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Evidence for the asymmetrical binding of *p*-chloromercuriphenyl sulphonate to the human erythrocyte nucleoside transporter

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Nucleosides cross the human erythrocyte membrane by a facilitated-diffusion process which is selectively inhibited by nanomolar concentrations of nitrobenzylthioinosine (NBMPR). The chemical asymmetry of the transporter was investigated by studying the effects of *p*-chloromercuriphenyl sulphonate (PCMBS) on uridine transport and high-affinity NBMPR binding in inside-out and right-side-out membrane vesicles, unsealed erythrocyte ghosts and intact cells. PCMBS was an effective inhibitor of the transporter (50% inhibition at 30 μM), but only when the organomercurial had access to the cytoplasmic membrane surface. PCMBS inhibition of NBMPR binding to ghosts was reversed by incubation with dithiothreitol. Both uridine and NBMPR were able to protect the transporter against PCMBS inhibition.

Introduction

Purine and pyrimidine nucleosides are transported across the erythrocyte membrane by a nucleoside-specific facilitated-diffusion process which is selectively inhibited by nanomolar concentrations of nitrobenzylthioinosine (NBMPR) and related 6-thiopurine ribonucleosides [1–5]. Physiologically, this transporter provides adenosine, and possibly inosine, for erythrocyte energy metabolism [4,6–9]. Inhibition of the transporter by NBMPR is reversible, and associated with high-affinity binding of inhibitor to the carrier (apparent K_d 0.1–1 nM) [10–12]. This binding is competitively blocked by transported nucleosides such as uridine and deoxycytidine and by the structurally unrelated nucleoside transport inhibitor dipyridamole.

For human erythrocytes, the apparent K_i value for uridine inhibition of NBMPR binding (1.25 mM) has been shown to be similar to the apparent K_m value for uridine equilibrium exchange (0.7–1.3 mM) [3,5,13,14]. The kinetics of NBMPR inhibition of erythrocyte nucleoside transport were studied in detail by Jarvis et al. [5]. NBMPR was found to be a competitive inhibitor of uridine zero-*trans* influx into nucleoside-permeable sheep erythrocytes (apparent K_i 1 nM) but a non-competitive inhibitor of uridine zero-*trans* efflux (apparent K_i 1.5 nM). These experiments suggest that the erythrocyte nucleoside transporter exhibits kinetic asymmetry with respect to its interaction with NBMPR.

At the molecular level, reversible and photoaffinity labelling studies with [^{3}H]NBMPR have identified the human and pig erythrocyte nucleoside transporters as band 4.5 polypeptides

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Abbreviations: PCMBS, *p*-chloromercuriphenyl sulphonate; NBMPR, nitrobenzylthioinosine; NBTGR, nitrobenzylthioguanosine.

(nomenclature of Steck [15]) with apparent molecular weights on sodium dodecyl sulphate polyacrylamide gels of 45 000–66 000 [16–19]. Reconstitution experiments have confirmed that isolated band 4.5 polypeptides are capable of catalysing carrier-mediated nucleoside transport [20], while radiation-inactivation studies estimate that the human erythrocyte transporter has an in situ molecular weight of 122 000, suggesting that the carrier exists in the membrane as a dimer [21,22].

The objective of the present series of experiments was to extend these molecular studies by investigating the chemical asymmetry of the human erythrocyte nucleoside transporter using the slowly-permeating organomercurial *p*-chloromercuriphenyl sulphonate (PCMBS). Our experimental strategy was to compare the effects of PCMBS on intact cells, unsealed erythrocyte ghosts and sealed inside-out membrane vesicles and sealed right-side-out membrane vesicles, using both NBMPR binding activity and uridine transport as assays for nucleoside transporter function. The results demonstrate that PCMBS is an effective inhibitor of erythrocyte nucleoside transport and NBMPR binding, but only when the organomercurial has access to the cytoplasmic membrane surface.

Materials and Methods

Materials. [2-^{14}C]Uridine (specific radioactivity 53 mCi/mmol) was obtained from Amersham International, Amersham, Bucks., U.K. [G-^{3}H]NBMPR (6-(4-nitrobenzyl)thio-9-β-D-ribofuranosylpurine) (specific radioactivity 17 Ci/mmol, > 98% radiochemically pure) was purchased from Moravek Biochemicals, Brea, CA, U.S.A, while nonradioactive nitrobenzylthioguanosine (2-amino-6-(4-nitrobenzyl)thio-9-β-ribofuranosylpurine, NBTGR) was a generous gift from Professor A.R.P. Paterson, Cancer Research Group (McEachern Laboratory), University of Alberta, Edmonton, Alberta, Canada. Other reagents were obtained as follows: nonradioactive uridine, PCMBS and dithiothreitol from Sigma Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K.: Dextran T-70 from Pharmacia Fine Chemicals, Hounslow, Middlesex, U.K. All other chemicals were of analytical grade.

Blood. Blood was withdrawn from healthy human volunteers by syringe into heparin. Erythrocytes were washed three times in 150 mM NaCl, 5 mM sodium phosphate (pH 8.0) by repeated centrifugation and resuspension. The buffy coat was discarded. To prepare erythrocyte ghosts, 2 ml of washed packed cells were haemolysed in 40 ml ice-cold 5 mM sodium phosphate (pH 8.0). Ghosts were harvested by centrifugation (30 000 × *g* for 10 min) and washed three times in 5 mM sodium phosphate. The final haemoglobin-free membrane pellet was resuspended in 2 ml of the same buffer.

Vesicle preparation. Inside-out vesicles were prepared by the method of Steck and Kant [23] as modified by Macintyre and Gunn [24] and Macintyre [25]. Briefly, 2 ml of ghost membranes (approx. 4 mg protein per ml) were diluted to 70 ml with ice-cold vesiculation medium containing 0.5 mM sodium phosphate and 0.5 mM EGTA (pH 8.4). This suspension was placed in a prewarmed water bath and incubated at 35°C for 15 min. Membranes were recovered by centrifugation (30 000 × *g* for 20 min), resuspended in 2 ml ice-cold vesiculation medium, and then passed five times through a No. 27 gauge needle attached to a 5 ml syringe to complete vesiculation. The homogenised vesicle suspension was layered onto 3 ml of Dextran T-70 barrier solution (4.5% (w/v) Dextran T-70 in 0.5 mM sodium phosphate, pH 8.0) in a 5 ml Beckman SW50L centrifuge tube and centrifuged at 30 000 × *g* for 1 h. Sealed inside-out vesicles were recovered from the barrier interface and washed twice in ice-cold 5 mM sodium phosphate, pH 8.0. The final vesicle pellet was resuspended in the same solution at a protein concentration of 2–4 mg per ml and stored at 0–4°C before use.

Right-side-out vesicles were also prepared according to the procedure of Steck and Kant [23] as modified by Cohen and Solomon [26]. Ghost membranes (5 ml) prepared as described above were diluted to 120 ml with 0.5 mM sodium phosphate (pH 8.0), and left on ice for 30 min. At the end of this time, 200 mM $MgCl_2$ was added to the suspension to give a final $MgCl_2$ concentration of 0.1 mM. Membranes were then pelleted by centrifugation (30 000 × *g*, 1 h) and the pellet kept at 4°C overnight. After overnight incubation, the membrane pellet was resuspended into 2 ml of 0.1

mM $MgCl_2$, 0.5 mM sodium phosphate (pH 8.0). The suspension was then homogenised by passing 5 times through a No. 27 gauge needle, and afterwards, was diluted into 5 ml of the same solution. This diluted suspension of vesicles was layered onto 8.5 ml of Dextran T-70 barrier solution (4.5% (w/v) Dextran T-70, 0.1 mM $MgSO_4$, 0.5 mM sodium phosphate, pH 8.0) in a 13.5 ml Beckman SW40 centrifuge tube and centrifuged at $30\,000 \times g$ for 4 h. Sealed right-side-out vesicles were recovered from the barrier interface, washed twice in 5 mM sodium phosphate (pH 8.0), and finally resuspended in the same buffer (2–4 mg protein/ml).

Vesicle sidedness was established by determining the accessibility of acetylcholinesterase, a marker for the extracellular membrane suface, and glyceraldehyde-3-phosphate dehydrogenase, a marker for the intracellular membrane surface, as described by Steck and Kant [23]. Our inside-out vesicle preparations typically contained 70–80% inside-out vesicles while the right-side-out vesicle preparations ranged from 90–98% pure.

PCMBS studies. Intact erythrocytes (10% haematocrit), ghosts and membrane vesicles (2 mg protein per ml) were treated with PCMBS on ice to minimise diffusion of the organomercurial through the lipid bilayer. After 30 min incubation, cells, membranes and vesicles were pelleted by centrifugation and resuspended in 20 vol. of the appropriate ice-cold medium (either 150 mM NaCl, 5 mM sodium phosphate (pH 8.0) or 5 mM sodium phosphate (pH 8.0)). Samples were recentrifuged and the washed pellets suspended in ice-cold medium in preparation for NBMPR binding and uridine transport assays.

NBMPR binding. High-affinity NBMPR binding to erythrocyte ghosts and membrane vesicles (both inside-out vesicles and right-side-out vesicles) was determined by a high-speed centrifugation procedure employing a Beckman Airfuge ultracentrifuge [22]. Portions of membrane or membrane vesicles (0.6 ml, 0.2–0.4 mg protein per ml) were equilibrated with a saturating concentration of [^{3}H]NBMPR (50 nM) at room temperature for 30 min in the absence and in the presence of 20 μM NBTGR as competing nonradioactive ligand. After incubation, duplicate 0.2 ml aliquots were removed and centrifuged at $120\,000 \times g$ for 10 min. Supernatants were carefully removed by suction and retained for radioactivity determination. The bottom half of each microcentrifuge tube was then placed in a scintillation mini-vial, and the membrane pellet dissolved in 0.5 ml 5% (w/v) Triton X-100. Radioactivity was determined by liquid scintillation spectrometry with appropriate quench correction. High-affinity (NBTGR-sensitive) NBMPR binding was calculated from the difference in pellet radioactivity measured in the absence and in the presence of NBTGR.

High-affinity NBMPR binding to intact erythrocytes was also measured at a saturating concentration of [^{3}H]NBMPR as described previously [12].

Uridine transport. Transport studies were performed at room temperature (22°C) using [2-^{14}C]uridine as the radioactive permeant. For intact erythrocytes, initial rates of NBTGR-sensitive uridine uptake (0.2 mM extracellular concentration, 6 s incubation) were measured by an oil-stop procedure employing *n*-dibutylphthalate [8].

Nucleoside uptake by membrane vesicles was assayed by a modified Millipore-filtration technique [24,27]. Briefly, uridine uptake was initiated by addition of 50 μl [^{14}C]uridine (0.4 mM, 10 μCi/μmol) in 5 mM sodium phosphate, pH 8.0 to an equal volume of membrane vesicle suspension (approx. 0.3 mg protein per ml). At the appropriate time thereafter, uptake was terminated by the rapid addition of 2 ml ice-cold stopping solution (20 μM NBTGR in sodium phosphate buffer), followed by immediate filtration under vacuum using Millipore HAWP 0.45 μm filters which had been prewashed with 2 ml stopping solution. Filters were rapidly washed four times with 2 ml aliquots of ice-cold stopping solution and the resulting washed filters transferred into 10 ml scintillation fluid for radioactivity determination. Blank values were obtained by processing samples in which ice-cold uridine solution was mixed with ice-cold membrane vesicles pretreated with 20 μM NBTGR and immediately diluted with stopping solution. Vesicle uridine uptake was calculated after subtraction of these blanks.

Protein determination. Protein was assayed by the method of Lowry et al. [28].

Results and Discussion

NBMPR binding

In a previous study [29] it was noted that pretreatment of intact fetal sheep erythrocytes with 1 mM PCMBS at 1°C for periods of up to 40 min had no significant effect on either high-affinity NBMPR binding activity or NBTGR-sensitive uridine zero-*trans* influx. In contrast, NBMPR binding to unsealed membrane ghosts prepared from these cells was inhibited 90% after 10 min exposure to organomercurial. These experiments suggested to us that the erythrocyte nucleoside transporter may exhibit chemical asymmetry with respect to its distribution of PCMBS-sensitive thiol groups. To investigate this possibility further, we compared the effects of PCMBS (0.01–0.2 mM) on high-affinity NBMPR binding to (a) intact human erythrocytes, (b) unsealed human erythrocyte ghosts, (c) sealed inside-out vesicles, and (d) sealed right-side-out vesicles (Fig. 1). As expected, these

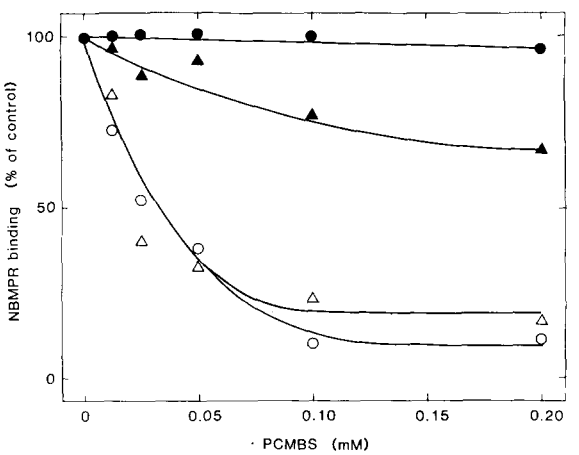

Fig. 1. *p*-Chloromercuriphenyl sulphonate inhibition of [^{3}H]nitrobenzylthioinosine binding to intact human erythrocytes, unsealed erythrocyte ghosts, inside-out and right-side-out membrane vesicles. Samples were pretreated with varying concentrations of PCMBS at 1°C for 30 min, washed free of excess inhibitor and assayed for high-affinity (NBTGR-sensitive) NBMPR binding activity using a Beckman Airfuge as described in the text. The inside-out and right-side-out vesicle preparations contained 71% and 95% sealed inside-out and right-side-out vesicles, respectively. Values are means of duplicate estimates and are expressed as percentages of control NBMPR binding activities measured in the absence of organomercurial. Symbols: ●, intact cells; ○, ghosts; ▲, right-side-out vesicles; △, inside-out vesicles.

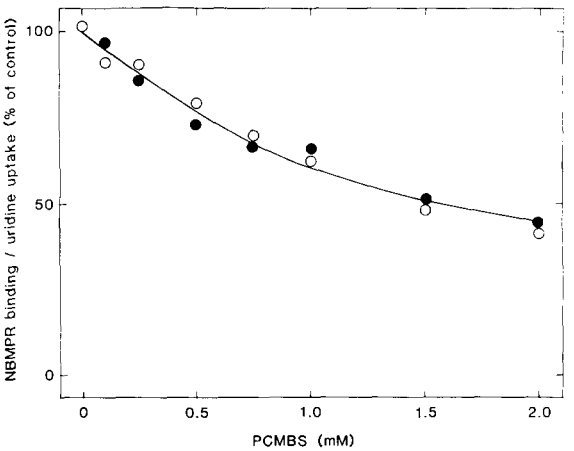

Fig. 2. Inhibition of [^{3}H]nitrobenzylthioinosine binding and [^{14}C]uridine uptake in intact human erythrocytes by high concentrations of *p*-chloromercuriphenyl sulphonate. Erythrocytes were pretreated with varying concentrations of PCMBS at 1°C for 30 min, washed free of excess inhibitor and assayed for high-affinity NBMPR binding activity and NBTGR-sensitive zero-*trans* uridine uptake (0.2 mM extracellular concentration). Values are means of duplicate estimates and are expressed as percentages of control activities measured in the absence of PCMBS. Symbols: ●, uridine transport; ○, NBMPR binding.

low concentrations of PCMBS had little effect on NBMPR binding to intact cells (5% inhibition at 0.2 mM PCMBS). Higher concentrations of PCMBS resulted in progressive inhibition of NBMPR binding activity (50% inhibition at 1.5 mM PCMBS) (Fig. 2). Similarly, NBMPR binding to right-side-out vesicles was relatively insensitive to PCMBS inhibition (30% at 0.2 mM PCMBS). However, these results contrast markedly with those obtained for ghosts and inside-out vesicles. NBMPR binding to these preparations was approx. 50-fold more sensitive to organomercurial than intact cells and right-side-out vesicles, 50% inhibition occurring at 20–30 μM PCMBS (30 min exposure at 1°C). At 0.2 mM PCMBS, NBMPR binding to inside-out vesicles and ghosts was inhibited by 85% and 90%, respectively.

The results presented in Fig. 3 demonstrate that uridine had the ability to protect ghosts against PCMBS inhibition, the degree of protection achieved being dependent upon the concentration of nucleoside present during exposure of membranes to PCMBS. The concentration of uridine required to give 50% protection was 0.3 mM. This

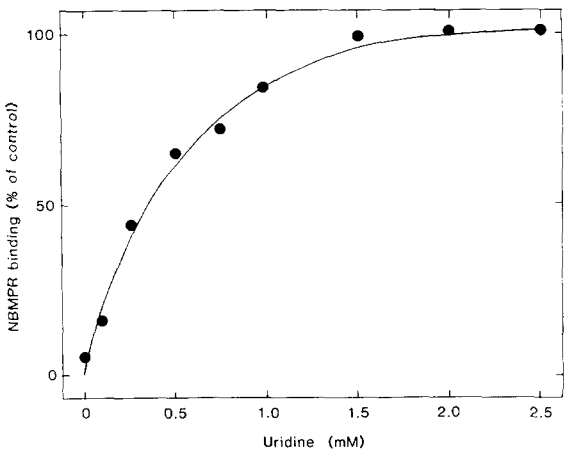

Fig. 3. Effect of uridine on *p*-chloromercuriphenyl sulphonate inhibition of [^{3}H]nitrobenzylthioinosine binding to human erythrocyte ghosts. Ghosts were preincubated with 0.1 mM PCMBS at 1°C for 30 min in the absence and in the presence of varying concentrations of uridine, washed free of excess inhibitor and nucleoside and assayed for high-affinity NBMPR binding activity. Values are means of duplicate estimates and are expressed as percentages of the NBMPR binding activity measured in the absence of PCMBS.

compares with estimates of 0.7–1.3 mM (22°C) for the apparent K_m of uridine equilibrium exchange in intact human erythrocytes and an apparent K_i value of 1.25 mM (25°C) for uridine inhibition of NBMPR binding [3,5,13,14]. Both the apparent K_m value for uridine equilibrium exchange and the apparent K_i for uridine inhibition of NBMPR binding might be expected to be lower at 1°C, the temperature we used for treatment of membranes with PCMBS (see Materials and Methods). Uridine concentrations above 1.5 mM gave complete protection against PCMBS inhibition.

We also investigated the ability of NBMPR itself to protect ghosts against PCMBS inhibition. NBMPR binding to the transporter is reversible. However, at 1°C the rate of dissociation of the transporter-inhibitor complex is very slow. We were therefore able to study the influence of site-bound NBMPR on PCMBS inhibition of transporter function in the absence of unbound NBMPR. In the experiment shown in Table I, unsealed ghosts were preincubated at room temperature in the presence and absence of sufficient nonradioactive NBMPR (50 nM) to occupy all the available NBMPR binding sites. Membranes were then washed free of unbound ligand prior to exposure to 0.1 mM PCMBS on ice for 30 min. As expected, PCMBS treatment of membranes in the absence of unbound ligand resulted in substantial inhibition of [^{3}H]NBMPR binding activity. This

TABLE I

p-CHLOROMERCURIPHENYL SULPHONATE INHIBITION OF [^{3}H]NITROBENZYLTHIOINOSINE BINDING TO HUMAN ERYTHROCYTE GHOSTS: EFFECTS OF DITHIOTHREITOL AND NITROBENZYLTHIOINOSINE PRETREATMENT

Membranes were incubated in the presence and absence of 50 nM nonradioactive NBMPR at room temperature for 30 min (A) and then washed free of unbound ligand using ice-cold medium. The membrane pellets were resuspended in ice-cold medium (±0.1 mM PCMBS) and left on ice for a further 30 min (B). Excess organomercurial was removed by washing and the membranes incubated at room temperature for 30 min in the presence and absence of 10 mM dithiothreitol (C). The membranes were washed once more to remove dithiothreitol and dissociated NBMPR and then assayed for high-affinity [^{3}H]NBMPR binding activity as detailed in Materials and Methods. –, denotes incubations without additions. Values are means of duplicate estimates.

	Incubation			[^{3}H]NBMPR binding (% control)
	A(22°C)	B(1°C)	C(22°C)	
1	–	–	–	100
2	–	–	dithiothreitol	102
3	–	PCMBS	–	4
4	–	PCMBS	dithiothreitol	74
5	NBMPR	–	–	104
6	NBMPR	–	dithiothreitol	105
7	NBMPR	PCMBS	–	76
8	NBMPR	PCMBS	dithiothreitol	76

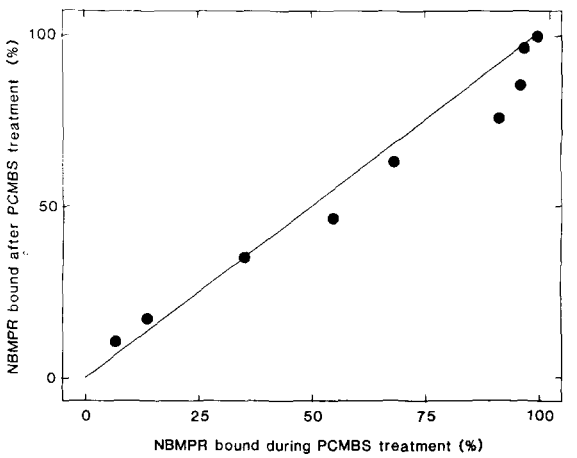

Fig. 4. Effect of nitrobenzylthioinosine on *p*-chloromercuriphenyl sulphonate inhibition of nucleoside transporter ligand binding activity. Human erythrocyte ghosts were equilibrated with varying concentrations of [^{3}H]NBMPR at room temperature and then washed free of unbound ligand using ice-cold medium. The washed membrane pellets were then suspended in ice-cold medium and aliquots were removed to determine the fraction of high-affinity NBMPR binding sites occupied by ligand in each sample. The remaining membranes were exposed to 0.1 mM PCMBS for 30 min at 1°C. Excess inhibitor was removed by washing and the ghosts were subsequently assayed for high-affinity NBMPR binding activity at room temperature as described in the text. The residual NBMPR binding activity of each sample was expressed as a percentage of the control high-affinity binding activity of the membranes measured in the absence of PCMBS and plotted against the corresponding value for the percentage of high-affinity NBMPR binding sites occupied by ligand during exposure to PCMBS.

inhibition was reversed to 74% of the control value by subsequent incubation of the PCMBS-treated ghosts with dithiothreitol, confirming that PCMBS was inhibiting transporter function by binding to membrane thiols. The results presented in Table I further demonstrate that site-bound NBMPR protected the transporter against PCMBS inhibition. This phenomenon was studied further using [^{3}H]NBMPR to accurately correlate the degree of protection achieved with the fraction of high-affinity NBMPR binding sites occupied by ligand during PCMBS treatment. In the experiment shown in Fig. 4, membranes were preincubated (30 min at room temperature) with graded concentrations of [^{3}H]NBMPR (1–50 nM) and then washed free of unbound ligand prior to exposure to 0.1 mM PCMBS at 1°C for 30 min. The amounts of [^{3}H]NBMPR bound to the transporter during PCMBS treatment ranged from 3–34 pmol/mg protein, corresponding to 9–100% of maximum binding activity. After PCMBS treatment, membranes were washed free of unreacted organomercurial and assayed for high-affinity NBMPR binding capacity. This was achieved by incubating membranes for a further 30 min at room temperature in the presence of excess (50 nM) [^{3}H]NBMPR (see Materials and Methods). The incubation period was sufficient to allow reequilibration of bound and free ^{3}H-ligand. The results demonstrate a linear relationship between NBMPR bound during PCMBS exposure and NBMPR binding activity remaining after PCMBS treatment. There was therefore a direct correlation between the degree of protection achieved and the number of high-affinity sites occupied by NBMPR. Thus, the fraction of sites capable of binding NBMPR after PCMBS treatment was equal to the fraction of sites occupied by NBMPR during exposure to PCMBS. In other words, NBMPR and PCMBS binding to the transporter were mutually exclusive.

Uridine transport

The NBMPR binding data presented in the preceding section suggest that PCMBS inhibits the

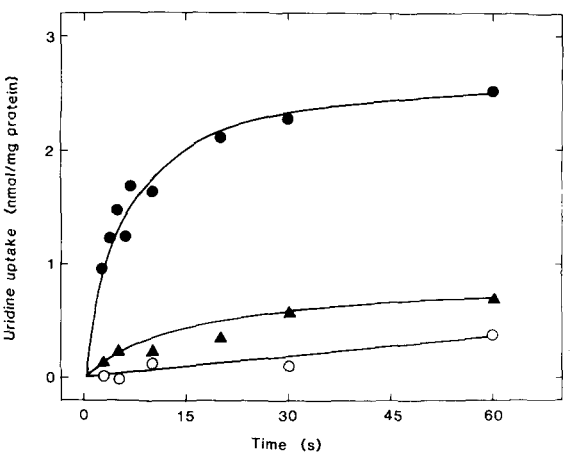

Fig. 5. Effects of *p*-chloromercuriphenyl sulphonate and nitrobenzylthioguanosine on [^{14}C]uridine uptake by human erythrocyte inside-out membrane vesicles. [^{14}C]uridine uptake (0.2 mM) was measured at room temperature by the Millipore-filtration technique described in Materials and Methods. Values are means of duplicate estimates. Symbols: ●, control; ▲, 0.1 mM PCMBS; ○, 20 μM NBTGR.

nucleoside transporter by binding to transporter thiol group(s) exposed to the cytoplasmic membrane surface. In agreement with this conclusion, we found that PCMBS was a relatively poor inhibitor of NBTGR-sensitive uridine zero-*trans* uptake by intact human erythrocytes, significant inhibition of transport activity only occurring at PCMBS concentrations in excess of 0.2 mM. Higher concentrations of organomercurial resulted in a parallel loss of uridine transport and NBMPR binding activity (Fig. 2).

As a further test of nucleoside transporter asymmetry, we investigated the ability of PCMBS to inhibit uridine transport in membrane vesicles prepared from human erythrocyte ghosts. The results presented in Figs. 5 and 6 demonstrate that both inside-out vesicles and right-side-out vesicles are capable of rapid uridine uptake at room temperature. The half-time for nucleoside equilibration was approx. 3 s for the two vesicle preparations (0.2 mM uridine). At equilibrium, inside-out vesicles contained 2.3 nmol uridine per mg protein compared with 2.4 nmol/mg protein for the right-side-out vesicle preparation. In both cases, uridine permeation was almost completely abolished in the presence of 20 μM NBTGR, the residual uptake in the presence of NBTGR giving only 15% and 9% equilibration after 60 s for inside-out vesicles and right-side-out vesicles, respectively. As far as we are aware, this is the first demonstration of nucleoside transport in erythrocyte membrane vesicles. NBTGR-sensitive uridine uptake by inside-out vesicles was almost completely inhibited by pretreatment of vesicles with 0.1 mM PCMBS (Fig. 5). In contrast, this concentration of organomercurial had no detectable effect on uridine uptake by right-side-out vesicles (Fig. 6).

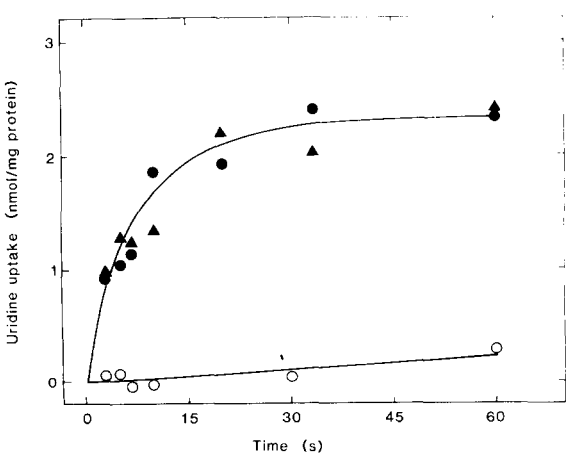

Fig. 6. Effects of *p*-chloromercuriphenyl sulphonate and nitrobenzylthioguanosine on [^{14}C]uridine uptake by human erythrocyte right-side-out membrane vesicles. [^{14}C]Uridine uptake (0.2 mM) was measured at room temperature by the Millipore-filtration technique described in Materials and Methods. Values are means of duplicate estimates. Symbols: ●, control; ▲, 0.1 mM PCMBS; ○, 20 μM NBTGR.

Conclusion

The results presented in this paper demonstrate that PCMBS inhibits uridine transport and NBMPR binding by interacting with nucleoside transporter thiol group(s) exposed to the cytoplasmic membrane surface. Both uridine and NBMPR had the ability to protect the transporter against PCMBS inhibition of NBMPR binding activity. The simplest interpretation of these results is that PCMBS, NBMPR and uridine bind to the same site on the nucleoside transporter, namely the nucleoside permeation site. This permeation site may exist in two orientations, previous kinetic studies indicating that NBMPR interacts preferentially with its outward-facing conformation [5,29]. In contrast, the present experiments suggest that PCMBS binds selectively to the inward-facing conformation of the site, as judged by the finding that the organomercurial requires access to the cytoplasmic membrane surface. It is therefore envisaged that the inward-facing conformation of the nucleoside permeation site possesses a thiol group reactive to PCMBS. This thiol group is presumably not exposed when the permation site is in its outward facing conformation (e.g. in the presence of NBMPR) or when the inward-facing conformation is occupied by substrate. Our results would, of course, be consistent with more complex models of the nucleoside transporter. One such model proposes the NBMPR inhibits carrier function by binding to a separate inhibitory allosteric site [30].

An important question is the extent to which the erythrocyte nucleoside transporter can be regarded as a model for nucleoside carriers in other, more complex cells. Nucleoside transporters sensitive to inhibition by 6-thiopurine ribonucleosides

such as NBMPR and NBTGR appear to be widely distributed in mammalian cells [31–33] and photoaffinity labelling experiments with [^{3}H]NBMPR have demonstrated that the nucleoside transporters from rat and guinea pig lung and liver, guinea pig heart and brain and cultured S49 mouse lymphoma cells have similar molecular weights on sodium dodecyl sulphate polyacrylamide gels as the erythrocyte system [34–38]. However, some lines of cultured cells such as the Walker 250 rat carcinoma and the Novikoff rat hepatoma have nucleoside transporters insensitive to nanomolar concentrations of NBMPR [39–41]. Other cultured neoplastic cells such as L1210 murine leukemia cells exhibit mixed NBMPR-sensitive and NBMPR-insensitive transport [41]. The mechanism of this NBMPR-insensitivity remains to be determined.

Interestingly, uridine zero-*trans* uptake by intact S49 cells resembles that in erythrocytes by being unaffected by low concentrations of PCMBS whereas the IC_{50} for PCMBS inhibition of uridine zero-*trans* uptake by Walker 250 cells is less than 25 μM [41]. A similar difference in PCMBS reactivity has been reported for the NBMPR-sensitive and NBMPR-insensitive nucleoside transporters in L1210 cells [41]. In contrast, the related organomercurials, *p*-hydroxymercuribenzoate and *p*-hydroxymercuriphenyl sulphonate, have been reported to inhibit both cultured cell transport systems when used at 37°C [42]. PCMBS may therefore have use in functional and molecular comparisons of NBMPR-sensitive and NBMPR-insensitive nucleoside transporters.

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