxanthine

Figure 5 shows the inhibition of $1 \mu\text{M}$ [^3H]adenosine uptake by hypoxanthine in the presence and absence of 100 nM NBMPR in ECV 304 cells. At high hypoxanthine concentrations (4 mM) the inhibition of adenosine influx in the absence of NBMPR was

approx. 30 %. The percentage of residual transport was similar to the magnitude of the NBMPR-sensitive nucleoside transporter (Figure 4). In contrast, when 100 nM NBMPR was added to the assay medium inhibition was almost complete. In addition, the K_i value ($290 \pm 14 \mu\text{M}$) obtained for hypoxanthine inhibition of adenosine transport in the presence of 100 nM NBMPR was similar to the K_m value ($320 \pm 10 \mu\text{M}$) for hypoxanthine uptake, suggesting that an NBMPR-insensitive nucleoside transporter mediates hypoxanthine entry into ECV 304 cells.

Kinetics of adenosine inhibition of hypoxanthine transport

To investigate the kinetics of inhibition of hypoxanthine uptake by adenosine, we examined the effect of various concentrations of adenosine in the presence of 100 nM NBMPR on hypoxanthine influx into ECV 304 cells. Figure 6 shows the results of one such experiment where the data are plotted as $1/v$ versus I (Dixon plot), where I is the inhibitor concentration. The plot was consistent with competitive inhibition and gave an apparent K_i value of $47 \mu\text{M}$.

DISCUSSION

The principal findings of this work are: (1) hypoxanthine is translocated across the plasma membrane of ECV 304 endothelial cells by a single saturable, medium-affinity transport system; and (2) this system is inhibited by nucleosides but not by purine and pyrimidine nucleobases and is equivalent to the NBMPR-insensitive nucleoside transporter.

As reviewed in the Introduction section, there are a number of different pathways by which hypoxanthine can enter animal cells. This study demonstrates for the first time that in the human endothelial cell line ECV 304, hypoxanthine entry is saturable, shows no dependency on the presence of sodium, and is mediated by a carrier that exhibits a medium affinity for the purine (apparent K_m $320 \pm 10 \mu\text{M}$). This affinity is similar to that observed in human erythrocytes [9,11], but in all other properties there is no resemblance between hypoxanthine influx in human erythrocytes and ECV 304 cells. Most notable is the lack of inhibition of hypoxanthine flux in ECV 304 cells by adenine and thymine, whereas nucleosides are potent and competitive inhibitors (apparent K_i $41 \pm 6 \mu\text{M}$ for adenosine). ECV 304 cells were shown to possess both NBMPR-sensitive and -insensitive nucleoside transport pathways, and NBMPR inhibited hypoxanthine influx at concentrations consistent with the involvement of the NBMPR-insensitive nucleoside transporter in the translocation of hypoxanthine influx. Moreover, blockade of the NBMPR-sensitive nucleoside transporter with 100 nM NBMPR, a condition that does not affect the operation of the NBMPR-insensitive nucleoside carrier, resulted in hypoxanthine totally inhibiting adenosine influx (Figure 5). Furthermore, the apparent K_i value for inhibition of the NBMPR-insensitive component of adenosine influx (apparent K_i $290 \pm 14 \mu\text{M}$) was identical to the apparent K_m for hypoxanthine influx in ECV 304 cells. These results are entirely consistent with the proposal that hypoxanthine entry into ECV 304 endothelial cells is mediated by the NBMPR-insensitive nucleoside carrier present in these cells.

The characteristics of hypoxanthine entry mediated by the NBMPR-insensitive nucleoside transporter differ in one important respect from those in previous studies that have indicated an involvement of this carrier in the transport of nucleobases. With the exception of Novikoff rat hepatoma cells, hypoxanthine influx via the NBMPR-insensitive nucleoside carrier in other mammalian cultured cells has a low affinity (range 2–3 mM) [12,16–18]. In contrast, the NBMPR-insensitive nucleoside trans-

porter systems in both ECV 304 cells and Novikoff cells [16,18] have a 10-fold higher affinity for hypoxanthine. The reason for this difference in affinity between different cells is not known. Nevertheless, these results indicate that isoforms of the NBMPR-insensitive nucleoside carrier exist with respect to their affinity for hypoxanthine.

The present results also raise questions as to how universal the involvement of the NBMPR-insensitive nucleoside carrier is in the transport of hypoxanthine by endothelial cells. For example, in early-passage human umbilical vein endothelial cells we did not detect significant NBMPR-insensitive adenosine transport [25], and our preliminary results have indicated that hypoxanthine influx in those cells occurs by a transporter selective for nucleobases [24]. Clearly additional studies will be required with endothelial cells derived from a number of different tissue beds to determine the possible heterogeneity in nucleobase transport in endothelial cells.

ECV 304 and early-passage human umbilical vein cells also differ in the number of NBMPR-sensitive nucleoside transporters present per cell. Assuming that each NBMPR-binding site represents a single NBMPR-sensitive nucleoside transporter, ECV 304 cells are estimated to have 2×10^5 sites/cell compared with 1.2×10^6 sites/cell for human umbilical vein endothelial cells [25]. Thus, it would appear that the nature of the spontaneous transformation in generating ECV 304 cells resulted not only in the detectable appearance of the NBMPR-insensitive nucleoside transporter but also in a decrease in the number of NBMPR-sensitive nucleoside carriers compared with normal umbilical vein endothelial cells.

This work was supported by the Wellcome Trust and the Nuffield Foundation. During this study N.O. was supported in part by the British Council. We thank Dr. David Morgan for providing us with ECV 304 cells, Luis Sobrevia for his helpful comments and Dulce Carvalho for her kind assistance.

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Received 11 March 1996; accepted 12 April 1996