Mechanism of Putrescine Transport in Human Pulmonary Artery Endothelial Cells

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ABSTRACT
Effective lung repair requires optimal replication of critical cell populations in the lung. Endogenous polyamines such as putrescine, spermine and spermidine play important roles in cell proliferation and differentiation, and may arise due to intracellular synthesis or transport into the cell. To determine the mechanism of polyamine transport in lung endothelial cells, the uptake of putrescine in human pulmonary artery endothelial cells was examined. Putrescine (7 nM) uptake into the cells approached equilibrium at 1 hr and was inhibited by methylglyoxal bis(guanyldihydrazone). Kinetic studies revealed that uptake occurred via both a high- and low-affinity system. The effect of several amines (700 μM) on the 15-min uptake of putrescine was examined and a rank order of inhibition was determined: methylglyoxal bis(guanyldihydrazone) > putrescine > spermine > spermidine > gentamicin > mepiperphenidol. α-Aminoisobutyric acid, a prototype system A amino acid, and tetraethylammonium, an organic cation, had no effect. N-ethylmaleimide inhibited transport 71%, whereas dinitrophenol did not. A reduction in temperature from 37°C to 5°C resulted in a 42% decrease in putrescine transport. Additionally, removing fetal bovine serum from the uptake medium reduced transport 38%. These data indicate that human pulmonary artery endothelial cells possess a specific transport system for polyamines. An improved understanding of this pathway in pulmonary endothelial cells may permit development of strategies to facilitate growth and repair of this critical cell population.

The endogenous polyamines putrescine, spermine and spermidine are ubiquitous cationic molecules that have multifarious roles in biological systems. Putrescine is a precursor for spermidine, which in turn is the precursor for spermine (for reviews see Tabor and Tabor, 1984; Pegg and McCann, 1988; Seiler and Heby, 1988). All three differ from one another by the addition of a single primary amino group (Put), two primary amino groups (Spd) or three primary amino groups (Spm). Putrescine is synthesized from ornithine and via a carboxylase the same or different transport systems. Additionally, removing fetal bovine serum from the uptake medium reduced transport 38%. These data indicate that human pulmonary artery endothelial cells possess a specific transport system for polyamines. An improved understanding of this pathway in pulmonary endothelial cells may permit development of strategies to facilitate growth and repair of this critical cell population.

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ABBREVIATIONS: α-AIBA, α-aminoisobutyric acid; CI, cytotoxic index; DOG, deoxyglucose; HPAC cells, human pulmonary artery endothelial cells; MGBG, methylglyoxyl bis(guanyldihydrazone); NEM, N-ethylmaleimide; R, release; TEA, tetraethylammonium.
epithelial cells, few studies have yet examined the importance of this transport system in pulmonary endothelial cells (Bogle et al., 1990). Therefore, to clarify the transport of polyamines in cells derived from the human pulmonary vasculature, HPAE cells in monolayer culture were used to determine if a specific transport mechanism for putrescine exists and if so, to determine the effect of various parameters and inhibitors on this transport system. By using cell cultures, we will be able to examine transport in a defined cell population. These results indicate that human pulmonary endothelial cells possess a specific transport system for polyamines, and this transport system can be significantly modified by a variety of competitive and noncompetitive inhibitors.

Methods

Isolation and culture of HPAE cells. HPAE cells were isolated, cultured and identified as previously described (Martin et al., 1989). Intact main pulmonary arteries were obtained as autopsy specimens from individuals who had no evidence of cardiovascular or pulmonary disease. All cells used in this study were derived from arteries that had been processed within 12 hr of the donor's death by clamping the artery, rinsing the luminal surface with normal saline followed by a 30-min exposure to 0.06% collagenase and saline followed by a 30-min exposure to 0.06% collagenase. The cells were harvested by gently scraping the luminal surface and collecting the detached cells in normal saline. Once the cells had been pelleted, they were resuspended in Medium 199 (M.A. Bioproducts, Walkersville, MD) containing penicillin (100 U/ml) and streptomycin (100 μg/ml) and supplemented with 20% fetal bovine serum (HyClone Laboratories, Logan, UT), endothelial cell growth supplement (50 μg/ml, Collaborative Research, Lexington, MA) and heparin (50 μg/ml). The harvested cells were then plated at high density into a Falcon Primaria cultured dish (Becton Dickinson Labware, Oxnard, CA). Once the HPAE cells became confluent, they were subcultured at a ratio of 1:5. The cultured cells were identified as human endothelial cells based on three criteria: 1) typical morphology of tightly packed polygonal-shaped cells; 2) presence of Factor VIII-related antigen; and 3) the presence of Weibel-Palade bodies, a finding characteristic of human endothelial cells (Martin and Kachel, 1989; Martin et al., 1989; Powis et al., 1990). All cells were used between 8 and 15 passages. Capacity to transport putrescine was not affected by the cell passage number.

[3H]Putrescine uptake by HPAE cells. Putrescine uptake was assayed using monolayer HPAE cells in 24-well Primaria dishes incubated in media containing 0.2 μCi [3H]putrescine/ml (7 nM) (American Corporation, Arlington Heights, IL). The number of counts per minute per well averaged from 500 to 1500. The media used in all experiments was RPMI 1640, with 25 mM HEPES (Gibco, Grand Island, NY) containing 10% fetal bovine serum (except in fig. 8, where there was no serum used in half of the experiment) (HyClone Laboratories), and penicillin and streptomycin.

To assess the rate of putrescine uptake, the cells were incubated in the labeled media for 0, 5, 15, 30 and 60 min at 37°C (fig. 1A) and for 0 and 15 min subsequently. The zero time point (t = 0) was used in order to obtain an estimate of nonspecific binding to the extracellular domain of the cells and was subtracted from the 15-min time point. This value represented 14.7 ± 1.2% of total uptake at 15 min (n = 34). The nonspecific binding at 15 min was examined by looking at putrescine uptake for 15 min at 5°C and compared to t = 0, 5°C, and to t = 0, 37°C. All values were found to be comparable. Presence of excess cold putrescine for 15 min at 37°C produced results similar to t = 0, 37°C. In addition, the nonspecific binding fraction did not increase as a function of increasing substrate concentration (kinetic studies). The transport of putrescine was examined under many different conditions: in the presence of drugs/chemicals that were potential transport competitors, in the presence of metabolic inhibitors, in different temperatures and in the absence of fetal bovine serum. The effect of the sulfhydryl modifier NEM was examined by treating cells with the agent for 15 min, washing them with 1 mM cysteine to remove any unreacted reagent and then examining putrescine uptake for 15 min. Pretreatment with the reagent, followed by examination of uptake, produced identical results to concomitant addition of NEM with putrescine. A cysteine control wash produced no effect on putrescine transport.

All of the uptake studies were terminated by removing the labeled media at the appropriate time, rapidly rinsing the cell monolayers (3×) with excess unlabeled media and solubilizing the cells in 0.25 N NaOH. For the zero time point, the labeled medium was added to cells and washed off immediately. The solubilized cell material was neutralized with HCl and transferred to 10 ml of scintillation cocktail (Scintiverse BD, Fisher Scientific, Itasca, IL) for determination in a Beckman LS8001 scintillation counter. Cell number was determined by averaging hemocytometer cell counts from four to six wells set up for each experiment. The number of cells per well was approximately 100,000.

Detection of cell damage using [3H]DOG. To determine if the effect of transport competitors or metabolic inhibitors on putrescine uptake was mediated by a toxic response, injury to HPAE cells was quantified by using a [3H]DOG cytotoxicity assay (Powis et al., 1990). HPAE cells at 80 to 90% confluency in 24-well Primaria culture dishes were radiolabeled with [3H]DOG (Amersham Corporation; specific activity of 17 Ci/mmol) at 1 μCi/ml for 15 hr. After incubation, HPAE cells were washed several times with media to remove any unincorporated [3H]DOG. Monolayer HPAE cells in 24-well primaria dishes were
incubated in the presence or absence of the transport competitors and metabolic inhibitors used in this study. After a 1-hr incubation period, the 24-well plates were centrifuged in plate carriers (Beckman, Arlington Heights, IL) at 300 × g. To determine the amount of [3H]DOG released by the cells, one-half of the supernatant was removed, followed by the addition of 2% Triton X-100 to release the intracellular label into the remaining supernatant. The supernatant and the corresponding supernatant/cell fractures were transferred to 10 ml of scintillation cocktail, and disintegrations per minute were subsequently determined in a Beckman LS3801 scintillation counter. The %R is 24/(A + B), where A represents the disintegrations per minute released into one-half of the supernatant and B represents the disintegrations per minute associated with the cells and remaining supernatant. The CI is defined as: CI = (%Rmax - %Rcontrol)/(%Rcontrol - %Rnormal) × 100, where R maximum represents the disintegrations per minute released from the control cells treated with 2% Triton X-100.

Every experiment was done in triplicate with at least three different preparations (each N represents a separate preparation, N ≥ 3). Data were analyzed by an analysis of variance and the differences in mean values were tested by using the Fisher’s test. A P value < 0.05 was taken as significant. Linear regression was done employing the Curve/line fitting program of cricket graph (Macintosh).

Results

The uptake of putrescine (7 nM) into HPAE cells was examined in the presence and absence of MGBG, an inhibitor of putrescine transport (Rannels et al., 1985a; Pegg and McCann, 1988) (fig. 1A). The uptake started to reach a plateau between 30 and 60 min. MGBG was inhibitory at all time points examined. When the difference between the uptake in the presence and absence of MGBG was assessed, the specifically mediated putrescine transport was obtained (fig. 1B). This value represents the transport of putrescine that is inhibitable by a competitor. The uptake was linear for the first 30 min and gave a linear regression correlation coefficient of 1.00. Therefore, 15 min was chosen as the time point at which the mechanism of putrescine transport was examined.

The specifically mediated uptake (15 min) of putrescine was plotted as a function of putrescine concentration and gave rise to two simple hyperbolic curves, indicating that the uptake could be described by Michaelis-Menton kinetics. The presence of two different curves, both of which achieve saturation, one at a low concentration (nM) and the other at a high concentration (μM), supports the existence of two separate pathways for uptake. Therefore, the kinetics of putrescine uptake was examined over these two ranges by employing Hanes-Woolf transformations (O’Flaherty, 1981). At lower concentrations (fig. 2A), putrescine uptake produced a Km value of 3.3 nM and a Vmax value of 8.2 fmol/15 min × 100,000 cells. The uptake in the presence of MGBG produced an increase in the Km to 5.1 nM and a decrease in the Vmax to 5.6 fmol/15 min × 100,000 cells. At higher concentrations (fig. 2B), the Km and Vmax values for putrescine uptake were 1.5 mM and 910 pmol/15 min × 100,000 cells, respectively. The presence of MGBG increased the Km and Vmax to 2.1 mM and 1000 pmol/15 min × 100,000 cells, respectively. In the nanomolar range, MGBG had an appreciable effect on both Km and Vmax, whereas in the micromolar range, MGBG had a greater effect on Km than on Vmax. It appears that these data are most consistent with MGBG, producing mixed inhibition of transport in the nanomolar range, but producing competitive inhibition in the micromolar range.

The effect of several chemical compounds on the uptake of putrescine was examined (fig. 3). The concentration of inhibitors was determined by choosing a maximal dose that was not capable of producing toxicity in a cell toxicity assay (see table 1 below). At nontoxic doses, MGBG was the most potent inhibitor of putrescine transport, blocking uptake 59%, although not statistically significant from the endogenous polyamines putrescine, spermine and spermidine, which blocked uptake 55, 49 and 44%, respectively (P < .05). It is possible that complete transport inhibition could occur at higher doses, but this was precluded by toxicity. Nonetheless, this may represent the maximal inhibition that was possible. The rest of the transport may be due to MGBG-insensitive uptake (passive diffusion and/or transport via a separate transport system). Less effective inhibitors were the polyamine antibiotic gentamicin, a weak substrate for the renal organic cation transport system (Sokol et al., 1989), and the classical organic cation transport inhibitor meperphenidol; the latter has been used as a probe for the organic cation transport system. These two compounds inhibited transport 35 and 23%, respectively (P < .05). TEA, another organic cation, and α-AIBA, a prototype system A amino acid, had no significant effect on putrescine transport. Interestingly, polyamines and α-AIBA shared a common transport system in neuroblastoma cells (Rinehart and Chen, 1984). It is notable to mention that meperphenidol and gentamicin, although capable of producing some inhibition of putrescine uptake, were statistically distinct from the potent inhibitor MGBG (P < .05). Dose-response curves for MGBG and spermidine inhibition of putrescine uptake were constructed (fig. 4). MGBG produced an IC50 value of 32 nM. In contrast, spermidine was less potent and had an IC50 value greater than 500 μM.

The effect of NEM and DNP on putrescine uptake was examined (fig. 5). As shown, NEM, a sulfhydryl modifier, blocked uptake 70% (P < .05), whereas DNP, an uncoupler of oxidative phosphorylation, had minimal effects (10%) (P > .05). A dose-response curve for NEM inhibition of transport was constructed and gave an IC50 value of 22 μM (fig. 6).

The effect of all of these agents was assumed to be specific because at the doses used, none elicited a toxic response in the tritiated deoxyglucose toxicity assay (P > .05) (table 1). The most toxic substance was 1.0 mM NEM (P < .05). Because this dose elicited a toxic response, lower concentrations were used.

The temperature dependence of putrescine transport was examined (fig. 7). As the temperature decreased from 37°C to 5°C, the control transport decreased 42% (P < .05). Similarly, there was a significant decrease in the MGBG-inhibitable transport from 53 to 27% of the control value (P < .05). However, even at the lower temperatures, MGBG was not capable of inhibiting the putrescine uptake completely.

The effect of fetal bovine serum in the incubation medium on the transport of putrescine was examined (fig. 8). The absence of serum produced a 32% decrease in transport (P < .05). In addition, the MGBG-inhibitable transport decreased 15% (P < .05).

Discussion

The results demonstrate that HPAE cells possess a specific transport system for polyamines. The uptake of the polyamine putrescine is time dependent, saturable, inhibited by members of the same chemical class, inhibited by the sulfhydryl modifier NEM and decreased both by lowering the incubation temper-
nature and by removing fetal bovine serum from the culture medium.

Several criteria are necessary in order to demonstrate that a specific transport system is present. The uptake must be shown to be time dependent (Balana-Fouce et al., 1989; De Smedt et al., 1989). Putrescine uptake into HPAE cells increased with time. In fact there was a time-dependent increase in both the total putrescine transport and transport in the presence of the inhibitor MGBG, indicating that there is mediated uptake occurring via a transport protein and a nonmediated uptake occurring via diffusion across the membrane.

The uptake must be concentration dependent, that is, saturable (De Smedt et al., 1989). Putrescine uptake is saturable and occurs via two transport systems, one with a high affinity

![Graph A](image1)

**Fig. 2.** Kinetic analysis of putrescine transport. Hanes-Woolf transformations are presented for putrescine transport at low (0.75-7.5 nM) (A) and at high (1-500 μM) concentrations (B). Data were generated in the presence (U) and absence (O) of 0.7 mM MGBG. A) Total transport: $y = 0.4064 + 0.1215x$ ($r = 0.98$); + MGBG: $y = 0.9113 + 0.1792x$ ($r = 0.93$). B) Total transport: $y = 1.686 + 0.001lx$ ($r = 0.99$); + MGBG: $y = 2.0508 + 0.001x$ ($r = 0.99$).

![Graph B](image2)

**Fig. 3.** Effects of organic cations and polyamines on putrescine transport. The transport of 7 nM putrescine was examined for 15 min in the presence of 0.7 mM concentrations of the following chemicals: α-AIBA, TEA, mepiperphenidol (Mepi), gentamicin, spermidine (Spd), spermine (Spm), cold putrescine (Put) and MGBG. The data are presented as femtomoles transported per 100,000 cells (means ± S.E.). *P < .05 vs. control.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Cytotoxic Index</th>
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<tbody>
<tr>
<td>NEM (1.0 mM)</td>
<td>5.5 ± 4.8</td>
<td></td>
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<tr>
<td>MGBG (0.7 mM)</td>
<td>3.6 ± 4.3</td>
<td></td>
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<tr>
<td>DNP (1.0 mM)</td>
<td>1.3 ± 1.0</td>
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<tr>
<td>Mepiperphenidol (0.7 mM)</td>
<td>0.8 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>α-AIBA (0.7 mM)</td>
<td>0.7 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>NEM (0.1 mM)</td>
<td>-0.1 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Putrescine (0.7 mM)</td>
<td>-0.6 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Gentamicin (0.7 mM)</td>
<td>-1.1 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>TEA (0.7 mM)</td>
<td>-3.2 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Spermidine (0.7 mM)</td>
<td>-3.5 ± 1.6</td>
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</tr>
<tr>
<td>DNP (0.1 mM)</td>
<td>-4.1 ± 4.0</td>
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*P < .05 vs. zero.

**TABLE 1**

Effect of chemicals (organic cations, polyamines, DNP, NEM) on HPAE viability

HPAE cells were treated with chemicals as indicated for 1 hr and then the cytotoxicity was assessed by examining [3H]DOG uptake (means ± S.E.).
Fig. 4. Dose-related influences of MGBG and spermidine on putrescine transport. The transport of 7 nM putrescine was examined for 15 min in the presence of increasing concentrations of MGBG (●) or spermidine (○). The data are presented as percents of control transport (means ± S.E.).

Fig. 5. Effect of NEM and DNP on putrescine transport. The transport of 7 nM putrescine was examined for 15 min in the presence of 0.1 mM concentrations of DNP and NEM. The data are presented as percents of control transport (means ± S.E.). *P < .05 vs. control.

Fig. 6. NEM dose-response curve. The transport of 7 nM putrescine was examined for 15 min in the presence of increasing concentrations of NEM. The data are presented as percents of control transport (means ± S.E.).

Fig. 7. Effect of temperature on putrescine transport. The transport of 7 nM putrescine was examined for 15 min at 37, 25 and 5°C. The data were obtained for total transport and transport in the presence of MGBG and are presented as percents of control transport at 37°C (means ± S.E.). *P < .05 vs. total transport at 37°C; †P < .05 vs. transport in presence of MGBG at 37°C.

Fig. 8. Effect of serum on putrescine transport. The transport of 7 nM putrescine was examined for 15 min in the presence and absence of 10% fetal bovine serum (FBS). The data were obtained for total transport and transport in the presence of MGBG and are presented as percents of control transport (means ± S.E.). *P < .05 vs. total transport; †P < .05 vs. transport + FBS.

(\(K_a = 3.2 \text{nM}, V_{max} = 8.6 \text{fmol/15 min} \times 100,000 \text{cells}\) and the second with a low affinity (\(K_a = 1.5 \text{mM}, V_{max} = 910 \text{pmol/15 min} \times 100,000 \text{cells}\)). In human umbilical vein endothelial cells, putrescine uptake had a \(K_i\) of 3.0μM and \(V_{max}\) of 19 pmol/hr/

\(\mu\)g protein (Morgan, 1990). It is possible that at the higher concentrations, putrescine might interact with a transport system responsible for the uptake of chemically related compounds (e.g., endogenous amines like organic cations). It is known that polyamines do overlap with organic cation transport in the kidney (Sokol et al., 1989; Sokol and Gates, 1990). However, when organic cation transport was examined by looking at TEA uptake in HPAE cells, the transport was quite low and not inhibitable by competitors (data not shown). Consistent with this observation, TEA had no effect on the putrescine uptake system (fig. 3). It is not likely that polyamines and organic cations share a common mechanism in these cells; however, the nature of the low-affinity putrescine transport system remains to be ascertained. It is possible that the low-affinity uptake of putrescine represents the allosteric modification by substrate of the high-affinity uptake system.

The uptake should be temperature dependent and transport should be affected by metabolic inhibitors like DNP and sulphydryl-modifying agents like NEM (Gawel-Thompson and Greene, 1988; Zimmerman et al., 1991). Total putrescine transport and MGBG-inhibitable transport were reduced by temperature. Putrescine uptake is blocked by NEM, an irreversible
transport in HPAE cells was not as extensive as inactivation transport requires inactivation of a second population of sulfhydryl modifier, which produced an IC\textsubscript{50} value of 22 \mu M. Because membrane proteins most likely possess sulfhydryl groups, the use of an agent which modifies these groups could inhibit transport. NEM produced a maximum inhibition of 71 ± 7%, which is slightly but not significantly greater than the inhibition of transport by MGBG (59 ± 4%). The inability to totally inactivate transport may be due to either nonmediated uptake via "leak" pathways or to uptake via MGBG-insensitive or NEM-insensitive pathways. NEM interacts with sulfhydryl groups present in hydrophobic environments (Glazer, 1970; Feeney et al., 1982). It is possible that total inactivation of transport requires inactivation of a second population of sulfhydryl groups. NEM was capable of inhibiting approximately 60 to 70% of transport over a 2 log concentration (1–100 \mu M). The sharper the dose-response curve, and the greater the percentage of inactivation, the higher the probability that a single chemical class is inactivated. Although NEM inactivation of putrescine transport in HPAE cells was not as extensive as inactivation of other transport systems (Sokol et al., 1986a,b; Zimmerman et al., 1991), the fact that nearly 70% of transport could be blocked by NEM supports the presence of essential sulfhydryl groups on the membrane protein. NEM, in contrast, had no inhibitory effect. Therefore, the role of oxidative phosphorylation in putrescine transport could not be established.

There is specificity of inhibition. Putrescine uptake was inhibited mostly by members of the same chemical class (i.e., polyamines were most effective in blocking uptake) (Rannels et al., 1985b). The synthetic polyamine MGBG was the most potent inhibitor. Equally effective blockers were cold putrescine, spermine and spermidine. The inability of high amounts of cold putrescine to block uptake greater than MGBG supports that the uptake of putrescine in the presence of competitors represents nonmediated transport. Excess cold putrescine should be capable of blocking radioisotopically labeled putrescine uptake occurring via a specific transport system.

The aminoglycoside antibiotic gentamicin was a weaker inhibitor of putrescine uptake in HPAE cells, as compared to the endogenous polyamines. Gentamicin is a nephrotoxic drug which interacts with both an organic cation and polyamine transport system in the kidney (Hauser and Cook, 1988; Sokol et al., 1989). Mepiperphenidol, a well-defined blocker of organic cation transport, also was a weak inhibitor of putrescine transport. Because no TEA transport was found in these cells (data not shown), it is unlikely that putrescine uptake occurred via this pathway. Therefore, it appears that mepiperphenidol, once used to identify organic cation transport specifically, has some affinity, albeit low, for the polyamine transport system. In contrast, TEA, an organic cation, had no effect. It is interesting to note that polyamines do have an effect on organic cation transport in other tissues (Sokol et al., 1989). Additionally, it is unlikely that putrescine shares a transport mechanism with amino acids because the prototypic system A amino acid had no effect on polyamine transport. In contrast, polyamines do share a common transport mechanism with system A amino acids in neuroblastoma cells (Rinehart and Chen, 1984).

Polyamine transport systems have been demonstrated in the lung (Karl and Friedman, 1983). Localization studies have shown that type II pneumocytes (epithelial cells) are a major site of putrescine uptake (Kameji et al., 1988; Saunders et al., 1989). However, it is not clear what role, if any, endothelial cells have in polyamine transport (Saunders et al., 1988). In this study, we demonstrated that putrescine was transported in HPAE cells via a high- and low-affinity uptake system, an observation consistent with the literature; many cells possess both high- and low-affinity uptake systems for putrescine (Lipton et al., 1988; De Smedt et al., 1989). In addition, dependence on time (fig. 1), temperature (fig. 7) and chemical specificity (fig. 3) are consistent with polyamine transport systems found in other parts of the lung and outside the lung (Pegg and McCann, 1988; Kameji et al., 1989).

The sulfhydryl modifier NEM inhibited putrescine transport (figs. 5 and 6). Although NEM has been shown to block polyamine transport in other cells (e.g., murine mesenchymal cells; Gawel-Thompson and Greene, 1988), the dose used was 50-fold higher than what we had used (0.1 mM). It is not clear whether their effect of NEM was direct or indirect due to cell injury caused by high doses. In our study, a nontoxic dose of NEM (table 1) blocked putrescine uptake (fig. 5). In contrast to NEM, the metabolic inhibitor DNP did not affect putrescine uptake at equimolar concentrations (0.1 mM). Others have found that higher doses of DNP did affect putrescine transport (Gawel-Thompson and Greene, 1988; De Smedt et al., 1989).

The presence of serum in the uptake medium had a facilitory effect on putrescine transport (fig. 8). A similar finding was found for spermidine uptake in type II pulmonary epithelial cells (Kameji et al., 1989). It appears that some constituent(s) in the serum is (are) required for putrescine uptake. The effect could be direct or indirect. Nevertheless, it is a rapid process because the cells were grown in media containing fetal bovine serum up until the 15-min uptake that was conducted in serum-free media. This observation suggests that there are factors (small molecules, growth factors, hormones, etc.) capable of modulating putrescine transport.

In conclusion, HPAE cells possess a specific transport system for polyamines. Understanding the mechanism of transport will be useful to study growth and repair in this critical cell population and can serve as a model system for studying effects of amine drugs and toxins.

**References**


mental Research Institute, Princeton, 08543.

SOKOL et al.


