Studies on the Transport of L-Tyrosine into an Adrenergic Clone of Mouse Neuroblastoma*

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SUMMARY

Studies on the transport of L-[U-14C]tyrosine into cultured mouse neuroblastoma clone NlE-115, which has high levels of tyrosine hydroxylase (EC 1.14.16.2), are presented. This transport was by a saturable process, exhibited marked exchanger mechanism similar to the leucine-preferring system which transport into this clone is by facilitated diffusion and by a mechanism similar to the leucine-preferring system which has been described by others.

The results are consistent with the conclusion that L-tyrosine transport into this clone is by facilitated diffusion and by a mechanism similar to the leucine-preferring system which has been described by others.

Tyrosine transport into an adrenergic nerve cell is required for the biosynthesis of catecholamines. Therefore, we have studied the properties of this amino acid transport in cultured mouse neuroblastoma cells as the first step in our studies on the biosynthesis of catecholamines. Our previous reports (1, 2) on transport of neurotransmitter precursors into cultured cells showed that tyrosine, phenylalanine, and choline were transported by saturable processes into adrenergic and cholinergic mouse neuroblastoma clones, glial cells, and fibroblasts and that this transport was characterized by similar kinetic and thermodynamic properties for each particular precursor.

For the studies of tyrosine transport presented here, we used the adrenergic mouse neuroblastoma clone NlE-115 (3), which has very high levels of tyrosine hydroxylase (EC 1.14.16.2), the first enzyme in the biosynthetic pathway to catecholamines. In this clone we showed that tyrosine hydroxylase activity was regulated with respect to cell division (4) and that this activity was stimulated by growing these cells in the presence of NADP, O2-dibutyryl adenosine 3':5'-monophosphate (5). NlE-115 also exhibits electrical activity which is similar to that seen in differentiated nerve cells.1 The data presented in this report offer further evidence that the transport of tyrosine into cultured mouse neuroblastoma cells is by facilitated diffusion (6) and in addition is similar to the leucine-preferring (7) system which has been described for other mammalian cells (7, 8).

METHODS AND MATERIALS

Cells and Culture Conditions

Mouse neuroblastoma clone NlE-115 (subculture 8-21) and rat glioma C-6 (passage 53-57) (9) were cultured in Dulbecco-Vogt modification of Eagle's medium (high glucose, no pyruvate, Grand Island Biological Co., Grand Island, N. Y.) (Medium I) supplemented with 10% fetal calf serum (Colorado Serum Co., Denver, Colo.) without antibiotics at 37° in an atmosphere of 10% CO2 and 95% humidified air. Cells were grown in flasks (75 cm²/250 ml, Falcon Plastics, Oxnard, Calif.) with 20 ml of medium prior to transfer into Linbro wells (Dispase trays, model FB 16-24TC, Becton, Dickinson, Inc., Wineland, N. J.) which required 1 ml of medium. Cells were negative for mycoplasma by bacteriological criteria.

Assays

Transport Assay—The standard assay was a modification of the microassay technique previously described (10). Approximately 2 days prior to assay, NlE-115 cells in flasks were subcultured with the use of a modified Rank's D1 solution (137 mm NaCl, 5.4 mm KCl, 0.17 mm NaHPO4, 0.22 mm KH2PO4, 5.5 mm glucose, and 5.9 mm sucrose, pH 7.2, 340 mosM) and plated into wells at approximately 1 X 10⁵ cells per well in 1 ml of Medium I with 10% fetal calf serum. For C-6 cells, 0.05% (w/v) trypsin in the modified Rank's D1 solution was used for subculturing. Unless otherwise stated, about 1 day prior to assay Medium I with 10% fetal calf serum was replaced by 1 ml of Medium I without tyrosine and 0% fetal calf serum, after washing the cells once with 0.5 ml of medium of the latter medium. The routine assay was performed at 37° in 0.2 ml of phosphate-buffered 943 mg % (w/v) sodium chloride solution containing the following: 110 mm NaCl, 5.3 mm KCl, 1.8 mm CaCl2, 1.0 mm MgCl2, 2.6 mm Na2HPO4, 2.5 mm glucose, and 78 mm sucrose. This solution was adjusted to pH 7.3 and an osmolality of 335 to 340 mosM. Just prior to assay, cells were washed once with 0.15 ml of phosphate-buffered saline at 37°. Except where noted elsewhere cells were incubated for 2 min in the presence of radioactively labeled L-[U-14C]tyrosine (Schwarz-Mann) which was freeze-dried and dissolved in phosphate-buffered saline maintained at 37°. The reaction was initiated by the addition of the radioactive iso-

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1 J. Tuttle and E. Richelson, unpublished observations.
tope and was terminated by aspiration of the incubation medium
with a Pasteur pipette connected to a vacuum line and two 0.5 ml
washes with phosphate-buffered saline at 4°C. For inhibitor studies
the compound to be tested was added about 15 s prior to the
addition of the [14C]tyrosine. Radioactivity and protein from the
wells were quantitatively harvested by adding 1.0 ml of
0.1 N NaOH to each well, tritutrating, and transferring this NaOH
solution to a scintillation vial. Radioactivity was determined after
the addition to the vial of 10 ml of scintillation fluid (Hy-
dromix, Yorktown Research, New Hyde Park, N. Y.). The
efficiency for counting as determined by an internal standard
([14C]toluene) was approximately 80%. Corrections for non-
specific transport were determined experimentally (11) by incubat-
ing the cells with radioactive tyrosine in the presence of 7.5 mM
L-phenylalanine. Non-specific radioactivity, which was deter-
mined with 7.5 mM L-phenylalanine, was used because of its ease of solution in phosphate-buffered saline.
Specific transport of tyrosine was calculated by subtracting the
non-specific transport from the total transport (amount of trans-
port occurring in the presence of L-phenylalanine). Where it was
appropriate, data were fitted to a straight line by a linear regres-
sion analysis.

**RESULTS**

**Effect of Culture Conditions on Tyrosine Transport**—For
the specific transport of tyrosine into NIE-115, we reported (1)
that the transport constant, $K_t$ (concentration of substrate at
one-half maximum velocity of transport), was higher in cells
cultured in Medium I supplemented with 10% fetal calf serum
as compared to Medium I without tyrosine and 0% fetal calf
serum. The results of further studies on this observation (Table I)
showed that the $K_t$ was higher in cells cultured in Medium I
with 10% fetal calf serum but that there was no significant difference in $K_t$ values for cells cultured in Medium I,
0% fetal calf serum, versus Medium I without tyrosine, 0% fetal
calf serum. The $V_{max}$ (maximum velocity of transport)
per cell was also higher in cells grown in Medium I with 10% fetal
calf serum. Since removal of serum from the medium
effectively stops cell division in these cells (4, 16), these results
probably reflect membrane changes associated with cell division
as studied in detail by others (17, 18).

Our $V_{max}$ values per cell and per mg of protein as well as our
$K_t$ values for the specific transport of tyrosine with the use of
Medium I without tyrosine, 0% fetal calf serum, were higher than
previously reported (1). We have no explanation for these
differences. We continued to use cells depleted of tyrosine in
our experiments in order to be assured of studying initial rates.

**Effect of Preloading Cells on Rate of Tyrosine Transport**—Pre-
incubation of NIE 115 cells in nonradioactive L-tyrosine (1 mM
or 0.1 mM) reproducibly caused an increase in the initial rate of
[14C]tyrosine transport (Fig. 1, A and B). An increase of lesser
degree was seen with preincubation in L-phenylalanine or
D-tyrosine. The slower rate of tyrosine transport caused by pre-

### Chemicals and Drugs

The following compounds were obtained from Sigma Chemical Co.
(St. Louis, Mo.): p and L tyrosine, aminoadipic acid, d-serine, d-aminovaleric acid, p-chloromercuribenzoate, p-chlorophenylalanine, 3,4-dinitrophenol, L-3-isodotyrosine, ouabain octohydrate, 2-phenyl-
pyruvic acid sodium salt, reserpine, sodium azide, 2,4,6-trinitro-
benzene sulfonic acid, and tyramine hydrochloride. Dopamine
hydrochloride and L-norepinephrine hydrochloride were obtained
from Regis Chemical Co. (Morton Grove, Ill.). L-3-Amino-
propionic acid was purchased from Aldrich Chemical Co., Inc.
(Milwaukee, Wis.). L-3,4-Dihydroxyphenylalanine was obtained
from Calbiochem (San Diego, Calif.). 4-Phenybutyric acid and
DL-phenylglycine were obtained from Eastman Kodak Co.
(Rochester, N. Y.). Morphine sulfate was obtained from Male-
linekrodt Chemicals (St. Louis, Mo.). We gratefully acknowledge
the gift of the following compounds, atropine, codeine maleate,
perphenazine (Shering Corp., Bloomfield, N. J.); chlorpromazine
hydrochloride, phenytoin (Smith, Kline & French Laboratories,
Philadelphia, Pa.); chlordiazepoxide hydrochloride, chlorprothixene, diazepam (Hoffmann-La Roche, Inc., Nutley,
N. J.); desipramine hydrochloride (USV Pharmaceutical Corp.,
Teckahoe, N. J.); haloperidol (McNeil Laboratories, Inc., Fort
Washington, Pa.); imipramine hydrochloride, opipramol (Ciba-
Geigy Corp., Summit, N. J.); promazine hydrochloride (Wyeth
Laboratories, Inc., Philadelphia, Pa.); and DL-2-hydroxy-3-
phenylpropionic acid (Dr. M. Walser, Department of Pharmacol-
yogy and Experimental Therapeutics, Johns Hopkins University
School of Medicine).
Mouse neuroblastoma clone NIE-115 (subculture 13) was incubated for 24 hours prior to the transport assay in the indicated culture media. The specific transport of tyrosine was determined as described under "Methods and Materials" with L-[U-14C]tyrosine (final specific activity 24 mCi per mmole) at five different concentrations (1.2 × 10⁻³ M to 12.5 × 10⁻³ M). All determinations were done in duplicate. The values for $V_{\text{max}}$ (maximum velocity of transport) and $K_t$ (concentration of substrate at one-half maximum velocity of transport) were determined from the intercepts of a Lineweaver-Burk plot of the data. Cell counts and proteins were determined as described under "Methods and Materials."

### Table I

| Culture medium for 24 hours prior to assay | Average number of cells per well | Average amount of protein per well | $V_{\text{max}}$ | $V_{\text{max}}$ per cell | $V_{\text{max}}$ per mg of protein | $K_t$ |
|------------------------------------------|----------------------------------|----------------------------------|----------------|--------------------------|---------------------------------|-------|
| Medium I, 10% fetal calf serum...         | $1.4 \times 10^6$ | µg | 470 | 2000 | 2.1 | 6200 | $17 \times 10^{-3}$ |
| Medium I, 0% fetal calf serum...          | $1.2 \times 10^6$ | µg | 280 | 1500 | 1.2 | 5400 | $5.7 \times 10^{-3}$ |
| Medium I without tyrosine, 0% fetal calf serum... | $1.1 \times 10^6$ | µg | 280 | 1600 | 1.5 | 5000 | $6.8 \times 10^{-3}$ |

Fig. 1. Effect of preloading cultured neuroblastoma and glial cells on the transport of tyrosine. A and B, clone NIE-115 (passage 18) was placed in Medium I without tyrosine and phenylalanine, 0% fetal calf serum, 24 hours prior to this experiment. Separate groups of cells in duplicate were incubated in 1 ml of phosphate-buffered saline for the control or 1 ml of phosphate-buffered saline containing one of the following: 1 mM L-tyrosine (O), 0.1 mM L-tyrosine (Δ), 1 mM L-phenylalanine (□), or 1 mM L-tyrosine (○). Every 30 min during this experiment the 1 ml of phosphate-buffered saline incubation solution for each condition was replaced with fresh solution to avoid changes due to evaporation. At the indicated time points, the 1 ml of phosphate-buffered saline solution was removed, and cells were tested for the transport of tyrosine at 5 × 10⁻³ M L-[U-14C]tyrosine (final specific activity 24 mCi per mmole) during a 30-s transport assay at 37° as described under "Methods and Materials." The number of cells per well averaged $1.4 \times 10^6$ and amount of protein per well averaged 210 µg.

For the control at zero time, the rate of tyrosine transport was 495 pmoles per min, and at 140 min it was 339 pmoles per min. The control was the same for Fig. 1A and B. C, rat glioma C-6 (subculture 56) was placed in Medium I without L-phenylalanine and L-tyrosine, 0% fetal calf serum, 24 hours prior to this experiment which was performed as described above for A and B except that the length of the tyrosine transport was 1 min. The average number of cells per well was $1.8 \times 10^5$. 1 mM L-tyrosine (□), 1 mM L-phenylalanine (Δ), and control (O). The curves are plotted through the average of duplicate values which are represented by the horizontal bars bracketing the symbols. For those points which are not bracketed, duplicate values fall within the dimensions of the symbol.

**Effect of Certain Inhibitors on Specific Transport of Tyrosine—**

p-Chloromercuribenzoic acid (0.1 mM), a reagent which attacks sulphydryl groups, was a potent inhibitor of tyrosine transport (Table II). L-Tyrosine (1 mM) added to the incubation medium with p-chloromercuribenzoic acid did not prevent the effect of this reagent (data not shown). The inability to inhibit tyrosine transport by 2,4,6-trinitrobenzenesulfonic acid, which also attacks sulphydryl groups (Table II), may reflect the fact that this reagent is active only at alkaline pH.

* Dr. I. Parikh, personal communication.
performed as described under “Methods and Materials” in the 2,4,6-Trinitrobenzene sulfonic acid solution. The rate of transport at 25°C and 4°C was, respectively, 68% and 16% of that at 37°C. The average number of cells per well was 1.1 X 10^6, and the average amount of protein per well was 830 μg. The rate of transport for the control averaged 430 pmoles per min. All points were determined in duplicate.

All points were determined in duplicate.

**Table II**

**Specific transport of tyrosine: effect of certain inhibitors**

Mouse neuroblastoma clone NIE-115 (subculture 11) was preincubated for 15 min at 37°C in 1.0 ml of phosphate-buffered saline solution in the presence (+) or absence (−) of the respective inhibitors with the use of 2.75 X 10^{-3} m L-[14C]tyrosine (final specific activity 44 mCi per mmole). The average number of cells per well was 1.1 X 10^6, and the average amount of protein per well was 830 μg. The rate of transport for the control averaged 430 pmoles per min. All points were determined in duplicate.

| Inhibitor | Prescubation period | Transport assay | Specific transport of L-tyrosine | % control |
|-----------|---------------------|-----------------|-------------------------------|----------|
| Ouabain, 1 mM | + | − | 86 | 86 |
| 2,4-Dinitrophenol, 1 mM | + | + | 95 | 95 |
| Sodium azide, 10 mM | − | + | 90 | 90 |
| 2,4,6-Trinitrobenzene sulfonic acid, 1 mM | + | − | 103 | 103 |
| p-Chloromercuribenzenesulfonic acid, 0.1 mM | + | + | 96 | 96 |

**Effect of Ions, pH, and Temperature**—The specific transport of tyrosine was not dependent to any major extent on the presence in the incubation medium of sodium, potassium, calcium, or magnesium ions (Table III). The small sodium dependency suggests that a small part of this tyrosine transport occurs by a sodium-dependent system (for example, the alanine-preferring (A) system (7)). There was no change in the rate of tyrosine transport at pH 6, 7, or 8.

Temperature had a marked effect on this transport such that the rate of transport at 25°C and 1°C was, respectively, 68% and 40% of that at 37°C. Since metabolic inhibitors did not affect this transport (Table II), the reduction in the rate of transport caused by a reduction in temperature may reflect physical changes in the membrane structure, an effect of temperature which changes the rate of sugar transport in Escherichia coli (10).

**Inhibition of Specific Transport of Tyrosine by Amines, Amino Acids, and Analogs**—Compounds were tested for their effects on the specific transport of tyrosine by using the Dixon method (20) to determine the inhibitor constant, K_i. A summary of the data is presented in Table IV. The most potent inhibitors of tyrosine transport (K_i values in the range of 2 to 7 X 10^{-3} m) were compounds with close structural analogy to tyrosine such as L-3,4-dihydroxyphenylalanine, L-3-idotyrosine, and L-phenylalanine (Fig 2). Compounds with K_i values in the range of 11 X 10^{-2} m to 30 X 10^{-2} m (Table IV) were not necessarily similar to tyrosine in structure although α amino acids predominated. Tyramine, which is a decarboxylation product of tyrosine, had reduced ability to inhibit tyrosine transport. Some stereo-

**Table III**

**Specific transport of tyrosine: dependence on ions**

Mouse neuroblastoma clone NIE-115 (subculture 12) was assayed for the specific transport of tyrosine as described under “Methods and Materials” with the use of 2.7 X 10^{-3} m L-[14C]tyrosine (final specific activity 44 mCi per mmole) in the indicated incubation media. Either sucrose or choline chloride was used to adjust medium to 340 mosm after the removal of the particular compound(s) from phosphate-buffered saline. Medium which lacked NaCl and Na_2HPO_4 was buffered with 2.0 mM KH_2PO_4 and contained 3.3 mM KCl to maintain the potassium concentration at 0.3 mM. The number of determinations for each condition was five. The average rate of transport for the control was 710 pmoles per min per mg of protein.

| Incubation medium | Specific transport of L-tyrosine as a percentage of control (± standard deviation) |
|-------------------|--------------------------------------------------------------------------------|
| Omissions         | Sucrose                     | Choline chloride                  |
| Sucrose           | 100                          | 101 (± 15)                        |
| NaCl, Na_2HPO_4   | 68 (± 10)                    | 81 (± 16)                         |
| NaCl, Na_2HPO_4   | 68 (± 10)                    | 81 (± 16)                         |
| KCl               | 95 (± 9)                     | 87 (± 7)                          |
| CaCl_2            | 86                            | 76 (± 7)                          |
| MgCl_2            | 99                            |                                  |

**Table IV**

**Inhibition of specific transport of tyrosine by amines, amino acids, and analogs: inhibitor constant (K_i) values**

Each compound was tested for its effect on the transport of L-[14C]tyrosine into mouse neuroblastoma clone NIE-115 at five different concentrations of inhibitor and two different concentrations of radioactive L-tyrosine. The K_i values were obtained from a plot of the data by the method of Dixon (20). The details of the transport assay are described under “Methods and Materials.”

| Compound                        | 10^8 X K_i in mmol |  |
|---------------------------------|-------------------|---|
| L-3-Iodotyrosine                | 2                 |  |
| L-3,4-Dihydroxyphenylalanine    | 5                 |  |
| L-Phenylalanine                 | 6                 |  |
| L-Tryptophan                    | 7                 |  |
| L-Methionine                    | 11                |  |
| D-Tyrosine                      | 14                |  |
| L-Leucine                       | 20                |  |
| L-Valine                        | 21                |  |
| L-Cysteine                      | 25                |  |
| L-Histidine                     | 25                |  |
| DL-2-Phenylglycine              | 25                |  |
| L-Leucine                       | 30                |  |
| Tyramine                        | 30                |  |
| L-Norepinephrine                | 35                |  |
| L-Isoleucine                    | 40                |  |
| 3-Phenylpropionic acid          | 40                |  |
| L-Alanine                       | 42                |  |
| DL-3-Amino-3-phenylpropionic acid | 50              |  |
confirmed competitive inhibition by L-alanine, L-leucine, L-phenylalanine, p-tyrosine, L-tryptophan, and L-valine. The following compounds at 10 × 10^{-5} M did not inhibit transport of L-[U-^{14}C]tyrosine at 2.5 × 10^{-5} M L-arginine, L-glutamic acid, L-glutamine, L-lysine, and L-proline.

The structures of some of the least potent inhibitors (K_i values in the range of 35 to 50 × 10^{-5} M) (Table IV and Fig. 2) indicate a correlation between the presence of an α amino group and the ability of a compound to inhibit transport of tyrosine. Thus, L-2-amino-3-phenylpropionic acid (L-phenylalanine) was a very potent inhibitor of L-tyrosine transport, but DL-3-amino-3-phenylpropionic acid and 3-phenylpropionic acid were weak inhibitors, and L-2-hydroxy-3-phenylpropionic acid did not inhibit this transport.

Studies on Exit of Tyrosine—The loss of radioactivity from cells preloaded with L-[U-^{14}C]tyrosine was much more rapid when nonradioactive tyrosine was present in the external medium at 37°C (Fig. 3). This stimulation of exit occurred with N1E-115 cells which were not depleted of tyrosine and with C-6 glioma cells but did not occur when preloaded cells were incubated at 4°C during the exit portion of the experiment. The stimulation of exit of L-[U-^{14}C]tyrosine from cells was dependent upon the concentration of nonradioactive tyrosine in the external medium (Fig. 4). Since the plateau in Fig. 4 occurred when 80% of the accumulated radioactivity had been excreted, the leveling off may reflect this limitation rather than the saturation of a process. However, the concentration of L-tyrosine in the external medium which gave half-maximum stimulation of exit was about 9 × 10^{-5} M, a value close to that found for the K_i (Table I), although not equivalent to a K_i as discussed below.

A number of compounds were tested for their effects on exit of radioactivity from cells which were preloaded for 4 min with

\[ v = \text{velocity of transport in absence of inhibitor, } I = \text{concentration of inhibitor.} \]
TABLE V

| Compound                        | Counts per min of \(^{3}C\)-radioactivity recovered from cells | \(\%\) control |
|---------------------------------|---------------------------------------------------------------|----------------|
| **Marked effect**               |                                                               |                |
| DL-3-Amino-3-phenylpropionic acid | 26                                                            |                |
| L-Cysteine                      | 20                                                            |                |
| L-3,4-Dihydroxyphenylalanine    | 18                                                            |                |
| L-Histidine                     | 21                                                            |                |
| L-3-Iodotyrosine                | 36                                                            |                |
| L-Isoleucine                    | 12                                                            |                |
| DL-Leucine                      | 13                                                            |                |
| L-Leucine                       | 19                                                            |                |
| L-Methionine                    | 15                                                            |                |
| L-Phenylalanine                 | 26                                                            |                |
| DL-2-Phenylglycine              | 20                                                            |                |
| DL-Tyrosine                     | 39                                                            |                |
| L-Tyrosine                      | 10                                                            |                |
| L-Tryptophan                    | 16                                                            |                |
| L-Valine                        | 18                                                            |                |
| **Moderate effect**             |                                                               |                |
| DL-Asparagine                   | 57                                                            |                |
| DL-p-Chlorophenylalanine        | 50                                                            |                |
| L-Glutamine                     | 58                                                            |                |
| Prechloroperazine               | 68                                                            |                |
| L-Threonine                     | 51                                                            |                |
| Trifluoperazine                 | 62                                                            |                |
| **Slight effect**               |                                                               |                |
| DL-Amphetamine sulfate          | 84                                                            |                |
| Chlorpromazine                  | 88                                                            |                |
| Phenthanamine                   | 74                                                            |                |
| L-2-Hydroxy-3-phenylpropionic acid | 96                                                          |                |
| Morphine sulfate                | 79                                                            |                |
| 3-Phenylpyruvic acid            | 89                                                            |                |
| Reserpine                       | 80                                                            |                |
| **No effect**                   |                                                               |                |
| Acetophenazine, DL-alanine, amitriptyline, L-arginine, chlor Diazepoxide, chlorprothixene, DL desipramine, diazepam, dopamine, L-glutamic acid, haloperidol, L-hydroxyproline, imipramine, L-lysine, mepazine, methaqualone, l norepinephrine, nortriptyline, opipramol, oxazepam, DL perphenazine, phenoxysalazine, 4-phenylbutyric acid, 3-phenylpropionic acid, L-proline, promazine, propranol, L-serine, taurine, trifluoperazine, tyramine | 100             | 4.7% of the recovered radioactivity was located with carrier L-3,4-dihydroxyphenylalanine. Only this layer chromatography was performed with this sample. The rate of tyrosine hydroxylation in intact cells is very slow, and, therefore, the formation of L-3,4-dihydroxyphenylalanine from L-tyrosine in living cells is not measurable with short incubation periods with the use of a low specific activity precursor.

The data indicate that tyrosine transport into mouse neuroblastoma clone N1E-115 cells is by facilitated diffusion (6). Thus, this transport of tyrosine is by a saturable process (Table I) (1, 2), is not energy-dependent (Table II), is inhibited by a reagent which attacks sulphydryl groups (Table II), is inhibited by molecules which are structurally analogous (Table IV, Fig. 2), and exhibits exchange with other molecules (Table V). In addition, the specific transport of tyrosine into these cultured cells is not dependent on sodium to a major degree and is not sensitive to pH (range 6 to 8). These latter characteristics, along with the very strong exchanging properties, are characteristics of the leu-


e. Richelson, unpublished observations.
cine-prefering (L) system described by Christensen and co-workers (7,8). The inhibition of tyrosine transport into NIE-115 by such compounds as L-isoleucine, L-leucine, L-methionine, L-tryptophan, and L-valine is consistent with the conclusion that tyrosine is transported into these cells by the L system and is somewhat similar to findings on the uptake of tyrosine by rat brain (22). N-Monomethylation of certain amino acids reduces or eliminates transport by the L system (23), and these results indicate the importance of the α-amino group for transport by this mechanism (24). Our results support this, since compounds which differed from L-phenylalanine in the α position of the molecule showed marked diminution in their ability to inhibit tyrosine transport (Table IV and Fig. 2).

The stimulation of exit of a radioactively labeled compound from inside a cell by the same nonradioactively labeled compound outside a cell has been termed the trans effect (25) and is evident in support of the mobile transport carrier hypothesis (6,25). This hypothesis which does not fit all the data on transport (26,27) states, in part, that the loaded and unloaded carriers cross the cell membrane and that the rate of transit of the loaded carrier can be different from that of the unloaded carrier. In the mathematical formulation for a simple symmetrical mobile carrier, Stein has shown (6) that the trans effect will occur only when  + 1, a condition which would be incompatible with the observation of the trans effect for tyrosine. Thus, the experimentally determined half-saturation concentration for stimulation of exit (Fig. 4) is not equivalent to a .

The competitive inhibition of tyrosine transport by phenylalanine is evidence for a proposed mechanism for the reduced levels of catecholamines and their metabolites in phenylketonuria (28,29,30). Recently, it was shown that in rats in vivo dopamine and its metabolite 3-methoxy-4-hydroxyphenylecetic acid are derived from tyrosine (31,32). The high concentrations of L-phenylalanine (millimolar range) obtained in the serum of phenylketonuric patients (33) is adequate to block a major portion of the transport of the catecholamine precursor, tyrosine, into the neuron and, therefore, reduce the synthesis of catecholamines.

That the transport of tyrosine into mouse neuroblastoma clone NIE-115 is similar to the leucine-prefering system described for other cell types corroborates our previous results (1,2) indicating a lack of specificity of tyrosine transport with cell type. It is also of interest to find that yet another cell type has this L system. However, an understanding of the characteristics of tyrosine transport will be useful to studies on the synthesis of catecholamines from the precursor tyrosine. Thus, for example, we have shown that in addition to being a potent inhibitor of tyrosine hydroxylase (34), 3-iodotyrosine is a potent inhibitor of tyrosine transport. With this knowledge of the characteristics of tyrosine transport, we have begun our studies on the hydroxylation of tyrosine after it has been transported into this clone.

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