Trans-stimulation and *trans*-inhibition of uridine efflux from human erythrocytes by permeant nucleosides

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The ability of nucleoside permeants to accelerate the efflux of uridine from human erythrocytes has been compared. In contrast to uridine, 2-chloroadenosine acted as a *trans*-inhibitor of uridine efflux from fresh human erythrocytes, and adenosine had little effect. These results are consistent with the lower maximum velocity for influx of 2-chloroadenosine and adenosine as compared with uridine and demonstrate that *trans* acceleration experiments do not discriminate between transported and non-transported permeants for the human erythrocyte nucleoside carrier.

INTRODUCTION

The nucleoside transporter of human erythrocytes is an example of a facilitated diffusion transport system which accepts as substrates a variety of nucleosides (Young & Jarvis, 1983). The properties of this system are consistent with those of a simple carrier, well defined, and similar to nucleoside transporters in many other cells (Plagemann & Wohlhueter, 1980; Paterson *et al.*, 1981; Young & Jarvis, 1983). In particular, the nucleoside carrier in human erythrocytes is a clear example of a nucleoside transport system totally inhibited by nanomolar concentrations of nitrobenzylthioinosine (NBMPR) (Cabantchik & Ginsburg, 1977; Jarvis & Young, 1980). As such, human erythrocytes have been used as a model system to determine whether nucleoside drugs enter cells by the NBMPR-sensitive saturable nucleoside transporter (Paterson *et al.*, 1981; Jarvis *et al.*, 1982).

A number of criteria have been established to distinguish between a transported and a non-transported nucleoside drug. These include saturable kinetics for drug permeation into cells, inhibition of the uptake of the drug by specific transport inhibitors such as NBMPR, and the ability of nucleoside analogues to accelerate uridine efflux from uridine-loaded human erythrocytes (*trans*-stimulation) (Paterson *et al.*, 1981). This last criterion has been used extensively because isotopically labelled nucleoside drug is not required and it has been assumed that transported nucleoside permeants when added to the extracellular compartment will always increase the outward transport of uridine from cells (Oliver & Paterson, 1971; Cass & Paterson, 1972; Plagemann & Wohlhueter, 1980; Paterson *et al.*, 1981). Indeed, a number of nucleoside analogues have been identified as permeants using this criterion (Cass & Paterson, 1972). In the present study, I have reassessed the ability of known nucleoside permeants to accelerate uridine efflux from human erythrocytes, a nucleoside not metabolized by these cells (Oliver & Paterson, 1971). Depending on the structure of the test nucleoside, both *trans*-stimulation and *trans*-inhibition of uridine efflux was observed. Therefore, the failure of a compound to accelerate the efflux of uridine from human erythrocytes is not sufficient grounds to discriminate between a transported and a

non-transported substrate for the nucleoside carrier in these cells.

MATERIALS AND METHODS

Chemicals

[5,6-³H]Uridine (sp. radioactivity 42 Ci/mmol) and [2,8-³H]adenosine (sp. radioactivity 48 Ci/mmol) were purchased from ICN, Irvine, CA, U.S.A. and 2-chloro[8-³H]adenosine (sp. radioactivity 18 Ci/mmol) was obtained from Moravsek Biochemicals, Brea, CA, U.S.A. NBMPR was a generous gift from Professor A. R. P. Paterson, Cancer Research Group (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada. All other reagents were from Sigma.

Erythrocytes

Blood from healthy human volunteers was collected into heparinized (Vacutainer) tubes and centrifuged at 1000 *g* for 10 min. The plasma and buffy coats were discarded, and the erythrocytes were washed three times with 20 vol. of a medium containing 140 mM-NaCl, 5 mM-KCl, 20 mM-Tris/HCl (pH 7.4 at 22 °C), 2 mM-MgCl₂ and 0.1 mM-EDTA (disodium salt). Out-dated human blood was obtained from the Blood Transfusion Service of the Canadian Red Cross Society.

Nucleoside transport

To determine the rate of the efflux of [³H]uridine from human erythrocytes, cells were first preloaded with [³H]uridine by mixing equal volumes (25 μ l) of cells (50% haematocrit) and [³H]uridine until equilibration between intracellular and extracellular uridine had occurred (2 h at 22 °C). The 'loaded' cells were recovered by centrifugation using a microcentrifuge (15 s, 12000 *g*), extracellular isotope was removed and 0.4 ml of medium with or without NBMPR (10 μ M) plus test compounds was immediately added to the cell pellet with continuous mixing (final haematocrit 3%). The initial rate of uridine efflux was measured by following the disappearance of [³H]uridine from the cell pellet. Incubations (3–30 s) were terminated by adding 0.4 ml of the incubation mixtures to 0.5 ml of ice-cold 'stopper' medium (containing 10 μ M-

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

NBMPR, a potent inhibitor of nucleoside transport) layered on top of ice-cold *n*-dibutyl phthalate contained in 1.5 ml microcentrifuge tubes. Tubes were immediately centrifuged (10 s, 12000 *g*) and the aqueous and *n*-dibutyl phthalate layers were removed by suction. Radioactivity associated with the cell pellet was determined as described previously by using an LKB/Wallac 1217 scintillation counter with automatic quench correction and d.p.m. conversion (Jarvis *et al.*, 1985). Transport rates were calculated after subtraction of the radioactivity in the test pellet from the radioactivity in the NBMPR pellet.

Initial rates of zero-*trans* nucleoside influx were determined by an 'inhibitor-oil-stop' method, the details of which have been published previously (Jarvis *et al.*, 1985).

RESULTS AND DISCUSSION

In preliminary experiments, the time course for the efflux of uridine (initial concentration 2 mM) from human erythrocytes was demonstrated to be linear during the first 30 s. In subsequent studies, incubation times of 10–15 s were chosen such that initial rates of efflux were measured.

The concentration effect of three nucleoside permeants, uridine, adenosine and 2-chloroadenosine, to accelerate the efflux of uridine (2 mM) from human erythrocytes is shown in Fig. 1. As expected, uridine dramatically accelerated the efflux of [³H]uridine, but adenosine had virtually no effect and 2-chloroadenosine inhibited the efflux of [³H]uridine. Similar results were also obtained with initial intracellular uridine concentrations of 1 mM (results not shown). According to previous criteria (Plagemann & Wohlhueter, 1980; Paterson *et al.*, 1981), these results would be interpreted to indicate that adenosine and 2-chloroadenosine are not transported permeants for the nucleoside transport system of human erythrocytes. However, other studies using isotopically labelled adenosine and 2-chloroadenosine have demonstrated that both nucleosides are permeants (Turnheim *et al.*, 1978; Paterson *et al.*, 1984; Jarvis *et al.*, 1985).

The phenomenon of *trans*-stimulation is dependent on the relative 'mobilities' of the empty carrier as compared with the loaded carrier. Therefore, if the rate of reorientation of the loaded carrier could be changed from greater than that of the empty carrier to a rate lower than that of the empty carrier the phenomenon of *trans*-stimulation would be replaced by that of *trans*-inhibition. The rates of reorientation for the simple carrier (the resistance parameters) are related to the reciprocals of the maximum velocity for transport (Lieb, 1982). Therefore, the maximum velocity for zero-*trans* influx of uridine, adenosine and 2-chloroadenosine for fresh human erythrocytes from four different subjects was compared at 22 °C. Human erythrocytes showed a four-fold difference in the transport rate for the three nucleosides at a saturating concentration of nucleoside (2.26 ± 0.03 , 1.03 ± 0.1 and 0.58 ± 0.12 mmol/min per litre of cell water for 2 mM-uridine, 1 mM-adenosine and 1 mM-2-chloroadenosine, respectively). From these results the resistance parameter for the nucleoside carrier in the loaded influx state (R_{21}) was calculated [0.44 ± 0.006 , 0.97 ± 0.01 and 1.72 ± 0.37 (mmol/min per litre of cell water)⁻¹ for uridine, adenosine and 2-chloroadenosine, respectively]. The resistance parameter for the unloaded carrier (R_{00})

Fig. 1. Effect of uridine, adenosine and 2-chloroadenosine on the initial rate of uridine efflux from human erythrocytes

Human erythrocytes from fresh blood were loaded with 2 mM-[³H]uridine and the initial rate of [³H]uridine efflux (time 0–10 s) into medium containing various concentrations of uridine (●), adenosine (■) and 2-chloroadenosine (▲) was determined. See the text for other experimental details. The arrow depicts the control value for uridine efflux into medium alone (86.4 mmol/h per litre of cells).

Table 1. Comparison of the effect of extracellular nucleosides on uridine efflux by erythrocytes from fresh and outdated blood

Erythrocytes were preincubated with 2 mM-[³H]uridine until equilibration had been reached and the cells were collected by centrifugation. The time course of uridine efflux from these cells was followed in the presence and absence of nucleosides (extracellular concentration 2 mM), as noted, and the initial rate of uridine efflux determined. Mean control values for uridine efflux for erythrocytes from fresh and outdated blood were 98.6 and 50.5 mmol/h per litre of cells, respectively. Values represent the mean of duplicate estimates from two different donors.

Extracellular nucleoside (2 mM)	Uridine efflux (% of control)	
	Fresh blood	Outdated blood
None	100	100
Uridine	447	324
Inosine	259	238
Adenosine	95	175
2-Chloroadenosine	29	67

has been previously determined to be equal to 0.84 ± 0.095 (mmol/min per litre of cell water)⁻¹ (Jarvis *et al.*, 1983; Young & Jarvis, 1983) and therefore R_{21} for uridine $< R_{21}$ adenosine = $R_{00} < R_{21}$ for 2-chloroadenosine. These parameters would predict that adenosine should have little or no effect on the accelerated efflux of uridine, whereas 2-chloroadenosine should cause a slowing of the exodus of uridine. The results of Fig. 1 show that this is indeed the case. Therefore, the structure of the nucleoside that drives the *trans* effect and its

corresponding maximum velocity for influx will determine whether the effect is positive or negative.

The inability of adenosine to accelerate the efflux of uridine (Fig. 1) is contrary to a previous report (Oliver & Paterson, 1971) which demonstrated that adenosine was able to stimulate uridine efflux by approx. 250% in erythrocytes from outdated stored blood. However, a more recent study (Jarvis *et al.*, 1983) has demonstrated that the kinetics of uridine transport in erythrocytes from fresh and outdated blood differ in terms of the symmetry of the transporter and the 'mobilities' of the loaded and unloaded carrier. Therefore, the ability of several nucleosides to accelerate the efflux of uridine in erythrocytes from fresh and outdated stored blood was compared (Table 1). In contrast to fresh erythrocytes, adenosine was able to stimulate the efflux of uridine from outdated stored erythrocytes. Similarly, uridine and inosine accelerated the exit of uridine from both fresh and outdated cells, but 2-chloroadenosine inhibited the exit of uridine from both types of cells.

In conclusion, the present results demonstrate that nucleoside permeants can either cause the *trans*-stimulation or the *trans*-inhibition of uridine efflux from human erythrocytes. These *trans* effects are also dependent on the source of the red blood cells. Therefore, the inability of a nucleoside to result in the acceleration of uridine efflux when present at the *trans* face will not discriminate between a transported and a non-transported nucleoside.

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REFERENCES

- Cabantchik, Z. I. & Ginsburg, H. (1977) *J. Gen. Physiol.* **69**, 75–96
- Cass, C. E. & Paterson, A. R. P. (1972) *J. Biol. Chem.* **247**, 3314–3320
- Jarvis, S. M. & Young, J. D. (1980) *Biochem. J.* **190**, 377–383
- Jarvis, S. M., Chapman, J. D., Ngan-Lee, J., Rutledge, K. A., Barr, P. J. & Paterson, A. R. P. (1982) *Cancer Res.* **42**, 4358
- Jarvis, S. M., Hammond, J. R., Paterson, A. R. P. & Clanachan, A. S. (1983) *Biochem. J.* **210**, 457–461
- Jarvis, S. M., Martin, B. W. & Ng, A. S. (1985) *Biochem. Pharmacol.* **34**, 3237–3241
- Lieb, W. R. (1982) in *Red Cell Membranes: A Methodological Approach* (Ellory, J. C. & Young, J. D., eds.), pp. 135–164, Academic Press, London
- Oliver, J. M. & Paterson, A. R. P. (1971) *Can. J. Biochem.* **49**, 262–270
- Paterson, A. R. P., Kolassa, N. & Cass, C. E. (1981) *Pharmacol. Ther.* **12**, 515–536
- Paterson, A. R. P., Harley, E. R. & Cass, C. E. (1984) *Biochem. J.* **224**, 1001–1008
- Plagemann, P. G. W. & Wohlhueter, R. M. (1980) *Curr. Top. Membr. Transp.* **14**, 225–330
- Turnheim, K., Plank, B. & Kolassa, N. (1978) *Biochem. Pharmacol.* **27**, 2191–2197
- Young, J. D. & Jarvis, S. M. (1983) *Biosci. Rep.* **3**, 309–322

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