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[^{3}H]Adenosine transport in DDT$_1$ MF-2 smooth muscle cells: inhibition by metabolites of propentofylline

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Abstract

Adenosine receptor signal transduction mechanisms have previously been characterized in Syrian hamster smooth muscle DDT$_1$ MF-2 cells but adenosine transport in these cells has not. DDT$_1$ MF-2 cells possess a high density (370000 sites/cell) of high affinity (K_d value of 0.26 nM) binding sites for [^{3}H]nitrobenzylthioinosine, a marker for the equilibrative and inhibitor-sensitive subtype of nucleoside transporters. Transport of [^{3}H]adenosine was insensitive to Na^+ and was inhibited by the nucleoside transport inhibitors nitrobenzylthioinosine, dilazep and dipyridamole with IC_{50} values of 1, 13 and 270 nM, respectively. Propentofylline, a neuroprotective compound that can inhibit nucleoside transporters, is rapidly metabolized in vivo to the racemate (±)-A720287. Based on recent findings that some transport inhibitors exhibit marked stereoselectivity, we tested the degree to which individual stereoisomers of (±)-A720287 affect adenosine transport. Propentofylline inhibited [^{3}H]adenosine transport in DDT$_1$ MF-2 cells with an IC_{50} value of 24 μM. (±)-A720287 and the individual stereoisomers (+)-833791 and (−)-844261 had similar potency to propentofylline for inhibition of [^{3}H]adenosine transport in DDT$_1$ MF-2 cells as well as in clonal mouse leukemia L1210/B23.1 cells, cells which possess only the equilibrative and inhibitor-sensitive subtype of nucleoside transporters. Thus, the neuroprotective effects of propentofylline may be due, in part, to the primary metabolites of propentofylline.

Keywords: Nitrobenzylthioinosine; Adenosine; Nucleoside transport; Propentofylline

1. Introduction

Adenosine is a product of ATP metabolism that can bind to and stimulate a family of cell surface receptors and produce a wide range of physiological effects. The concentration of adenosine in the extracellular space available to interact with its receptors is regulated by nucleoside transporters that catalyze the movement of nucleosides across plasma membranes. At least seven functionally distinct nucleoside transport processes have been characterized from various cell types and species (Cass, 1995) and these processes are broadly classified into two groups: Na^+-dependent and Na^+-independent. Na^+-dependent nucleoside transport processes are coupled to movements of Na^+ across cell membranes and are capable of concentrating nucleosides intracellularly while the Na^+-independent transporters are equilibrative processes that can move nucleosides in either direction across cell membranes.

The best-characterized nucleoside transporter is the equilibrative process that is sensitive to inhibition by low nanomolar concentrations of the nucleoside analogue nitrobenzylthioinosine; this widely distributed transporter has been termed *es* (equilibrative, sensitive) (Cass et al., 1974; Belt, 1983; Vijayalakshmi and Belt, 1988). In many cell types, binding of [^{3}H]nitrobenzylthioinosine corresponds stoichiometrically to inhibition of nucleoside transport, thus [^{3}H]nitrobenzylthioinosine is a high affinity and selective marker for *es* transporters (Cass et al., 1974). Inhibitors of equilibrative transporters, such as nitrobenzylthioinosine, dipyridamole and dilazep, block the carrier-mediated transfer of adenosine across cell membranes. By inhibiting the removal of adenosine from the interstitium, where adenosine has access to its specific receptors, transport inhibitors have been proposed to enhance and/or

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prolong the receptor-mediated effects of endogenous adenosine (Van Belle, 1988; Geiger and Fyda, 1991; Marangos et al., 1990; Jacobson et al., 1991). In support of this, propentofylline inhibits nucleoside transport processes at concentrations that are associated with neuroprotection in vivo (Parkinson et al., 1993) and it has been proposed that the elevation of endogenous adenosine levels produce, or at least contribute to, the neuroprotective effects of propentofylline (Parkinson et al., 1994). However, propentofylline is rapidly reduced in vivo to the racemic mixture (±)-A72 0287 (Macdonald et al., 1986) and other nucleoside transport inhibitors like draflazine and R88016, the enantiomers of (±)-R75 231, have been shown to be stereoselective in blocking adenosine transport (Beukers et al., 1994; Van Belle et al., 1993).

Clonal Syrian hamster DDT_1 MF-2 smooth muscle cells have been used extensively to investigate signal transduction pathways for adenosine A_1 and A_2 receptors (Ramkumar et al., 1990; Gerwins and Fredholm, 1992; Gerwins et al., 1990). The purpose of this study was to characterize the nucleoside transport processes in these cells and evaluate the inhibitory effects of propentofylline and its hydroxy-metabolites. We found that DDT_1 MF-2 cells possess a high density of low capacity *es* transporters that are inhibited with nearly equal potency by low micromolar concentrations of propentofylline, (±)-A72 0287, (+)-A83 3791 and (−)-A84 4261.

2. Materials and methods

2.1. Materials

[^{3}H]Adenosine, [^{3}H]nitrobenzylthioinosine, ^3H_2O (5 mCi/ml) and [^{14}C]polyethylene glycol (20 mCi/g) were from DuPont Canada (Mississauga, Ontario). Dipyridamole, adenosine, uridine, thymidine, *N*-methyl-D-glucammonium and Triton X-100 were purchased from the Sigma Chemical Co. (St. Louis, MO). Nitrobenzylthioinosine was purchased from Research Biochemicals International (Natick, MA). Dulbecco's modified Eagle's medium and fetal bovine serum were obtained from Gibco BRL (Burlington, Ontario). Dilazep was provided by F. Hoffmann-La Roche (Basel, Switzerland). Propentofylline, (±)-A72 0287, (+)-A83 3791 and (−)-A84 4261 were provided by Dr. K.A. Rudolphi, Hoechst (Frankfurt, Germany).

2.2. Cell culture

DDT_1 MF-2 smooth muscle cells, originally isolated from a steroid-induced leiomyosarcoma of Syrian hamster vas deferens (Norris et al., 1974), were obtained from the American Type Culture Collection. Cells were grown in suspension at 37°C in 5% CO_2/95% humidified air and maintained as exponentially proliferating cultures at cell densities of 3×10^4 to 3×10^5 cells/ml in Dulbecco's modified Eagle's medium supplemented with 4.5 g/l glucose, supplemented with 10% fetal calf serum, and 2 mM L-glutamine (Gerwins et al., 1990).

2.3. [^{3}H]Nitrobenzylthioinosine binding

Cells were harvested, washed twice ($100 \times g$, 5 min) and resuspended in physiological buffer (Na^+ buffer; in mM: Tris, 20; K_2HPO_4, 3: NaCl, 120; $MgCl_2$, 1.0; $CaCl_2$, 1.2; glucose, 10; to pH 7.4 with HCl). Saturation binding experiments were performed using [^{3}H]nitrobenzylthioinosine at concentrations of 0.01–5 nM and using 25 000 cells per ml assay volume. Site-specific binding of [^{3}H]nitrobenzylthioinosine was the difference between binding in the absence and presence of dilazep (100 μM) or unlabelled nitrobenzylthioinosine (1 μM). Cells were incubated (22°C) with radioligand for 1 h, a time period that allowed equilibrium binding to be achieved (data not shown), and reactions were terminated by filtration through Whatman GF/B filters using a Brandel cell harvester. [^{3}H]Nitrobenzylthioinosine concentrations were corrected for ligand depletion. [^{3}H]Nitrobenzylthioinosine binding constants (K_d and B_{max}) were obtained using nonlinear regression analysis.

Inhibition of [^{3}H]nitrobenzylthioinosine binding was determined using 0.5 nM [^{3}H]nitrobenzylthioinosine and 8–10 concentrations of the nucleoside transport inhibitors nitrobenzylthioinosine, dipyridamole or dilazep. Inhibition constants (K_i values) for inhibition of [^{3}H]nitrobenzylthioinosine binding were obtained by nonlinear regression analysis and application of the Cheng and Prusoff equation, $K_i = IC_{50}/(1 + [L]/K_d)$ (Cheng and Prusoff, 1973).

2.4. [^{3}H]Adenosine accumulation measurements

Cells were harvested by centrifugation ($100 \times g$ for 10 min), washed twice and resuspended (10^6 cells/ml) in either Na^+ buffer or *N*-methyl-D-glucammonium buffer (NMG^+ buffer) containing (in mM) Tris, 20; K_2HPO_4, 3; *N*-methyl-D-glucamine, 120; glucose, 10; $MgCl_2$, 1.0; $CaCl_2$, 1.2; to pH 7.4 with HCl. Osmolarity of the buffers was adjusted, as necessary, to 300 ± 10 mosM.

[^{3}H]Adenosine uptake was determined by an oil-stop centrifugation method (Harley et al., 1982). Briefly, a reaction mixture (100 μl) containing 1 μM [^{3}H]adenosine (2.5 μCi/ml) in either Na^+ or NMG^+ buffer was layered over 200 μl oil (85 parts silicon oil: 15 parts paraffin oil) in a microcentrifuge tube. Uptake was initiated by rapid addition of cell suspensions (100 μl) to reaction mixtures and was terminated by centrifugation of cells through oil ($16\,000 \times g$, 30 s). Supernatant fractions were aspirated, tubes were washed three times with water, oil was removed, and pellets were dissolved in 5% Triton X-100 for determination of radioactivity. Intracellular water volume

was the difference between the total water space of cell pellets, determined with ^3H_2O (1 μCi/ml) and the extracellular space, determined with [^{14}C]polyethylene glycol (2 μCi/ml), and was (mean ± S.E.M.) 1.24 ± 0.08 pl/cell (n = 65). Linear regression was used to calculate the initial rates of [^{3}H]adenosine uptake.

2.5. Adenosine transport kinetics

The concentration dependence of adenosine uptake was determined by incubating cells with graded concentrations of [^{3}H]adenosine (0.5–100 μM; 2.5 μCi/ml) for 1 s or 15 s. Comparisons were made between cells in Na^+- or NMG^+-containing buffer. The differences between the 1 s (linear with concentration of [^{3}H]adenosine, data not shown) and the 15 s values were analyzed by nonlinear regression to obtain kinetic constants (K_m and V_{max} values) for [^{3}H]adenosine influx.

2.6. Inhibition of [^{3}H]adenosine transport

Inhibition of [^{3}H]adenosine transport was determined using 1 μM [^{3}H]adenosine and 8–10 concentrations of the nucleoside transport inhibitors, nitrobenzylthioinosine, dipyridamole, dilazep, propentofylline, (±)-A72 0287, (+)-A83 3791 or (−)-84 4261. Cells were preincubated (15 min) with inhibitor prior to initiation of transport assays, which were 15 s. Inhibition of [^{3}H]adenosine transport by the nonisotopic nucleoside permeants adenosine, uridine and thymidine was also evaluated. Cells were not preincubated with nucleoside to avoid intracellular accumulation prior to initiation of uptake. Uptake intervals of 15 s were used. IC_{50} values for inhibition of [^{3}H]adenosine transport were obtained using nonlinear regression analysis.

2.7. Mouse leukemia L1210/B23.1 cells

Clonal mouse leukemia L1210/B23.1 cells were maintained as exponentially proliferating cultures at cell densities of 0.5–5 × 10^5 cells/ml in RPMI 1640 culture medium supplemented with 10% heat-inactivated horse serum. The nucleoside transport properties of these cells have been described previously (Vijayalakshmi et al., 1992; Parkinson et al., 1993).

[^{3}H]Nitrobenzylthioinosine binding assays were performed as described above using 1 × 10^5 cells per assay and 8–10 concentrations of the inhibitors propentofylline, (±)-A72 0287, (+)-A83 3791 and (−)-A84 4261. Inhibition of [^{3}H]adenosine transport was performed as described for DDT_1 MF-2 cells except that 6 s uptake intervals were used.

2.8. Data analysis

[^{3}H]Adenosine transport measurements were in triplicate and [^{3}H]nitrobenzylthioinosine binding measurements were in duplicate. Each experiment was performed at least three times and all values are reported as mean ± S.E.M. unless otherwise indicated. Nonlinear regression was performed using the software package GraphPad PRISM.

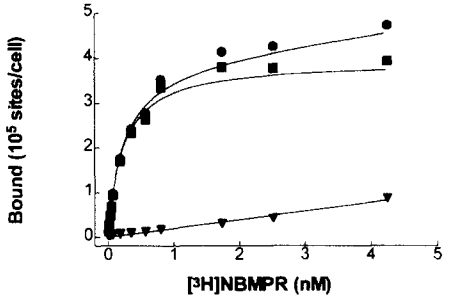

Fig. 1. Concentration dependence of the binding of [^{3}H]nitrobenzylthioinosine to DDT_1 MF-2 cells. The difference between the total (●) and non-specific (▼, determined in the presence of dilazep, 100 μM) is the specific binding component (■). Points are means of two determinations; data are from one experiment representative of five.

3. Results

The binding of [^{3}H]nitrobenzylthioinosine to DDT_1 MF-2 cells was saturable, with a K_d value of 0.26 ± 0.06 nM and a B_{max} value of 370 000 ± 38 000 sites per cell (n = 5) (Fig. 1). Concentration-dependent inhibition of the binding of [^{3}H]nitrobenzylthioinosine by nucleoside transport inhibitors resulted in a rank order of potency of nitrobenzylthioinosine > dilazep > dipyridamole (Table 1).

Initial rates of uptake were determined following exposure of cells to 1 μM [^{3}H]adenosine for time intervals of up to 30 s (Fig. 2). Accumulation of [^{3}H]adenosine was found to be linear, with initial rates of uptake for cells in Na^+ buffer of 0.59 ± 0.16 pmol/μl cell water/s (n = 7). A similar rate of uptake, 0.63 ± 0.11 pmol/μl cell water/s (n = 6), was apparent for cells in NMG^+ buffer. Accumulation of [^{3}H]adenosine in either buffer was abolished by 1 μM nitrobenzylthioinosine (Fig. 2), 10 μM dipyridamole or 10 μM dilazep (data not shown). To test further for Na^+-dependent transport, the concentration dependence of [^{3}H]adenosine influx was compared between cells in Na^+ and NMG^+ buffer and the kinetic constants, K_m and V_{max}, were not significantly different (Table 2).

Table 1
Inhibition constants for inhibition of [^{3}H]nitrobenzylthioinosine binding to DDT_1 MF-2 cells by nucleoside transporter inhibitors

Inhibitor	K_i ± S.E.M. (n)
Nitrobenzylthioinosine	0.6 ± 0.3 nM (3)
Dilazep	4.6 ± 1.9 nM (3)
Dipyridamole	153 ± 25 nM (3)

Inhibition of site-specific [^{3}H]nitrobenzylthioinosine binding was determined using 0.5 nM [^{3}H]nitrobenzylthioinosine and and 8–10 concentrations of nucleoside transport inhibitors. K_i values were obtained using nonlinear regression and application of the Cheng and Prusoff equation.

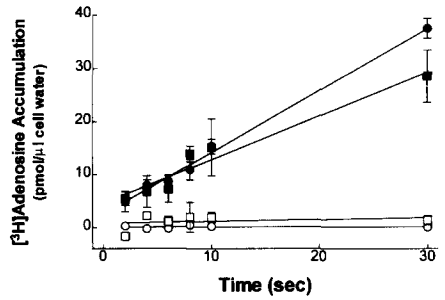

Fig. 2. Time course of uptake of [^{3}H]adenosine (1 μM) using rapid time intervals. DDT_1 MF-2 cells were prepared in Na^+ buffer (□, ■) or NMG^+ buffer (○, ●) and incubated for 15 min in the absence (■, ●) or presence (□, ○) of nitrobenzylthioinosine (10 μM). Cells were then added to [^{3}H]adenosine to initiate uptake. Symbols are means of triplicates; S.E.M. bars are shown unless obscured by the symbols. Data are from one experiment representative of at least six.

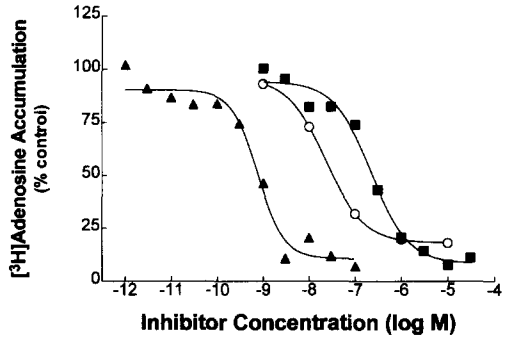

Fig. 3. Concentration-dependent inhibition of cellular accumulation of [^{3}H]adenosine by nucleoside transport inhibitors. DDT_1 MF-2 cells were incubated for 15 min in the absence (control) or presence of graded concentrations of nitrobenzylthioinosine (▲), dilazep (○) or dipyridamole (■) then exposed for 15 s to [^{3}H]adenosine (1 μM) in the continued presence of inhibitors, as described in the text. Points are means of triplicates from one experiment representative of at least four.

Since [^{3}H]adenosine uptake was linear for at least 30 s, a time interval of 15 s was used to evaluate concentration-dependent inhibition of [^{3}H]adenosine (1 μM) accumulation into DDT_1 MF-2 cells by nucleoside transport inhibitors and permeants. Nitrobenzylthioinosine, dilazep and dipyridamole, inhibited [^{3}H]adenosine uptake with IC_{50} values of 1 ± 0.2 nM ($n = 4$), 13 ± 7 nM ($n = 4$) and 270 ± 111 nM ($n = 7$), respectively (Fig. 3). Concentration-dependent inhibition of [^{3}H]adenosine accumulation

Table 2
Kinetic constants for [^{3}H]adenosine uptake into DDT_1 MF-2 cells

Cell treatment (n)	$K_m \pm$S.E.M. (μM)	$V_{max} \pm$S.E.M. (pmol/μl cell water/s)
Na^+ buffer (3)	12.0 ± 1.7	4.6 ± 1.1
NMG^+ buffer (3)	9.5 ± 0.8	3.4 ± 1.4

Cells were harvested and resuspended in Na^+ or NMG^+ buffer. Uptake of [^{3}H]adenosine during 14 s intervals was determined using [^{3}H]adenosine concentrations of 0.5–100 μM. K_m and V_{max} values were determined using nonlinear regression analysis.

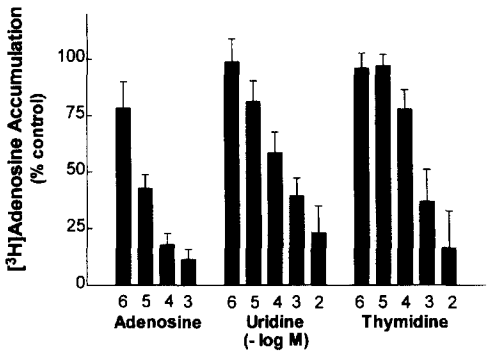

Fig. 4. Concentration-dependent inhibition of cellular accumulation of [^{3}H]adenosine. DDT_1 MF-2 cells were incubated for 15 s with [^{3}H]adenosine (1 μM) in the absence (control) or presence of graded concentrations of unlabelled adenosine, thymidine or uridine as described in the text. The data are means ± S.E.M. of 3–7 experiments, each performed in triplicate.

was also observed with the nonisotopic nucleosides adenosine, uridine and thymidine; IC_{50} values were 4 ± 1.7 μM ($n = 4$), 93 ± 34 μM ($n = 7$) and 170 ± 20 μM ($n = 3$), respectively (Fig. 4).

Propentofylline was shown previously to inhibit nitrobenzylthioinosine-sensitive adenosine transport in cells derived from the mouse leukemia L1210 cell line (Parkinson et al., 1993) and this finding was confirmed in the DDT_1 MF-2 cells (Fig. 5). In vivo, propentofylline is metabolized to the racemic compound (±)-A72 0287 (Macdonald et al., 1986) which consists of the (+) isomer 83 3791 and the (−) isomer 84 4261. All three compounds were equally potent to propentofylline as inhibitors of [^{3}H]adenosine transport (Fig. 5). The IC_{50} values obtained were 24 ± 5 μM, 22 ± 7 μM, 27 ± 11 μM and 26 ± 12 μM for propentofylline, (±)-A72 0287, (+)-A83 3791 and (−)-A84 4261, respectively. To confirm our finding that the hydroxy-metabolites of propentofylline are equally potent to propentofylline, we used another cell line, mouse leukemia L1210/B23.1, which has only nucleoside trans-

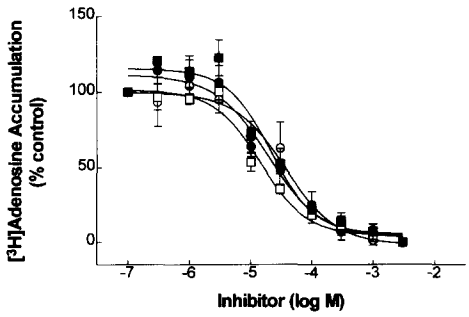

Fig. 5. Concentration-dependent inhibition of cellular accumulation of [^{3}H]adenosine. DDT_1 MF-2 cells were incubated for 15 min in the absence (control) or presence of graded concentrations of propentofylline (■), (±)-A72 0287 (□), (+)-A83 3791 (●) or (−)-A84 4261 (○) then exposed for 15 s to [^{3}H]adenosine (1 μM) in the continued presence of inhibitors, as described in the text. The data are means ± S.E.M. of 3–4 experiments, each performed in triplicate.

Table 3
Inhibition of [^{3}H]nitrobenzylthioinosine binding and [^{3}H]adenosine transport in clonal mouse leukemia L1210/B23.1 cells

Compound	[^{3}H]Nitrobenzylthioinosine binding $K_i \pm$S.E.M. (μM)	[^{3}H]Adenosine transport $IC_{50} \pm$S.E.M. (μM)
Propentofylline	14±0.3	14±8
(±)-A72 0287	12±6	9±3
(+)-A83 3791	8±3	8±3
(−)-A84 4261	10±2	7±2

Inhibition of site-specific [^{3}H]nitrobenzylthioinosine binding was determined using 0.5 nM [^{3}H]nitrobenzylthioinosine and 8–10 concentrations of inhibitors. K_i values were obtained using nonlinear regression analysis and application of the Cheng and Prusoff equation. Inhibition of [^{3}H]adenosine transport was determined using 6 s uptake intervals, 1 μM [^{3}H]adenosine and 8–10 concentrations of inhibitors. IC_{50} values were obtained using nonlinear regression analysis.

porters of the *es* subtype (Vijayalakshmi et al., 1992). Each of the compounds tested, propentofylline, (±)-A72 0287, (+)-A83 3791 and (−)-A84 4261, had similar potency for inhibition of [^{3}H]nitrobenzylthioinosine binding and inhibition of [^{3}H]adenosine transport (Table 3). In L1210/B23.1 cells, as in DDT_1 MF-2 cells, the reduced forms (±)A72 0287, (+)-A83 3791 and (−)A84 4261 were of similar potency to the parent compound propentofylline (Table 3).

4. Discussion

As many as seven functionally distinct nucleoside transport processes have been described in cells from a variety of species and tissues. These transporters fall into two broad categories: Na^+-dependent and -independent processes (Cass, 1995). [^{3}H]nitrobenzylthioinosine binds with high affinity and selectivity to one subtype that is Na^+-independent and is termed the *es* transporter (Vijayalakshmi and Belt, 1988). Binding studies with this radioligand indicated that DDT_1 MF-2 cells have a high abundance of *es* transporters. The identification of these binding sites as functional transporters is supported by the finding that the structurally dissimilar transport inhibitors dipyridamole and dilazep inhibited [^{3}H]nitrobenzylthioinosine binding.

Dipyridamole exhibits species differences in affinity for nucleoside transporters with high affinity (1–10 nM) for human, dog and guinea pig, moderate affinity (100–500 nM) for mouse and relatively low affinity (1–5 μM) for rat tissues (Hammond and Clanachan, 1985; Lee and Jarvis, 1988a,b). It is not known whether the heterogeneity in affinity for transporters by dipyridamole is due to differences in the primary structure of the *es* transporters among these species. The affinity of dipyridamole for [^{3}H]nitrobenzylthioinosine binding sites in DDT_1 MF-2 cells suggests that the *es* transporters in hamster are similar to those in mouse.

Initial rates and kinetic constants for uptake of [^{3}H]adenosine for DDT_1 MF-2 cells in Na^+-containing and Na^+-replacement buffer were compared and found to be similar. Thus, our data do not provide evidence for the presence of Na^+-dependent transport in these cells, although the presence of a small component that was not detectable with our assays cannot be ruled out.

Assuming each high affinity [^{3}H]nitrobenzylthioinosine binding site corresponds to a functional transporter, the translocation capacity for each transporter was 9.3 molecules/s. This value is approximately 14–20-fold lower than the values reported for [^{3}H]uridine uptake into erythrocytes from several species (Jarvis et al., 1982), 32-fold lower than the value reported for [^{3}H]adenosine uptake into cultured human umbilical vein endothelial cells (Sobrevia et al., 1994) but similar to the values (7.3–14.1 molecules/transporter/s) reported for [^{3}H]uridine uptake into rat erythrocytes, hepatocytes and kidney cells (Plagemann and Wohlhueter, 1985).

[^{3}H]Adenosine accumulation was completely inhibited by the transport inhibitors nitrobenzylthioinosine, dilazep and dipyridamole with similar inhibition constants to those obtained for inhibition of [^{3}H]nitrobenzylthioinosine binding. To date, two forms of equilibrative nucleoside transporters have been characterized which are distinguished based on their sensitivity to inhibition by nitrobenzylthioinosine. The *es* (equilibrative, sensitive) process is inhibited by low nanomolar concentrations and the *ei* (equilibrative, insensitive) process is either not inhibited or requires micromolar concentrations of nitrobenzylthioinosine (Cass, 1995; Vijayalakshmi and Belt, 1988). In our studies, nitrobenzylthioinosine completely inhibited [^{3}H]adenosine transport with nanomolar affinity, thus the presence of an *ei* transporter was not apparent.

Propentofylline is a xanthine compound that reduces neuronal damage following cerebral ischemia (DeLeo et al., 1987). Although its precise mechanism of action is not completely understood, it is able to inhibit cellular uptake of adenosine (Ohkubo et al., 1991; Parkinson et al., 1993), elevate interstitial concentrations of adenosine (Andiné et al., 1990) and decrease extracellular glutamate levels (Hagberg et al., 1990). These effects are consistent with potentiation of the receptor-mediated effects of adenosine. However, propentofylline undergoes rapid chemical reduction in vivo to the racemic mixture (±)-A72 0287 (Macdonald et al., 1986), thus it is important to examine the effects of the resulting stereoisomers in addition to the parent compound especially in light of the stereoselectivity exhibited by the enantiomers of (±)-R75 231 (Van Belle et al., 1993; Beukers et al., 1994). Previously, we reported that (±)-A72 0287 inhibited [^{3}H]nitrobenzylthioinosine binding in rat brain with similar affinity to propentofylline (Parkinson and Fredholm, 1991). In this study we found that the racemic mixture, (±)-A72 0287, and the stereoisomers, (+)-83 3791 and (−)-84 4261, inhibit [^{3}H]adenosine uptake by *es* transporters as effectively as propento-

fylline, thus stereoselective inhibition of nucleoside transport varies among inhibitory compounds. Since inhibition of adenosine transport appears to be important for the neuroprotective effects of propentofylline, the hydroxy-metabolites may also provide neuroprotection.

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