Characterization of the Polyamine Transport System in Mouse Neuroblastoma Cells

EFFECTS OF SODIUM AND SYSTEM A AMINO ACIDS*

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The biochemical properties of polyamine transport system have been studied in detail in NB-15 mouse neuroblastoma cells in culture by measuring the uptake of [14C]putrescine under various experimentally imposed pharmacological conditions. Putrescine uptake in the NB-15 mouse neuroblastoma cells appeared to be a sodium-dependent process. Iso-osmotic displacement of Na+ in the assay medium with either choline or Li+ resulted in a linear decrease of putrescine uptake. Gramicidin, a channel-former ionophore, inhibited putrescine uptake by more than 90% at 20 \( \mu \text{M} \). N\-Ethylmaleimide at 5 \( \mu \text{M} \) or p-chloromercuribenzene sulfonate at 50 \( \mu \text{M} \) completely abolished putrescine uptake. Conversely, oxidized glutathione at 10 \( \mu \text{M} \) or 5,5'-dithiobis-(2-nitrobenzoic acid) at 5 \( \mu \text{M} \) gave a 1.3–1.4-fold stimulation after a 1-h incubation. This polyamine transport system appeared to be subjected to adaptive regulation. Polyamine antimetabolites such as \( \alpha \)-difluormethyl ornithine stimulated putrescine uptake whereas preloading of cells with polyamines inhibited putrescine uptake. Preloading cells with neutral amino acids that belong to sodium-dependent transport System A stimulated putrescine uptake whereas preloading of cells with polyamines inhibited putrescine uptake by more than 8–10-fold. These results suggested that the polyamine transport system in NB-15 mouse neuroblastoma cells was sodium dependent and shared some characteristics common to other known sodium-dependent transport systems. These characteristics included (a) sensitivity to ionophores, (b) sensitivity to sulfhydryl reagents, and (c) sensitivity to intracellular contents of substrate molecules. Our data also indicated that polyamine transport may be regulated by transport System A amino acids.

Polyamines (putrescine, spermidine, and spermine) are naturally occurring organic cations ubiquitously distributed in nature (1–3). The importance of polyamines in cellular growth regulation and in many metabolic events has been well documented (for reviews see Refs. 1–5). One of the earliest biochemical events during growth stimulation is a rapid increase of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17), the rate-controlling enzyme in polyamine biosynthesis (1–5).

While cellular polyamines appear to be produced mainly via de novo synthesis, the existence of a specific polyamine transport system in both prokaryotes and eukaryotes (7–10) have been demonstrated. The notion that the polyamine transport system may have a physiological role is supported by the finding that polyamine transport correlates with growth state (7–10). For example, Pohjanpello (7) reported that putrescine uptake is greatly increased in human fibroblasts stimulated to proliferate. Kano and Oka (8) have shown that polyamine transport in mouse mammary explants can be stimulated by insulin and prolactin, hormones that stimulate growth of the explant.

It has been reported that putrescine is an essential component in a serum-free hormone-supplemented chemically defined medium used for culturing cells (11, 12). This finding also underscores the possible physiological importance of the polyamine transport system since putrescine in the medium presumably has to be taken up into the cell via a polyamine transport system.

During the course of differentiation of mouse neuroblastoma cells, we have observed changes of polyamine metabolism (13). Chief among these changes is a significant decrease in the rate of putrescine uptake (14). The decrease can be detected as early as 6 h after the initiation of cell differentiation and represents one of the earlier changes of membrane events associated with the differentiation of mouse neuroblastoma cells (14).

This result, together with the consideration that the polyamine transport system may have an important physiological role, led us to further characterize the polyamine transport system in mouse neuroblastoma cells. In the present study we described our findings on putrescine uptake in NB-15 mouse neuroblastoma cells. Our results indicated that this transport was (i) a Na+-dependent process; (ii) sensitive to sulfhydryl reagents such as pCMBS; (iii) sensitive to intracellular polyamine content; and (iv) stimulated by amino acids that belong to Na+-dependent transport systems, particularly asparagine.

EXPERIMENTAL PROCEDURES

Cell Culture—NB-15 mouse neuroblastoma cells were grown as monolayer cultures in Dulbecco's modified Eagle medium (with 4500 mg of glucose/liter) supplemented with 10% fetal calf serum under conditions previously described (14). The growth medium also contained 50 units/ml of penicillin and 50 \( \mu \text{g} / \text{ml} \) of streptomycin. The cell culture was maintained in a humidified Forma water-jacketed CO\textsubscript{2} incubator (95% air, 5% CO\textsubscript{2}) at 37°C.

Transport Assay—Unless otherwise indicated, confluent mono-

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layer cultures in 35-mm culture dishes were rinsed twice with pre-
warmed (37°C) Earle's balanced salt solution and all subsequent
incubations were carried out in Earle's balanced salt solution. Trans-
port was initiated by adding 0.1 μCi/ml of [3H]-labeled polyamine or
0.5 μCi/ml of [14C]-labeled amino acid to the cell culture. Initial rates
of uptake were measured over time intervals of 20 min for putrescine,
15 min for spermine, 5 min for α-aminoisobutyric acid, and 0.5 min
for leucine. Uptake was carried out at 37°C in a tissue culture
incubator for all compounds except leucine, which was measured at
23°C. Transport was terminated by rapidly rinsing the cells twice
with ice-cold phosphate-buffered saline containing 5 mM of the un-
labeled substrate. The cells were then scraped into 0.5 ml of 20 mM
Tris-HCl (pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride.
The cells were disrupted by sonication (Heat Systems Sonicator
equipped with microtip) and aliquots of the cell homogenate solubi-
lized in Aquasol for radioactivity counting using a Beckman LS 7000
liquid scintillation counter. Protein content was determined by Low-
ry's method (15).

Quantitation of Intracellular Polyamines—The method of Seiler
and Wiechman (16) was used to prepare dansyl derivatives of poly-
amines extracted from cell homogenates as previously described (17).
Dansylated polyamines were quantitated by either thin layer chro-
matography on high pressure liquid chromatography. This layer chro-
matography was performed on GHLF silica gel plates (Analtech,
Newark, DE) in a chloroform/methanolamine system (100:20, v/v). Na+,
K+ and α-amino groups of polyamines were well resolved on this sys-
tem as described previously (17).

Materials—Putrescine, spermidine, LTBN, gramicidin, pCMBS,
N-ethylmaleimide, and other fine biochemicals were obtained from
Sigma. MGBG was from Aldrich. α-Amanin was from Boehringer
Mannheim (New York). α-Difluoromethyl ornithine was a generous
gift of Dr. P. McCann (Merrel Co., GH). Moneansin and A23187 were
kindly provided by Dr. R. Hamill of Eli Lilly Co. The radioisotopes
[1,4-14C]putrescine (113 mCi/mmol) and [1S]spermine tetrahydro-
chloride (122 mCi/mmol) were obtained from Amer sham/Searle.
[4,5-3H]Leucine (96.5 Ci/mmol) and α-amino[methyl-3H]-isobutyric
acid (10 Ci/mmol) were obtained from New England Nuclear.

RESULTS

Effect of Extracellular Na⁺ on Putrescine Uptake in Cultured
Mouse Neuroblastoma Cells—The transport of some impor-
tant nutrient molecules such as amino acids that belong to
transport System A and D-glucose into mammalian cells has
been shown to be dependent upon extracellular Na⁺ (19, 20).
In the present study, the possibility of a Na⁺ requirement for
putrescine uptake was examined by iso-osmotic displacement
of Na⁺ with choline in the incubation medium. As illustrated
in Fig. 1, putrescine uptake decreased with decreasing extra-
cellular Na⁺ concentration, suggesting that putrescine uptake
was a Na⁺-dependent process. Under identical experimental
conditions, the transport of α-aminoisobutyric acid, usually a
substrate for transport System A (19), showed Na⁺ depend-
ency, whereas the transport of leucine, an approximate stand-
ard substrate for the transport System L (19), showed full
Na⁺-independency in mouse neuroblastoma cells (Fig. 1). The
iso-osmotic displacement experiment was repeated using Li⁺
to replace Na⁺; similar Na⁺ dependency of putrescine uptake
was observed (data not shown).

Effect of Ionophores on Putrescine Uptake in Cultured
Mouse Neuroblastoma Cells—If the Na⁺ electrochemical
gradient is involved in the putrescine transport process, dissipa-
tion of this gradient with specific antibiotics should inhibit
putrescine uptake. Results summarized in Table I suggested
that this is indeed the case. Two classes of ionophores, mobile
ion carriers and channel formers, with different cation speci-
ficity, were used to test their effect on putrescine uptake.
Among the ionophores tested, gramicidin was the most potent
inhibitor of putrescine uptake. At 20 nM it almost completely
abolished putrescine uptake. Gramicidin is a channel-forming
ionophore which is approximately 1000-fold more efficient as
compared to carrier ionophores in transporting cations across
their electrochemical gradient (21). In addition, gramicidin
does not discriminate between Na⁺ and K⁺ and hence can set
both Na⁺ and K⁺ electrochemical potential differences across
the membrane to 0 (22). The extreme effectiveness of gрами-
cidin in abolishing putrescine uptake supported the notion
that Na⁺ electrochemical gradient was required for putres-
mine uptake.

Effect of Sulfhydryl Reagents on Putrescine Uptake in Cul-
tured Mouse Neuroblastoma Cells—Many studies have shown
that the Na⁺-dependent amino acid transport System A and the Na⁺-dependent carbohydrate transport system have func-
tional —SH groups which are sensitive to sulfhydryl reagents
(23-26). These observations, together with the apparent Na⁺
dependency of putrescine transport led us to investigate the
effect of sulfhydryl reagents on putrescine uptake (Table II).
A 10-min incubation of mouse neuroblastoma cells with
reduced sulfhydryl compounds such as GSH and DTT, re-
resulted in a slight decrease (~10%) of putrescine uptake.
Conversely, treatment with oxidized sulfhydryl reagents such
as GSSG and DTNB gave a slight increase (~10%) of putre-
scine uptake. However, if the preincubation time was extended
from 10 min to 1 h, both the reduced (GSH and DTT) and

FIG. 1. The sodium dependency of putrescine uptake in NB-
15 mouse neuroblastoma cells. The transport rates of [14C]putre-
scine (0.1 μCi/ml, 1 μM), α-amino[methyl-3H]-isobutyric acid (0.5 μCi/
ml, 5 μM), and [3H]leucine (0.5 μCi/ml, 10 μM) in confluent NB-15
cells were measured, respectively, for 20, 5, and 0.5 min in an Earle's
balanced salt solution where NaCl was displaced iso-omotically by
choline chloride (100 mM). At the end of the incubation period, cells
were washed and cell-associated radioactivity was determined as de-
described under "Experimental Procedures." Each point represents
the average of triplicate measurements. Standard error is within the
range of 10% for each point.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Cation specificity</th>
<th>Putrescine uptake</th>
<th>Per cent inhibition nmol/mg protein/20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.53 ± 0.018</td>
<td>0</td>
</tr>
<tr>
<td>Glicicidin (20 nM)</td>
<td>Na⁺, K⁺</td>
<td>0.014 ± 0.001</td>
<td>97</td>
</tr>
<tr>
<td>Valinomycin (2 μM)</td>
<td>K⁺</td>
<td>0.23 ± 0.006</td>
<td>97</td>
</tr>
<tr>
<td>Moneans (2 μM)</td>
<td>Na⁺, K⁺</td>
<td>0.029 ± 0.012</td>
<td>22</td>
</tr>
<tr>
<td>A23187 (2 μM)</td>
<td>Ca⁺Τ</td>
<td>0.125 ± 0.002</td>
<td>62</td>
</tr>
</tbody>
</table>
oxidized (GSSG and DTNB) sulfhydryl reagents gave a similar degree of stimulation of putrescine uptake (1.3-1.4-fold). The stronger oxidation of GSH and DTT (27) may explain why after 1 h of incubation, GSH and DTT stimulated putrescine uptake to the same extent as GSSG and DTNB. These data suggested that sulfhydryl groups may be involved in putrescine uptake and that the disulfide form (i.e. -S-S-) of the transport system may be a more favorable conformation for putrescine uptake. This notion is also supported by the results of polyamines in ascitic tumor cells (28), and trypanosomes (29). washed and incubated with various reagents in Earle’s balanced salt solution and putrescine uptake was measured as described under “Experimental Procedures.” Each value represents the average of triplicate measurements. Standard error is less than 10% of the indicated values. C—C, no addition; ○—○, +1 mM α-difluoromethylornithine; Δ—Δ, +1 μM MGBG.

![FIG. 2. The effects of α-difluoromethylornithine and MGBG on putrescine uptake. A sparse culture of NB-15 mouse neuroblastoma cells (24 h after seeding at 10⁴ cells/cm² in 35-mm dishes) was treated with 1 mM α-difluoromethylornithine (DFMO) or 1 μM MGBG. At 1, 2, and 3 days after treatment, the cells were washed twice with Earle’s balanced salt solution and putrescine uptake was measured as described under “Experimental Procedures.” Each point represents the average of triplicate measurements. Standard error is less than 10% of the indicated values. C—C, no addition; ○—○, +1 mM α-difluoromethylornithine; Δ—Δ, +1 μM MGBG.](http://www.jbc.org/)

**FIG. 2.** The effects of α-difluoromethylornithine and MGBG on putrescine uptake.

**TABLE II**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Preincubulation time</th>
<th>Uptake nmol/mg protein/20 min</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>10 min</td>
<td>0.377 ± 0.001</td>
<td>100</td>
</tr>
<tr>
<td>GSH (10 mM)</td>
<td>10 min</td>
<td>0.332 ± 0.015</td>
<td>88</td>
</tr>
<tr>
<td>DTT (10 mM)</td>
<td>10 min</td>
<td>0.336 ± 0.022</td>
<td>89</td>
</tr>
<tr>
<td>GSSG (10 mM)</td>
<td>10 min</td>
<td>0.433 ± 0.012</td>
<td>115</td>
</tr>
<tr>
<td>DTNB (5 μM)</td>
<td>10 min</td>
<td>0.401 ± 0.038</td>
<td>106</td>
</tr>
<tr>
<td>GSH (1 mM)</td>
<td>1 h</td>
<td>0.494 ± 0.011</td>
<td>131</td>
</tr>
<tr>
<td>DTT (1 mM)</td>
<td>1 h</td>
<td>0.506 ± 0.014</td>
<td>134</td>
</tr>
<tr>
<td>GSSG (10 mM)</td>
<td>1 h</td>
<td>0.528 ± 0.030</td>
<td>140</td>
</tr>
<tr>
<td>DTNB (5 μM)</td>
<td>1 h</td>
<td>0.543 ± 0.015</td>
<td>144</td>
</tr>
<tr>
<td>NEM (5 μM)</td>
<td>5 min</td>
<td>0.021 ± 0.001</td>
<td>5</td>
</tr>
<tr>
<td>pCMBS (50 μM)</td>
<td>10 min</td>
<td>0.021 ± 0.02</td>
<td>6</td>
</tr>
<tr>
<td>pCMBS (50 μM)</td>
<td>10 min</td>
<td>0.323 ± 0.013</td>
<td>88</td>
</tr>
</tbody>
</table>

*Cells were first treated with 50 μM pCMBS for 10 min, washed, and then treated with 10 mM DTT for another 10 min before putrescine uptake assay.*

Effect of Amino Acids on Putrescine Uptake in Cultured Mouse Neuroblastoma Cells—We have previously reported that asparagine is the most effective amino acid in stimulating ornithine decarboxylase activity in mouse neuroblastoma cells maintained in a salts/glucose medium (41). This observation has since been extended to other cultured cell lines (42-44). It was also found that all the amino acids, including α-aminobutyric acid, that were effective in stimulating ornithine decarboxylase activity, belong to the Na”-dependent transport System A (45). Apparently, these amino acids are giving the cell a signal that an increased level of polyamines is needed. The cell then responds by increasing the level of ornithine decarboxylase activity which is the rate-controlling enzyme for the biosynthesis of polyamines (46). Since the cells can...
accomplish the same result by accumulating polyamines from the external environment, the possibility that amino acids, particularly the System A amino acids, may regulate polyamine transport was investigated. In Table IV, the amino acids tested were grouped into four classes according to their respective transport systems (19). Among them, asparagine was found to be the most effective in stimulating putrescine uptake. The time course and dose-response curve of the effect of asparagine on putrescine uptake are shown in Fig. 3. Putrescine uptake in neuroblastoma cells increased to near maximal rate after a 1-h incubation with asparagine and reached a plateau value by 2 h (Fig. 3A). The concentrations of asparagine needed to give a half-maximal and maximal stimulation to putrescine uptake were 0.5 and 5 mM, respectively. In addition to asparagine, the data in Table IV indicated that other neutral amino acids requiring Na+ for transport into cells, such as glutamine, α-(methylamino)isobutyric acid, serine and alanine, were all effective in stimulating putrescine transport. All these amino acids gave a more than 8-fold stimulation of putrescine uptake after a 2.5-h preincubation. Neutral amino acids such as leucine and 2-aminonorbornane-2-carboxylic acid, whose transport into cells was Na+ independent, were either much less effective or ineffective. Basic amino acids such as lysine and arginine were slightly inhibitory, while acidic amino acids such as aspartic acid and taurine were either ineffective or only slightly stimulatory. The use of α-(methylamino)isobutyric acid, a non-metabolizable model substrate for the sodium-dependent transport System A, suggested that the stimulation of putrescine uptake by System A amino acids was independent of metabolites of these amino acids. Our studies on the effect of protein synthesis inhibitor on putrescine uptake indicated that a 3-h period of incubation with either cycloheximide (5

TABLE III
Subconfluent cultures of NB-15 mouse neuroblastoma cells were incubated with putrescine, spermine, or α-difluoromethyl ornithine (DFMO) in fresh Dulbecco's medium (serum free) for time periods as indicated. Cells were then washed three times with Earle's balanced salt solution to remove extracellular polyamines. Putrescine uptake was determined as described under "Experimental Procedures." Each value for putrescine uptake represents the average of three separate experiments. The values for polyamine content are the average of duplicated determinations. Results on putrescine uptake are expressed in means ± standard deviations.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Incubation time</th>
<th>Putrescine uptake</th>
<th>%</th>
<th>Polyamine content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>nmol/mg protein/20 min</td>
<td></td>
<td>nmol/mg protein</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>0.47 ± 0.03</td>
<td>100</td>
<td>0.70</td>
</tr>
<tr>
<td>Putrescine (2 mM)</td>
<td>22</td>
<td>0.20 ± 0.02</td>
<td>43</td>
<td>0.89</td>
</tr>
<tr>
<td>Spermine (2 mM)</td>
<td>22</td>
<td>0.01 ± 0.001</td>
<td>4</td>
<td>0.82</td>
</tr>
<tr>
<td>DFMO (1 mM)</td>
<td>22</td>
<td>1.23 ± 0.05</td>
<td>262</td>
<td>0.56</td>
</tr>
</tbody>
</table>

*Asparagine and glutamine may also use Na+ dependent System N, serine and alanine may use Na+ dependent System ASC (see Refs. 19 and 20).

![FIG. 3. The effect of asparagine on putrescine uptake in NB-15 mouse neuroblastoma cells.](http://www.jbc.org/)

A, time course. confluent cultures of NB-15 cells were washed with Earle's balanced salt solution and incubated in the same buffer with the indicated amount of asparagine (0.5 mM). Putrescine uptake was measured at the indicated times as described under "Experimental Procedures." O—O, no addition; □— □, 0.5 mM asparagine. Each point represents an average of triplicate measurements. B, dose-response curve. confluent cultures of NB-15 cells were washed two times with Earle's balanced salt solution and incubated in the same buffer with the indicated amount of asparagine. Transport was initiated 2 h later by the addition of [3H]putrescine (0.1 nCi/ml). The data points represent the average of triplicate measurements. Standard error is less than 10% of the indicated values.
The Na⁺ electrochemical gradient in animal cells and tissues has been shown to be a major source of energy in the active transport of many neutral amino acids (System A and System ASC amino acids) and D-glucose (19, 20). The coupling mechanism between the Na⁺ electrochemical gradient and those transport systems has also been extensively studied and the co-transport of Na⁺ and amino acids or sugars have been demonstrated (48). In the present studies, the observations that iso-osmotic displacement of Na⁺ with choline or Li⁺ resulted in an inhibition of putrescine uptake (Fig. 1) and that gramicidin (20 nM) abolished putrescine uptake (Table I) strongly suggested a sodium dependency of polyamine transport. The coupling mechanism, however, remains to be elucidated.

Many earlier studies have used the sulfhydryl reagent N-ethylmaleimide to probe the sodium-dependent transport systems of amino acids and carbohydrates (49, 50). The permeant nature of N-ethylmaleimide, however, makes it difficult to distinguish its interaction with membrane components from its effects on cellular metabolism (51, 52). The use of pCMBS, a rather impermeable sulfhydryl reagent due to its sulfonate group, presumably overcomes this problem (50). The impermeant nature of pCMBS, its potency in inhibiting putrescine uptake, and the reversal of this inhibition by DTT (Table II), provided strong evidence that sulfhydryl groups of certain membrane components, most likely the transporter protein itself, may be important in polyamine transport. In this study we showed that DTT and GSH slightly inhibited putrescine uptake (10%) whereas DTNB and GS-SG slightly increased putrescine uptake (10–20%) (Table II). Similar effects of these reagents on 2-aminoisobutyric acid uptake in thymocytes have been reported (53). Our data seemed to suggest that a dynamic equilibrium of —SH and —S—S— groups is involved in modulating the activity of the polyamine transport system. It has previously been reported that insulin binding causes the formation of disulfide covalent complex of insulin and its receptors in rat adipocytes (54). It will be of interest to investigate whether the stimulatory effect of insulin on putrescine uptake in human fibroblasts (7) and mouse neuroblastoma cells² can be accounted for by this mechanism.

Adaptive regulation has been shown to be an important regulatory mechanism for many sodium-dependent transport systems (30–33). The results summarized in Table III illustrate a qualitative inverse relationship between putrescine uptake and cellular content of total polyamines, suggesting the operation of an adaptive regulation mechanism for the polyamine transport system. Since the distribution of each individual polyamine inside the cell, either in the free or bound form, is not clear at this moment, we cannot determine whether it is the free form or bound form of cellular polyamines that is responsible for the adaptive regulation of the polyamine transport system in mouse neuroblastoma cells. It should also be noted that, in the present study, polyamine depletion was achieved by treatment of cells with α-difluoromethyl ornithine, MGBG, and other analogs, as chemotherapeutic drugs for cancer and other hyperplasia diseases (55, 56) and (b) the

presence of polyamines in body fluids including circulating blood (4).

Among the amino acids that stimulated putrescine uptake, \( \alpha \)-(methylamino)suberic acid, a nonmetabolizable amino acid analogue, is generally considered as an exclusive model substrate for Na\(^+\)-dependent transport System A (reviewed in Ref. 19). Other effective amino acids have also been shown either partially or preferentially to utilize transport System A in many cell types studied (reviewed in Ref. 19). We therefore identified those effective amino acids as System A amino acids (Table IV). It should be noted, however, that Kilberg et al. (26) have recently found that while asparagine is a substrate for both Na\(^+\)-dependent System A and System N, glutamine appears to be only a substrate for System N in rat hepatocytes. In light of this study and the fact that alanine and serine can be substrates for both Na\(^+\)-dependent System A and System ASC (19), the association of the stimulation of putrescine uptake with transport System A may not be exclusive in mouse neuroblastoma cells.

The rather specific stimulatory effects of asparagine and other System A amino acids on putrescine uptake (Fig. 3 and Table IV), together with our previous findings that asparagine and other System A amino acids are effective in stimulating ornithine decarboxylase activity in animal cells (41, 44, 45) pointed to a possible role of these amino acids in the homeostasis of cellular polyamines. It will also be of interest to see whether the effects of asparagine (or other System A amino acids) on these two biochemical events, namely, polyamine transport and ornithine decarboxylase induction, are mechanistically related.

Mutual influence between sodium-dependent amino acid transport and sodium dependent sugar transport has been reported (57). Evidence has been presented that such transport interaction is via a common Na\(^+\) electrochemical gradient (57). Since polyamine transport also appeared to be a sodium-dependent process, we examined whether the stimulation of putrescine uptake by the System A amino acids could be explained by a transport interaction mechanism. Preliminary studies showed that putrescine uptake did not result in a concomitant efflux of loaded amino acids, and preloading of cells with putrescine did not cause any stimulation of amino acid uptake (data not shown). Hence, a transport interaction (trans-stimulation) mechanism seemed unlikely to account for the stimulating effect of System A amino acids on putrescine uptake.

Many recent studies have emphasized a possible correlation between transport System A and the growth state of cells in tissue culture (58–60). For example, Boerner and Saier (58) have examined various nutrient transport systems in MDCK cells under different experimentally imposed regulatory conditions and found that the activity of transport System A consistently correlates with growth rate. Similar studies carried out by Tramacere et al. (59) led them to propose that the transport System A may be a “target” upon which different conditions of regulation converge. In light of these studies and the abundant literature evidence in support of an important role of polyamines in growth regulation (1–5), the possibility that System A amino acids, particularly asparagine, may be coupled to polyamine metabolism via regulation of ornithine decarboxylase activity (41) and polyamine transport (Table IV) deserves further considerations. Many previous studies have highlighted the control of polyamine metabolism, especially through regulation of ornithine decarboxylase, by hormones and growth factors (4, 61). The present finding (Table IV) and our previous reports (41, 42, 45), emphasize that polyamine metabolism could also be regulated by nutrients.

In conclusion, the polyamine transport system in mouse neuroblastoma cells appeared to be sodium dependent (Fig. 1 and Table I). Its activity was found to be linked to cellular content of polyamines (Table III) and System A amino acids (Table IV). The finding that free sulfhydryl groups at plasma membranes were needed for putrescine uptake (Table II) suggested that protein(s) may be involved in polyamine transport. We believe that further study of polyamine transport system, both at structural and mechanistic levels, will shed light on its physiological significance.

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REFERENCES
Characterization of the polyamine transport system in mouse neuroblastoma cells.
Effects of sodium and system A amino acids.
C A Rinehart, Jr and K Y Chen


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