Kinetics of nitrobenzylthioinosine binding to the human erythrocyte nucleoside transporter

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The kinetics of [^3H]nitrobenzylthioinosine binding to human erythrocyte membranes was studied. The pseudo-first-order association was linear and consistent with a simple bimolecular reaction mechanism between nitrobenzylthioinosine and the nucleoside-transport mechanism. Dissociation of the [^3H]nitrobenzylthioinosine complex at 22°C was also linear (apparent $k_{-1} \approx 0.20 \text{ min}^{-1}$). Adenosine was a competitive inhibitor of equilibrium high-affinity [^3H]nitrobenzylthioinosine-binding activity (apparent K_i 0.1 mM). Dissociation of the [^3H]nitrobenzylthioinosine–membrane complex was faster in the presence of adenosine and uridine, and this effect was proportional to the nucleoside concentration. Nucleoside concentrations less than 1 mM had no significant effect on the dissociation rate constant. In contrast, dissociation was slower in the presence of high concentrations (micromolar) of dipyrindamole. Low concentrations of dipyrindamole (2–200 nM) and nitrobenzylthioinosine concentrations as high as 2.5 μM had no effect on the rate of [^3H]nitrobenzylthioinosine dissociation. These results are discussed in terms of possible distinct inhibitor and permeation sites, and are suggested to be consistent with both a single-site model for the binding of nitrobenzylthioinosine and permeant to the same site, or an allosteric-site model in which permeant and inhibitor bind to different sites.

Specific inhibitors of transport systems have proved to be extremely useful as probes for the kinetic and molecular properties of transporters. Nitrobenzylthioinosine is a potent inhibitor of nucleoside transport, a non-concentrative ‘facilitated diffusion’ process in most cell types (for reviews see Paterson *et al.*, 1981; Jarvis & Young, 1982a; Young & Jarvis, 1983). As such, NBMPR has been used (1) to determine quantitatively the numbers of functional nucleoside-transport sites present in different cell types (Jarvis & Young, 1980; Cass *et al.*, 1981; Jarvis *et al.*, 1982a), (2) to investigate changes in transport activity during ontogeny, reticulocyte maturation and the cell cycle (Cass *et al.*, 1979; Wiley *et al.*, 1982; Jarvis & Young, 1982b), (3) to aid measurements of transport rates (Jarvis *et al.*, 1982b, 1983; Harley *et al.*, 1982), (4) to determine the specificity of transport systems (Wohlhueter *et al.*, 1978), (5) to isolate the membrane protein(s) responsible for nucleoside

transport and to investigate their molecular properties (Jarvis & Young, 1981; Jarvis *et al.*, 1980; Young *et al.*, 1983), and (6) to manipulate *in vivo* the tissue distribution of nucleoside drugs (Lynch *et al.*, 1981a,b; Kolassa *et al.*, 1982). As a consequence of these broad uses of NBMPR, and the possibility of clinical applications, there is considerable interest in the mechanism by which this compound inhibits nucleoside transport.

NBMPR inhibition of nucleoside transport is associated with tight, but reversible, high-affinity binding of inhibitor to functional specific sites on the cell membrane (apparent K_d 0.1–1 nM) (Cass *et al.*, 1974, 1981; Jarvis & Young, 1980, 1982a,b; Jarvis *et al.*, 1982a,b). This interaction of NBMPR with the membrane has been mainly studied under equilibrium binding conditions. Transported nucleosides are competitive inhibitors of high-affinity NBMPR binding, and the apparent K_i for uridine inhibition of NBMPR binding by human erythrocytes is similar to the apparent K_m of uridine equilibrium exchange transport (Cass & Paterson, 1976; Jarvis *et al.*, 1982b). Kinetic studies with

Abbreviation used: NBMPR, nitrobenzylthioinosine 6-[(4-nitrobenzyl)thio]-9- β -D-ribofuranosylpurine.

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erythrocytes have demonstrated that NBMPR is an apparent competitive inhibitor of uridine influx and uridine equilibrium exchange efflux but an apparent non-competitive inhibitor of uridine efflux (Eilam & Cabantchik, 1977; Jarvis *et al.*, 1982b). We have previously proposed that much of the above evidence favours the simple view that the erythrocyte NBMPR-binding site is identical with the nucleoside-permeation site of the carrier and that it is largely located on the outer surface of the erythrocyte cell membrane (Jarvis & Young, 1982b). However, the data are also consistent with the hypothesis that NBMPR binds to an allosteric site.

In order to assess the possibility of distinct inhibitor and permeation sites, we have investigated the effect of nucleoside substrates and inhibitors on the kinetics of NBMPR dissociation from human erythrocyte membranes. In addition, the kinetics of NBMPR association were studied. The results are consistent with either the simple model of NBMPR binding to the permeation site of the nucleoside transporter (Jarvis & Young, 1982b) or NBMPR and nucleoside permeants binding to separate sites.

Materials and methods

Preparation of erythrocyte membranes

Whole blood from healthy human volunteers was collected into heparinized evacuated tubes. Erythrocytes were washed three times with a medium containing 140 mM-NaCl, 20 mM-Tris/HCl buffer, pH 7.4 at 22°C, 2 mM-MgCl₂ and 0.1 mM-EDTA, and the plasma and buffy coats were discarded. Haemoglobin-free erythrocyte membranes, prepared by the method of Dodge *et al.* (1963), were resuspended in 5 mM-sodium phosphate buffer, pH 7.4 at 22°C, and stored at -70°C for up to 3 weeks.

Dissociation kinetics of [³H]NBMPR binding

Human erythrocyte membranes (final protein concn. 0.5 mg/ml) were incubated with graded concentrations of [³H]NBMPR (final equilibrium concn. 0.1–2.0 nM) for 30 min at 22°C. Incubations were terminated by centrifugation at 45 000 *g* for 15 min, and portions of the supernatant were retained for radioactivity determinations. The membrane pellets were washed once with 10 ml of ice-cold 5 mM-sodium phosphate buffer, pH 7.4, and resuspended at a protein concentration of 1 mg/ml. The [³H]NBMPR-labelled membranes were kept on ice for periods of up to 6 h. Control experiments demonstrated that the amount of [³H]NBMPR bound to the human erythrocyte membranes remained constant during this period (see also Jarvis & Young, 1980). Dissociation was initiated by adding 50 µl of ice-cold [³H]NBMPR-labelled membranes to 60 ml of 5 mM-sodium phosphate buffer,

pH 7.4, at 22°C containing no addition or 5 nM-NBMPR or graded concentrations of adenosine, uridine and dipyridamole in the presence or in the absence of 5 nM-NBMPR. The medium was continuously stirred, and at various time intervals (0.5–30 min) after the addition of the membranes 5 ml portions were removed and filtered immediately through glass-fibre filters (Whatman GF/C, which were washed with ice-cold buffer before sample filtration) under suction. The filters were washed twice with 4 ml portions of ice-cold 5 mM-sodium phosphate buffer, pH 7.4. The entire procedure was completed within 10 s. The filters were dried and counted for radioactivity with 8 ml of Scinti Verse Bio-HP (Fisher Scientific Co., Fair Lawn, NJ, U.S.A.). Dissociation from non-specific binding sites was determined by labelling the erythrocyte 'ghosts' with [³H]NBMPR in the presence of 5 µM-NBMPR and then measuring the rate of dissociation by the above procedure. Control experiments showed that the dissociation of non-specific binding reached a steady-state rate after 1 min of incubation and remained constant throughout the experiment. Therefore non-specific binding was determined as a routine separately at the end of the assay and was typically less than 2% of the total binding. Specific binding was the difference between total binding and non-specific binding.

The above procedure for measuring the dissociation kinetics was designed such that the re-binding of [³H]NBMPR was prevented (see Fig. 1). Therefore the results obtained were evaluated by using the following equation derived from a simple bimolecular reaction:

$$\ln \left(\frac{B_t}{B_e} \right) = -k_{-1} \cdot t \quad (1)$$

where B_e is the specific binding at time zero, B_t is the specific binding at time t , and k_{-1} is the dissociation rate constant.

Association kinetics of [³H]NBMPR binding

To measure the association kinetics of [³H]NBMPR binding, the experimental conditions were designed to be pseudo-first-order, that is the concentration of free radioactive ligand, $[L]$ in eqn. (2) is constant throughout the reaction. In order to achieve such conditions, a low protein concentration in a relatively large volume of [³H]NBMPR was used. Binding was started by adding 50 µg of erythrocyte 'ghosts' to 70 ml of [³H]NBMPR (0.1–2.0 nM) at 22°C. After various time intervals (0–20 min) 5.0 ml portions of the reaction mixture were removed and filtered as described above, and the bound radioactivity was determined. Non-specific binding was measured in the presence of 5 µM-NBMPR, and specific binding is defined as the difference in binding in the absence and in the presence of 5 µM-NBMPR.

To analyse the association kinetics, the values obtained were plotted with the use of the integrated rate equation (for derivation see Bennett, 1978):

$$\ln \left(\frac{B_e - B_t}{B_e} \right) = -(k_{+1}[L] + k_{-1}) \cdot t \quad (2)$$

where B_e and B_t are the concentrations of bound receptor at equilibrium and at time t , k_{+1} and k_{-1} are the association and dissociation rate constants respectively, and $[L]$ is the free radiolabelled-ligand concentration at steady state. The percentage of ligand bound at the lowest concentration of [^3H]-NBMPR used (0.1 nM) was less than 5% (results not shown), and therefore $[L]$ in eqn. (2) can be considered to be constant throughout the experiment.

Equilibrium binding of [^3H]NBMPR

Equilibrium binding of NBMPR to human erythrocyte membranes was determined at 22°C as previously described (Jarvis & Young, 1980, 1982a; Jarvis *et al.*, 1982b).

Chemicals

[G- ^3H]NBMPR (sp. radioactivity 16 Ci/mmol) was purchased from Moravsek Biochemicals, Brea, CA, U.S.A., and was >98% radiochemically pure. NBMPR was generously given by Dr. A. R. P. Paterson, Cancer Research Unit (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada. Dipyrindamole {2,2',2'',2'''-(4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine-2,6-diyl-dinitrilo)tetraethanol} was obtained from Boehringer Ingelheim (Canada), Burlington, Ontario, Canada.

Results

Dissociation kinetics

In preliminary experiments the dissociation of bound [^3H]NBMPR was measured by resuspending labelled membranes in 1000-fold excess of 5 mM-sodium phosphate buffer, pH 7.4, at 22°C (Fig. 1). When the data from this experiment were plotted in accordance with eqn. (1) a curvilinear plot was observed, suggestive of either the presence of two or more components of dissociation or the re-binding of free dissociated [^3H]NBMPR. To test this latter possibility, NBMPR at a concentration sufficient to saturate the high-affinity NBMPR-binding sites (5 nM) was added to the buffer to prevent re-binding by displaced [^3H]NBMPR (Fig. 1). The resulting dissociation curve was linear and the apparent dissociation rate constant (0.25 min⁻¹) was similar to that of the initial phase of dissociation in the presence of buffer alone (see Fig. 1). Therefore all further dissociation experiments, unless noted otherwise, were performed in the presence of 5 nM-NBMPR. The rate of dissociation was similar at three [^3H]NBMPR concentrations tested (0.1, 0.19

Fig. 1. Time course of dissociation of [^3H]NBMPR-erythrocyte membrane complex

Human erythrocyte membranes (0.5 mg/ml) were incubated with [^3H]NBMPR (final equilibrium concn. 0.67 nM) at 22°C for 30 min. [^3H]NBMPR-labelled membranes were washed free of excess [^3H]NBMPR and dissociation was started by the addition of 50 μl of labelled membranes to 60 ml of 5 mM-sodium phosphate buffer, pH 7.4, containing no addition (■), 5 nM-NBMPR (□) or 10 mM-uridine plus 5 nM-NBMPR (●). The percentage of the remaining specifically bound [^3H]NBMPR after various times was plotted in accordance with eqn. (1) in the Materials and methods section. At time zero there was 23 pmol of [^3H]NBMPR bound per mg of protein.

and 2.0 nM), demonstrating that the dissociation rate constant is independent of the initial percentage of high-affinity NBMPR-binding sites bound (results not shown).

Fig. 1 also demonstrates that the rate of [^3H]NBMPR dissociation from erythrocyte membranes in the presence of high concentrations of uridine (10 mM) plus 5 nM-NBMPR increased 2-fold as compared with the control. In order to investigate

Fig. 2. Effect of adenosine and uridine on the kinetics of $[^3\text{H}]\text{NBMPR}$ dissociation from human erythrocyte membranes

Human erythrocyte membranes were labelled with $[^3\text{H}]\text{NBMPR}$ as described in Fig. 1 legend. At time zero, dissociation was initiated in the presence of various concentrations of adenosine ($\blacktriangle$) or uridine ($\blacksquare$) in the presence of 5 nM-NBMPR. The per-

this phenomenon further, we studied the effects of various concentrations of two transported permeants, adenosine and uridine, on the rate of $[^3\text{H}]\text{NBMPR}$ dissociation (Fig. 2). These nucleosides were chosen because their kinetic properties for the nucleoside transporter are well characterized and adenosine appears to have the highest affinity (apparent K_m 25–100 μM) of the physiological nucleosides for the carrier (Young, 1978; Harley *et al.*, 1982). Increasing concentrations of both uridine and adenosine in the presence of 5 nM-NBMPR resulted in increases in the dissociation rates of $[^3\text{H}]\text{NBMPR}$ from human erythrocyte membranes (Fig. 2). This effect was directly proportional to the nucleoside concentration over the range 0–20 mM and was independent of nucleoside substrate. Similar results were obtained in the absence of NBMPR (results not shown).

In a previous study (Jarvis *et al.*, 1982b) we demonstrated that uridine was a competitive inhibitor of high-affinity NBMPR binding to human

centage of the remaining specifically bound $[^3\text{H}]\text{NBMPR}$ after various times was plotted in accordance with eqn. (1) and the apparent dissociation rates (apparent k_{off}) were determined and re-plotted versus the nucleoside concentration. Results are the means of results for three experiments.

Fig. 3. Effect of adenosine on equilibrium $[^3\text{H}]\text{NBMPR}$ binding to human erythrocyte membranes

The reciprocals of $[^3\text{H}]\text{NBMPR}$ bound to high-affinity sites in the presence of various concentrations of adenosine at 22°C are plotted against the respective reciprocals of the free equilibrium concentrations of $[^3\text{H}]\text{NBMPR}$. Extracellular adenosine concentrations (mM) were 2.4 ($\bullet$), 0.97 (Δ), 0.47 ($\blacktriangle$), 0.24 ($\square$) and 0 ($\blacksquare$). The inset shows a plot of the apparent K_d values, as derived from the double-reciprocal plot, versus initial adenosine concentrations. Apparent K_i was 0.10 mM.

erythrocyte membranes (apparent inhibitor constant $\cdot 0.9 \text{ mM}$ at 25°C). In the present study the ability of adenosine to inhibit high-affinity NBMPR binding to human erythrocyte membranes was investigated in detail. Fig. 3 shows the effect of various concentrations (0–2.4 mM) of adenosine on high-affinity NBMPR binding, plotted as the reciprocal of specific bound $[^3\text{H}]\text{NBMPR}$ versus the reciprocal of the equilibrium free concentration of inhibitor. Adenosine was an apparent competitive inhibitor of $[^3\text{H}]\text{NBMPR}$ binding with an apparent inhibition constant of 0.10 mM . The linearity of the re-plot of apparent K_d values versus adenosine concentration (inset to Fig. 3) provides further evidence of simple competitive inhibition of binding activity.

Dipyridamole is another potent inhibitor of nucleoside transport activity in a variety of cell types (Paterson *et al.*, 1980; Plagemann *et al.*, 1978; Jarvis *et al.*, 1982b). Inhibition of the mammalian erythrocyte transporter was apparently competitive, with an apparent K_i value of 2–22 nM (Jarvis *et al.*, 1982b; Hammond *et al.*, 1981; S. M. Jarvis, unpublished work). Figs. 4(a) and 4(b) demonstrate that dipyridamole (initial concentration 1–10 μM) decreased the rate of dissociation of $[^3\text{H}]\text{NBMPR}$ from human erythrocyte membranes. The time required for 50% dissociation increased from 3.3 min for the control (5 nM-NBMPR) to 14.5 min for 2.5 μM -dipyridamole (Fig. 4a). Similar results were also obtained when the dipyridamole solutions contained 20 nM-NBMPR (results not shown). No significant decrease in dissociation rate constants (apparent k_{off}) was observed at dipyridamole concentrations less than 200 nM. In contrast with dipyridamole, adenosine and uridine, NBMPR, even at high concentrations (0.1–10 μM), had little effect on the apparent rate of dissociation (Fig. 4b).

Association kinetics

The forward reaction between $[^3\text{H}]\text{NBMPR}$ and human erythrocyte membranes was studied under pseudo-first-order conditions. Three concentrations of $[^3\text{H}]\text{NBMPR}$ (0.1, 0.2 and 2 nM, representing low, half saturation and saturation concentration) were incubated with human erythrocyte 'ghosts' at 22°C and the rate of complex-formation was measured as a function of time (Fig. 5). Binding of $[^3\text{H}]\text{NBMPR}$ reached a steady state within about 5 min after the reaction was initiated, and the time required to reach 50% saturation was approx. 0.15, 1.2 and 1.6 min with 2 nM-, 0.2 nM- and 0.1 nM- $[^3\text{H}]\text{NBMPR}$ respectively. Kinetic analysis, in accordance with eqn. (2), yielded linear plots (results not shown), consistent with a simple bimolecular association. The average apparent association rate constant (k_{+1}), estimated by least-squares fit, was $1.7 \times 10^9 \pm 0.3 \times 10^9 \text{ M}^{-1} \cdot \text{min}^{-1}$. Dividing the rate con-

Fig. 4. Effect of NBMPR and dipyridamole on the rate of dissociation of $[^3\text{H}]\text{NBMPR}$ from human erythrocyte membranes.

(a) Human erythrocyte membranes (0.5 mg/ml) were incubated with $[^3\text{H}]\text{NBMPR}$ (final equilibrium concn. 0.40 nM) at 22°C for 30 min. $[^3\text{H}]\text{NBMPR}$ -labelled membranes were washed free of excess $[^3\text{H}]\text{NBMPR}$ and dissociation was initiated by the addition of 50 μl of labelled membranes to 60 ml of 5 mM-sodium phosphate buffer, pH 7.4, containing 20 nM-NBMPR ($\blacktriangledown$), 1 μM -dipyridamole ($\blacktriangle$), 2.5 μM -dipyridamole ($\square$) or 10 μM -dipyridamole ($\blacksquare$). The percentage of the remaining specifically bound $[^3\text{H}]\text{NBMPR}$ after various times was plotted in accordance with eqn. (1) in the Materials and methods section. (b) The apparent dissociation rates (apparent k_{off}) were determined from experiments similar to those for (a) and plotted as a function of inhibitor concentration (μM). Symbols: $\bullet$, dipyridamole; Δ , NBMPR. Results are the means of results for three experiments.

stant for dissociation (k_{-1} ; see Figs. 2 and 4) by the association rate constant (k_{+1}) yielded an equilibrium dissociation constant of 0.12 nM, a value similar to that determined by equilibrium binding analysis with the same batch of membranes (apparent K_d 0.13 nM; see Fig. 3).

Fig. 5. Time course of [^3H]NBMPR binding to human erythrocyte membranes

Human erythrocyte membranes ($50\mu\text{g}$) at 22°C were incubated with 70ml of 0.1nM ($\blacksquare$), 0.2nM ($\bullet$) and 2.0nM ($\blacktriangle$) [^3H]NBMPR at time zero. Samples were removed and filtered at the indicated time, and the specifically bound [^3H]NBMPR was determined as described in the Materials and methods section.

Discussion

The experiments described in the present paper were designed to test the possibility of distinct NBMPR-inhibitor-binding and substrate-permeation sites for the erythrocyte nucleoside transporter. The approach we employed was to investigate the kinetics of NBMPR dissociation from human erythrocyte membranes by dilution in the presence or in the absence of nucleoside permeants and inhibitors. Changes in the rate of dissociation may be suggestive of site-to-site interactions. In addition, the kinetics of association were studied.

To investigate the association kinetics of [^3H]NBMPR, binding was carried out under pseudo-first-order conditions, i.e. the concentration of ligand was much larger than the concentration of binding sites. Under such conditions the association curves, plotted in accordance with eqn. (2), were linear, consistent with a simple bimolecular reaction mechanism between NBMPR and the nucleoside transporter. The rate constant ($1.7 \times 10^9 \pm 0.3 \times 10^9 \text{M}^{-1} \cdot \text{min}^{-1}$) was in the same range as the 'on' rate constant for uridine as determined for uridine transport across the human erythrocyte membrane at 22°C (Young & Jarvis, 1983).

Dissociation also appeared to be a simple first-order process. The initial apparent rate constant for

dissociation was the same when determined either by dilution or by dilution plus 5nM -NBMPR (Fig. 1). The addition of unlabelled NBMPR (concentrations up to 1000-fold higher than the apparent K_d for equilibrium binding of NBMPR) had no significant effect on the rate of dissociation (Fig. 4b). Higher concentrations of NBMPR ($5\text{--}10\mu\text{M}$) increased the rate of [^3H]NBMPR dissociation by 10–20% (Fig. 4b). The kinetic measurements of NBMPR association and dissociation are in close agreement with the equilibrium binding measurements, i.e. $k_{-1}/k_{+1} = K_d$.

The above results suggest a simple ligand–protein model for NBMPR binding to the erythrocyte nucleoside transporter and are in agreement with earlier equilibrium binding studies (Cass *et al.*, 1974; Jarvis & Young, 1980; Hammond *et al.*, 1981). These studies demonstrated a single class of high-affinity NBMPR-binding sites in mammalian erythrocytes with a Hill coefficient of near unity. In addition, uridine and deoxycytidine blocked NBMPR binding to human and nucleoside-permeable sheep erythrocyte membranes (Jarvis *et al.*, 1982b). The present results also indicate that adenosine is an apparent competitive inhibitor of high-affinity NBMPR binding to membranes prepared from human erythrocytes. The apparent K_i value for adenosine inhibition of NBMPR binding was 0.1mM , a value approx. 10-fold lower than the apparent K_i for uridine inhibition of binding, but in agreement with the higher affinity of adenosine for the nucleoside transporter (Young, 1978; Harley *et al.*, 1982). These results are consistent with the notion that NBMPR competes directly with nucleosides for the permeation site of the transport system. However, the results presented in Figs. 1 and 2 demonstrating that the nucleoside permeants, uridine and adenosine, markedly increased the rate of dissociation would appear to indicate permeant interactions at a transporter site other than the NBMPR-binding site. Nevertheless, these effects of adenosine and uridine would, at first sight, appear to be unrelated to the apparent affinities of these nucleosides for the nucleoside-transport system. For example, at concentrations equal to the apparent K_i values for adenosine and uridine inhibition of NBMPR binding (0.1 and 1.2mM respectively), no significant increase in the dissociation of [^3H]NBMPR was observed (Fig. 2). Furthermore, the apparent concentration-dependence for the increase in dissociation rate was similar for adenosine and uridine, despite the latter nucleoside possessing a 10-fold lower affinity for the nucleoside transporter. In addition, the concentration-dependence curves for the effects of nucleosides on the dissociation rate were non-saturable in the concentration range $0\text{--}20\text{mM}$, suggesting that the nucleosides are not interacting with a finite number of binding sites.

Thus, although high concentrations of nucleosides apparently increase the rate of [^3H]NBMPR dissociation, these effects may be unrelated to the nucleoside-transport properties of these permeants, suggesting that the observed enhancement may be due to a non-specific interaction of high concentrations of nucleosides with the carrier. Alternatively, the finding that high concentrations of permeants are necessary to enhance the dissociation of bound [^3H]NBMPR may also be interpreted in terms of an allosteric model of the nucleoside transporter in which NBMPR bound to its modifier site markedly decreases the affinities of nucleosides for the permeation site. Therefore the present data do not distinguish between a non-specific effect of high concentrations of ligand and a real, but low-affinity, effect at a second site.

In contrast with adenosine and uridine, dipyridamole decreased the [^3H]NBMPR dissociation rate (Fig. 4). However, as was the case for nucleosides, no significant decrease was observed at the apparent K_i value for inhibition of NBMPR binding (approx. 2 nM; Hammond *et al.*, 1981; S. M. Jarvis, unpublished work). Decreases in the dissociation rate constant were only observed at dipyridamole concentrations 100-fold greater than the apparent K_i value (Fig. 4b). These effects of dipyridamole on the dissociation rate may be due either to non-specific interactions or to dipyridamole binding to a site other than the NBMPR-binding site (see the above discussion).

In conclusion, the data presented in this paper are consistent with two differing models for the mechanism by which NBMPR interacts with the nucleoside transporter. In the simple model, NBMPR is proposed to compete with nucleosides for the permeation site of the nucleoside-transport system (Jarvis & Young, 1982b; Young & Jarvis, 1983). In the second model, NBMPR is proposed to bind to an allosteric site that is distinct from the permeation site.

Note added in proof (received 18 October 1983)

High concentrations of uridine (20 and 40 mM) have recently been reported to increase the rate of dissociation of site-bound [^3H]NBMPR from cultured hamster cells (Koren *et al.*, 1983). These results were suggested to indicate that uridine binds to a site separate from the NBMPR-binding site.

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