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 mM. N'-Ethylmaleimide at 5 mM or N-chloromercuribenzenesulfonate at 50 µM completely abolished putrescine uptake. Conversely, oxidized glutathione at 10 mM or 5,5'-dithiobis-(2-nitrobenzoic acid) at 5 µM 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 α-difluoromethyl 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. 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.

While cellular polyamines appear to be produced mainly via de novo synthesis, the existence of a specific polyamine transport system in both prokaryotes (6) 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 describe 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 µg/ml of streptomycin. The cell culture was maintained in a humidified Forma water-jacketed CO2 incubator (95% air, 5% CO2) at 37°C.

Transport Assay—Unless otherwise indicated, confluent mono-

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1 The abbreviation used are: pCMBS, p-chloromercuribenzenesulfonate; DTNB, N,5'-dithiobis-(2-nitrobenzoic acid); MGBG, methylglyoxal bis(guany1hydrazone); GSH, reduced glutathione; DTT, di-thiothreitol; GSSG, oxidized glutathione.
Polyamine Transport and Sodium Dependency

Addition Cation specificity Putrescine uptake Per cent inhibition

| Addition | Cation | Putrescine uptake | Per cent inhibition |
|----------|--------|------------------|-------------------|
| None     |        |                  |                   |
| Glicinid (20 nM) | Na⁺, K⁺ | 0.014 ± 0.001 | 97                |
| Valinomycin (2 μM) | K⁺ | 0.231 ± 0.006 | 31                |
| Monensien (2 μM) | Na⁺, K⁺ | 0.262 ± 0.012 | 22                |
| A23187 (2 μM) | Ca²⁺ | 0.125 ± 0.002 | 62                |

FIG. 1. The sodium dependency of putrescine uptake in NB-15 mouse neuroblastoma cells. The transport rates of [1⁴C]putrescine (0.1 μCi/ml, 1 μM), α-amino[ethyl-³H]sobutycic 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-osmotically by choline chloride (100% NaCl corresponds to 116 mM). At the end of incubation period, cells were washed and cell-associated radioactivity was determined as described under "Experimental Procedures." Each point represents the average of triplicate measurements. Standard error is within the range of 10% for each point.

TABLE 1

The effects of ionophores on putrescine uptake in NB-15 mouse neuroblastoma cells

Confluent cultures of NB-15 cells were preincubated with the individual ionophores for 20 min in Earle's balanced salt solution prior to the addition of [¹⁴C]putrescine. Putrescine uptake was measured for another 20 min and the radioactivity was determined as described under "Experimental Procedures." Each value represents the mean ± standard deviation of three separate experiments.

Effect of Extracellular Na⁺ on Putrescine Uptake in Cultured Mouse Neuroblastoma Cells—The transport of some important 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 extracellular Na⁺ concentration, suggesting that putrescine uptake was a Na⁺-dependent process. Under identical experimental conditions, the transport of α-aminoobutyric acid, usually a substrate for transport System A (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, dissipation 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 specificity, 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 Na⁺, K⁺ gradients (22). The extreme effectiveness of gramicidin in abolishing putrescine uptake supported the notion that Na⁺ electrochemical gradient was required for putrescine uptake.

Effect of Sulhydryl Reagents on Putrescine Uptake in Cultured Mouse Neuroblastoma Cells—Many studies have shown that the Na⁺-dependent amino acid transport System A and the Na⁺-dependent carbohydrate transport system have functional —SH groups which are sensitive to sulphydryl reagents (23–26). These observations, together with the apparent Na⁺ dependency of putrescine transport led us to investigate the effect of sulphydryl reagents on putrescine uptake (Table I). A 10-min incubation of mouse neuroblastoma cells with reduced sulphydryl compounds such as GSH and DTT, resulted in a slight decrease (~10%) of putrescine uptake. Conversely, treatment with oxidized sulphydryl reagents such as GSSG and DTNB gave a slight increase (~10%) of putrescine uptake. However, if the preincubation time was extended from 10 min to 1 h, both the reduced (GSH and DTT) and
oxidized (GSSG and DTTB) sulphydryl reagents gave a similar degree of stimulation of putrescine uptake (1.3-1.4-fold). The autooxidation 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 DTTB. These data suggested that sulphydryl 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).

The autooxidation of GSH and DTT (27) may explain why the blocking of free sulphydryl groups was responsible for the inhibition of putrescine uptake. Effects of Cellular Polyamine Content on Putrescine Uptake in Cultured Mouse Neuroblastoma Cells—It has been recently reported that polyamine antimetabolites such as α-difluoromethyl ornithine and MGBG can increase cellular uptake of polyamines in ascitic tumor cells (28), and trypanosomes (29).

These results suggest that polyamine transport may be subject to adaptive regulation. Adaptive regulation, linking transport activity to the intracellular content of pertinent substrates, has been described in a variety of tissues and cells in culture as a mechanism affecting the transport of neutral amino acids through System A (30-33). α-Difluoromethyl ornithine, a specific suicidal inhibitor for ornithine decarboxylase (34), depletes the cells of putrescine and spermidine (35, 36). MGBG specifically inhibits S-adenosylmethionine decarboxylase and, therefore, the biosynthesis of spermidine and spermine (37). These two drugs were used in the experiment illustrated in Fig. 2 to study the effect of polyamine depletion on putrescine uptake in the mouse neuroblastoma cells. The rate of putrescine uptake in cells treated with 1 mM α-difluoromethyl ornithine for 2 days was 2.4-2.8-fold higher than that of the control and remained high at day 3. In contrast, MGBG (1 μM) gave a transient stimulation of putrescine uptake on day 1 but became inhibitory on day 3. MGBG, although known as a specific inhibitor of S-adenosylmethionine decarboxylase, has also been shown to cause an increase of cellular putrescine (38) and to affect mitochondrial activity (39). These side effects of MGBG could contribute to the observed inhibition on putrescine uptake after a long period of incubation.

To examine whether increase in cellular content of polyamines may affect putrescine transport, cells were preloaded with individual polyamines. Extracellular polyamines in the medium were then removed by washing and the uptake of [14C]putrescine was determined. The results are summarized in Table II. Preloading of cells with either putrescine (2 mM) or spermine (2 mM) resulted in a decrease of putrescine uptake, with spermine being more effective than putrescine in inhibiting this putrescine uptake. Analysis of cellular content of polyamines indicated that the preloading of cells with spermine gave a higher total cellular polyamine content than loading with putrescine. In both cases, the cellular putrescine content did not differ significantly from that of the control. This result is consistent with the notion that cellular putrescine has a high turnover rate (40). The results in Table III suggested that putrescine uptake was more dependent on total cellular polyamine content rather than on the cellular putrescine content alone.

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

![Figure 2](http://www.jbc.org/Downloaded from)
The effects of intracellular polyamine contents on putrescine uptake in NB-15 mouse neuroblastoma cells

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 III

| Addition | Incubation time | Putrescine uptake | % | Polyamine content |
|----------|----------------|-------------------|---|-----------------|
|          | h | nmol/mg protein/20 min |       | Putrescine | Spermine | Spermine | Total |
| None | 1 | 0.47 ± 0.03 | 100 | 0.70 | 2.30 | 1.55 | 4.55 |
| Putrescine (2 mM) | 22 | 0.20 ± 0.02 | 43 | 0.67 | 2.95 | 1.55 | 4.97 |
| Spermine (2 mM) | 1 | 0.04 ± 0.001 | 9 | 1.00 | 4.35 | 2.50 | 7.90 |
| DFMO (1 mM) | 22 | 0.01 ± 0.001 | 4 | 0.82 | 3.15 | 1.70 | 5.67 |

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-aminobornane-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 IV

**Effects of amino acids on putrescine uptake in NB-15 mouse neuroblastoma cells**

Confluent cultures of NB-15 mouse neuroblastoma cells were incubated with various amino acids (0.5 mM) for 2.5 h at 37°C in Earle's balanced salt solution and followed by the addition of [1-14C]putrescine. The uptake of [1-14C]putrescine was measured over a 20-min period at 37°C. Values represent the average of triplicate determinations and results are expressed as means ± standard deviations. Per cent stimulation is defined as [(V - V₀)/(Vₘₐₓ – V₀)] x 100, where Vₘₐₓ, V₀, and Vₙ represent putrescine uptake measured in the presence of amino acids, asparagine, and neither addition, respectively.

| Transport system | Addition | Putrescine uptake | Per cent stimulation |
|------------------|----------|-------------------|---------------------|
| A                | None     | 0.052 ± 0.001     | 100                 |
|                  | Asparagine | 0.559 ± 0.029     | 100                 |
|                  | Glutamine  | 0.428 ± 0.020     | 74                  |
|                  | α-(Methylamino) isobutyric acid | 0.483 ± 0.019 | 87                  |
|                  | Serine    | 0.467 ± 0.017     | 82                  |
|                  | Alanine   | 0.458 ± 0.026     | 80                  |
| L                | Leucine   | 0.209 ± 0.025     | 31                  |
|                  | 2-Aminobornane-2-carboxylic acid | 0.093 ± 0.008 | 8                   |
| L₀               | Lysine    | 0.047 ± 0.016     | −1                  |
|                  | Arginine  | 0.032 ± 0.005     | −4                  |
|                  | α-Difluoromethyl ornithine | 0.057 ± 0.013 | 1                   |
| β₀               | Aspartic acid | 0.087 ± 0.011 | 7                   |
|                  | Taurine   | 0.179 ± 0.016     | 25                  |

*Asparagine and glutamine may also use Na⁺-dependent System N, serine and alanine may use Na⁺-dependent System ASC (see Refs. 19 and 20).
The results summarized in Table V indicated that amino acids must be allowed to accumulate inside the cell in order to be effective in stimulating putrescine uptake. Putrescine uptake was actually slightly inhibited (~3%) if it was measured immediately after the addition of asparagine or leucine. This slight inhibition was likely to be due to their competition for the same Na⁺ electrochemical gradient.

**DISCUSSION**

Previous studies of the polyamine transport system in human fibroblasts (7, 9), mouse mammary gland (8), mouse fibroblasts (10), and mouse neuroblastoma cells (14, 47) have established that (a) all three naturally occurring polyamines share a specific transport system which is distinct from any other known nutrient transport systems; (b) polyamine transport is a saturable, temperature-dependent process; maximal rate occurs at 37 °C (47); (c) polyamine transport can be stimulated by serum (7), growth factors (9), and hormones (8); and (d) putrescine uptake is inhibited by dinitrophenol, an uncoupler of oxidative phosphorylation (8, 14). Although micromolar or submicromolar concentrations of polyamine in the medium can be concentrated into mammalian cells which generally contain polyamines in the millimolar concentration range, the cationic nature of the polyamines makes it difficult to ascertain if polyamine uptake is via an active transport process (7, 9, 14).

To gain a better understanding of the significance of polyamine transport in cell physiology we decided to study the biochemical and physical properties of the polyamine transport system in greater detail. The results recounted in this paper suggested that polyamine transport in mouse neuroblastoma cells is a Na⁺-dependent process and appears to share some characteristics common to other Na⁺-dependent transport processes.

**Table V**

| Addition     | Preincubation time | Uptake | Per cent stimulation |
|--------------|--------------------|--------|----------------------|
|              | h                  | nmol/mg protein/20 min |                     |
| None         | 0                  | 0.050  | 0                    |
| Leucine      | 0                  | 0.080  | 7                    |
| Asparagine   | 0                  | 0.468  | 100                  |
| Asparagine*  | 0                  | 0.490  | 103                  |
| Leucine      | 0                  | 0.036  | -3                   |
| Asparagine   | 0                  | 0.036  | -3                   |

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 pememeant 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 nature, 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 (10-20%) whereas DTNB and GSSG 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 cells 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 instead of using the more conventional serum- and nutrient-deprivation methods (30-33). Unlike neutral amino acids or glucose, the efflux of cellular polyamines in a serum- and nutrient-free medium was too slow to be an effective method for depleting cellular polyamines.

2 K. Y. Chen and C. A. Rinehart, Jr., unpublished data.
presence of polyamines in body fluids including circulating blood (4).

Among the amino acids that stimulated putrescine uptake, \(\alpha\)-(methy1amino)isobutyric acid, a nonmetabolizable amino acid analogue, is generally considered as an exclusive model substrate for Na\(^+\)-dependent 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 homoeostasis 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

1. Cohen, S. S. (1971) Introduction to Polyamines. pp. 1-179, Prentice-Hall, Englewood Cliffs, NJ
2. Bachrach, U. (1973) Function of Naturally Occurring Polyamines, pp. 1–211, Academic Press, New York
3. Tabor, C. W., and Tabor, H. (1976) Annu. Rev. Biochem. 45, 285-306
4. Russell, D. H., and Durie, B. G. M. (1978) Polyamines as Biochemical Markers of Normal and Malignant Growth, pp. 1-178, Raven Press, New York
5. Jané, J., Foso, H., and Raina, A. (1977) Biochim. Biophys. Acta 473, 241–263
6. Tabor, C. W., and Tabor, H. (1966) J. Biol. Chem. 241, 3714–3723
7. Polymapelo, P. (1976) J. Cell Biol. 68, 512–520
8. Kano, K., and Oka, T. (1976) J. Biol. Chem. 251, 2785–2800
9. DiPasquale, A., White, D., and McGuire, J. (1978) Exp. Cell Res. 110, 317–323
10. Bethell, D. R., and Pegg, A. E. (1981) J. Cell. Physiol. 109, 461–468
11. Ham, R. G. (1965) Proc. Natl. Acad. Sci. U. S. A. 53, 288–295
12. Bottenstein, J. E., and Sato, H. (1976) Cell. Physiol. Membr. Tramp. 45, 501–530
13. Chen, K. Y., and Liu, A. Y-C. (1983) Adv. Polyamine Res. 4, 743–755
14. Chen, K. Y., and Rinehart, C. A., Jr. (1981) Biochem. Biophys. Res. Commun. 101, 243–249
15. Lowy, O. H., Roussiberg, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
16. Seiler, C., Dall'Asta, U. S. A., Federspiel, A., and Wiechman, F. (1979) J. Biol. Chem. 255, 929–936
17. Poretz, R. A. Niederman, and G. Strauss for helpful discussions during the course of this work.


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31. Gazzola, G. C., Dall’Asta, V., and Guidotti, G. G. (1981) J. Biol. Chem. 256, 3191–3198
32. Logan, W. J., Klip, A., and Gagalang, E. (1982) J. Cell. Physiol. 112, 229–236
33. Gazzola, G. C., Franchi-Gazzola, R., Ronchi, P., and Guidotti, G. G. (1973) Biochem. Biophys. Acta 311, 292–301
34. Metcalf, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P., and Veret, J. P. (1978) J. Am. Chem. Soc. 100, 2551–2553
35. Prakash, N. J., Scheckter, P. J., Mamont, P. S., Grove, J., Koch-Weser, J., and Sjoerdsm, A. (1980) Life Sci. 26, 181–194
36. Sunkara, S. S., Prakash, N. J., Mayer, G. D., and Sjoerdsm, A. (1983) Science (Wash. D.C.) 219, 561–563
37. Williams-Ashman, H. G., and Schenone, A. (1972) Biochem. Biophys. Res. Commun. 46, 288–293
38. Porter, C. W., Bergeron, R. J., and Stolowich, N. J. (1982) Cancer Res. 42, 4072–4078
39. Dave, C., Pathak, S. N., and Porter, C. W. (1978) Adv. Polyamine Res. 1, 153–171
40. Snyder, S. H., Kreuz, D. S., Medina, V. J., and Russell, D. H. (1976) Ann. N. Y. Acad. Sci. 171, 749–771
41. Chen, K. Y., and Canellakis, E. S. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3791–3795
42. Chen, K. Y., and Liu, A. Y.-C. (1983) Biochim. Biophys. Acta 755, 244–252
43. Costa, M. (1979) Life Sci. 25, 2113–2124
44. Bachrach, U. (1980) Biochem. J. 188, 387–392
45. Vicepts-Madore, D., Chen, K. Y., Tsou, H.-R., and Canellakis, E. S. (1982) Biochim. Biophys. Acta 717, 905–915
46. Pegg, A. E., and Williams-Ashman, H. G. (1968) Biochem. J. 108, 533–539
47. Rinehart, C. A., Jr. (1983) Ph.D. thesis, Rutgers University
48. Schultz, S. G. (1978) in Physiology of Membrane Disorders (Andréoli, T. E., Hoffman, J. F., and Fanestil, D. D., eds) pp. 273–286, Plenum Medical Book Co., New York
49. Aledort, L. M., Troup, S. B., and Weed, R. I. (1968) Blood 31, 471–479
50. Rothstein, A., Knauf, P. A., Cabantchik, Z. I., and Balehin, M. (1973) in Drugs and Transport Process (Caldwell, B. S., ed) pp. 53–72, University Park Press, Baltimore
51. Webster, J. L. (1966) in Enzyme and Metabolic Inhibitors, Vol. 2, pp. 633–653, 729–865, Academic Press, New York
52. Schaeffer, J. F., Preston, R. L., and Curran, P. F. (1973) J. Gen. Physiol. 62, 131–146
53. Kwock, L. (1981) J. Cell. Physiol. 106, 279–282
54. Clark, S., and Harrison, L. C. (1982) J. Biol. Chem. 257, 12239–12244
55. Siimes, M. A., Seppanen, P., Alhonen-Hongisto, L., and Jänne, J. (1981) Int. J. Cancer 28, 567–570
56. Jänne, J., Alhonen-Hongisto, L., Kanyaho, K., and Seppanen, P. (1983) Adv. Polyamine Res. 4, 17–32
57. Mauer, H., Sigrist-Nelson, K., and Hopfer, U. (1975) J. Biol. Chem. 250, 7392–7396
58. Boerner, P., and Sauer, M. H., Jr. (1982) J. Cell. Physiol. 113, 240–246
59. Tramacere, M., Borgheti, A. F., and Guidotti, G. G. (1977) J. Cell. Physiol. 93, 425–434
60. Oxender, D. L., Lee, M., and Cecchini, G. (1977) J. Biol. Chem. 252, 2680–2693
61. Canellakis, E. S., Vicepts-nadore, D., Kyriakidis, D. A., and Heller, J. S. (1979) Curr. Top. Cell. Regul. 15, 156–202
Characterization of the polyamine transport system in mouse neuroblastoma cells.
Effects of sodium and system A amino acids.
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