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P. R. Myers
W. G. Shain, Jr.

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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P. R. MYERS
W. G. SHAIN, JR.

D. O. CARPENTER
Chairman
Neurobiology Department

LaWAYNE R. STROMBERG, MD
Colonel, USA
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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ABSTRACT

The transport, metabolism and pharmacology of the putative neurotransmitter dopamine by a somatic cell hybrid were studied. Dopamine uptake was investigated using NX31 and N18TG2 in exponential growth phase, and NX31 grown in the presence of dibutyryl cyclic adenosine monophosphate. The cell line N18TG2 is a clonal line of the mouse neuroblastoma C1300, and NX31 is a somatic cell hybrid between N18TG2 and mouse sympathetic ganglion cells. NX31 in exponential growth phase displayed a saturable high affinity uptake system and an apparently nonsaturable low affinity uptake system. The K_m for the saturable component was 31.6 μM with a V_{max} of 1.4 pg/μg per min (9.2 nmoles/g per min). Dopamine uptake in NX31 grown in dibutyryl cyclic adenosine monophosphate has a K_m of 40.2 μM and a V_{max} of 3.9 pg/μg per min (25.8 nmoles/g per min). The parent N18TG2 accumulates dopamine by a nonsaturable linear process. Dopamine transport in NX31 is sensitive to temperature and drugs known to inhibit dopamine uptake in vivo. The uptake in NX31 in exponential growth phase and NX31 grown in dibutyryl cyclic adenosine monophosphate is not dependent on sodium. The data demonstrate for the first time the presence of a saturable catecholamine uptake system in a cultured cell line. The K_m of the dopamine carrier is not significantly different after cells are treated with dibutyryl cyclic adenosine monophosphate; however, the maximal velocity is increased.

I. INTRODUCTION

Neurotransmitter uptake is a well-documented means of terminating synaptic activity and maintaining intraneuronal transmitter levels. Uptake studies have primarily been done using systems in vivo, tissue explants, or purified tissue preparations. Advances in cell culture methods have made available large homogeneous populations of cells without interfering structural barriers or glial tissues. These cell lines in turn offer unique advantages for the kinetic and developmental characterization of neurotransmitter transport. In this study we have utilized a somatic cell hybrid which incorporates properties of both a dividing neuroblastoma and nondividing sympathetic neuronal population in order to investigate the kinetic and developmental characteristics of a catecholamine uptake system.

Neurotransmitter uptake studies using cultured cells have centered largely on gamma-aminobutyric acid,[9,12,18,23] or glutamic acid[7] using glial cell lines or nervous tissue explants. Membrane transport studies of catecholamines in cultured cells have not been reported. Coyle et al.[2] have reported dopamine uptake in explants of substantia nigra that increased with time in culture. Silberstein et al.[20] and Hanbauer et al.[5] have studied norepinephrine uptake in superior cervical ganglia in organ culture.

In this report we have investigated the kinetics and pharmacology of membrane transport of 3, 4-dihydroxyphenylethylamine (dopamine) in the neuroblastoma clone N18TG2 and in the somatic cell hybrid NX31, a hybrid line that resulted from the fusion of N18TG2 and mouse sympathetic ganglion cells. NX31 was grown in exponential growth phase and in the presence of dibutyryl cyclic adenosine monophosphate, a

compound known to initiate morphological and biochemical modulation, in order to investigate possible transitions in kinetic properties during differentiation.

II. EXPERIMENTAL METHODS

Materials. Cells were grown in modified F12 medium.[24] The following compounds were used: fetal calf serum: GIBCO; $N^6, O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphoric acid (dibutyryl cyclic adenosine monophosphate): Sigma; all catecholamines and their metabolites: Sigma; Dowex 50W-X8: Bio Rad Laboratories; N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid: Sigma; ^{3}H-3,4-dihydroxyphenylethylamine (^{3}H-dopamine; 1,2-^{3}H; 0.018-0.019 mg/mCi per ml): New England Nuclear; d-amphetamine sulphate, reserpine, amantadine, 6-hydroxydopamine and ouabain: Sigma; benztropine mesylate: Merck, Sharp, & Dohme; imipramine: Geigy.

Cell lines. NX31 is a somatic cell hybrid that resulted from the fusion of mouse neuroblastoma N18TG2 and mouse sympathetic ganglion cells by Greene et al.[4] The N18TG2 parent cells used in this study were obtained from Dr. Marshall Nirenberg, Laboratory of Biochemical Genetics, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland. Early subcultures 18 and 19 N18TG2 were used. Cells were grown in Falcon dishes or Bellco roller bottles. Unless otherwise stated, N18TG2 were grown using modified F12 medium and NX31 grown with modified F12 medium supplemented with hypoxanthine (100 μM), aminopterin (0.4 μM) and thymidine (16 μM). All media contained 5 percent fetal calf serum. Cells were maintained at 37°C and 5 percent carbon dioxide-95 percent air in a water saturated atmosphere.

Dopamine uptake experiments. NX31 are sensitive to medium changes, pH, and temperature. Variation of any of these factors significantly alters physiological characteristics. Chalazonitis et al.[1] have found this to be the case for electrophysiological parameters and we have also found this to be true for dopamine uptake. All uptake experiments were carried out in F12 medium at 37°C unless otherwise stated. The day prior to experiments, approximately 10^5 cells were seeded into 35-mm Falcon dishes with growth medium free of aminopterin, since cells grown in the presence of this drug failed to accumulate dopamine. The time between plating and experiments allowed cells to attach to the bottom of the dish. Dopamine uptake experiments carried out with NX31 in suspension revealed little uptake. Caution was taken to ensure that there were no temperature fluctuations during uptake incubations and that the cells were maintained at 36-37°C. During incubations the dishes were gently swirled.

For uptake studies using cells grown in the presence of dibutyryl cyclic adenosine monophosphate, cells were exposed to medium containing 1 mM drug at least 8 days prior to the experiment. Medium containing the drug was replaced every other day with fresh medium.

^{3}H-dopamine was combined with nonradioactive dopamine to obtain resultant stock concentrations to be used for the incubations. The radioactive dopamine was tested for purity by thin-layer chromatography and was routinely found to be greater than 90 percent in purity. After the incubations, the growth medium was removed and cells were washed with 2 ml of Konigsberg's modification[11] of Hanks' saline (37°C) buffered with N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid to a pH of 7.4 and containing .02 mg ascorbic acid per ml. In experiments where cells were incubated

with radioactive isotope, nonradioactive dopamine (1 mM) was included in the saline wash. This wash was removed and cold (4°C) saline (pH = 6.8) containing 0.3 percent bovine serum albumin and 1 mM dopamine was added. In the presence of cold saline, the cells detached from the bottom of the dish and were subsequently collected by passing the suspension through a 2.5-Millipore glass prefilter (number AP2002500). Microscopic examination of the filtrate showed that no cells were lost through the filter. The dish was rinsed twice with cold saline (total volume of 5 ml) and the rinses were also passed through the filter. The filter was transferred to vials for analysis in a liquid scintillation spectrometer (Nuclear-Chicago, Mark II). Efficiencies were determined by the external standard ratio method. Background binding to the filter was determined by filtering a solution containing ^{3}H-dopamine, washing the filter, and then counting the filter, as described for experimental samples. In most cases background was less than 20 cpm. Uptake was expressed as micrograms dopamine taken up per milligram protein per minute. Protein was determined by the method of Lowry et al.[13]

To ascertain the effects of various pharmaceuticals on uptake, cells were prepared for uptake experiments as described above except that they were preincubated with the drug under consideration prior to adding the ^{3}H-dopamine. Results are expressed as percent inhibition of uptake relative to control uptake levels.

For the experiments to determine the effect of low sodium concentrations on the uptake of dopamine in NX31, F12 medium was modified as follows: N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer (3.4 g/l) replaced sodium bicarbonate and choline chloride replaced sodium chloride. KCl and $CaCl_2$ were replaced by their hydroxide salts using equal equivalents of potassium and calcium. The nonsodium salts

of all other medium constituents were used. The osmolarity was brought up to 320 mosmoles with 31 g/l glucose. The final pH was 7.3. Fetal calf serum that had been dialyzed against water at 4°C overnight was added (1 percent v/v). Analysis by flame photometry indicated that the sodium concentration was less than 80 μM. Seventy-five minutes prior to uptake incubations, growth medium was removed and cells were washed with sodium-free medium for experimental samples or medium with normal sodium concentration (150 mM) for controls. An aliquot of experimental or control medium was then added to the dishes.

Metabolism of accumulated dopamine. The metabolism of newly accumulated dopamine was investigated using thin-layer and column chromatography. NX31 cells were seeded into 60-mm Falcon dishes 1 day prior to the experiments. ^{3}H-dopamine concentrations ranged from 1.24 to 6.2 μM (10-50 μCi/ml). After a 15-min incubation, the medium was removed and the cells were washed once at 37°C with 4 ml modified Hanks' saline containing 1 mM dopamine and then rapidly rinsed with distilled water to remove salts. Dishes were scraped and washed twice with ethanol-0.1 N HCl (95:5 v/v) containing dopamine, noradrenaline, and 3,4-dihydroxyphenylacetic acid (0.25 mg/10 ml) plus homovanillic acid and 3-methoxytyramine (1.25 mg/10 ml) as carriers. The combined washes (total of 2 ml) were homogenized and centrifuged. The supernatant was removed and the pellet washed with 1 ml ethanol-HCl without carriers and recentrifuged. The supernatant fractions were combined and dried with nitrogen gas. The resultant residue was resuspended in 0.5 ml ethanol-HCl and 10 μl were spotted on MN300 cellulose thin-layer chromatographic plates (Brinkman). Three solvent systems were employed for separation of the catecholamines and their

metabolites: isopropanol-1 N acetic acid-water (35:10:10); ethyl acetate-acetic acid-water (10:3:6); and N-butanol-5 N acetic acid (100:35). Improved separations were obtained when plates were prerun with the solvent. Chromatographs were developed with ethylenediamine[17] to form the catecholamine fluorescent derivatives. Fluorescent cellulose spots were scraped off the plates and analyzed by scintillation spectrometry.

Column chromatography using Dowex 50W-X8 was done according to the method outlined by Snyder and Taylor.[21] The remaining sample volume from the thin-layer chromatography analysis was subjected to column chromatography by evaporating the ethanol-HCl and redissolving the residue in 400 μl of 0.1 M sodium acetate (pH = 4.7). A total of 0.05 mg of dopamine, noradrenaline, and 3,4-dihydroxyphenylacetic acid plus 0.35 mg homovanillic acid and 3-methoxytyramine were applied to a column. Most of the homovanillic acid and 3,4-dihydroxyphenylacetic acid were not bound to the Dowex and appeared in the first wash. Noradrenaline was then eluted with 6 ml of 1.2 N HCl, dopamine with 6 ml of 2 N HCl and 3-methoxytyramine with 6 ml of 3 N HCl. The catecholamine or metabolite present in each fraction was determined by thin-layer chromatography and the relative amounts of radioactivity were determined by scintillation spectrometry.

Autoradiography. Autoradiography of NX31 exposed to ^{3}H-dopamine was done by freeze-drying cells grown on glass slides and coating them with Kodak NTB2 Nuclear Track Emulsion by a roller method. After a 7-day exposure in the dark at 4^{o}C, slides were developed according to the method outlined by Kodak, stained, dehydrated, and mounted.

III. RESULTS

Transport of dopamine. The experimental approach was to investigate and compare dopamine transport in NX31 and N18TG2 in exponential growth phase, and NX31 that had been grown in the presence of dibutyryl cyclic adenosine monophosphate. Figure 1 illustrates dopamine uptake versus time for NX31 in exponential growth phase. At 5 μM dopamine, uptake is linear through 10 minutes and begins to saturate after 30 minutes. Therefore, incubation times of 10 minutes were used in most of the experiments.

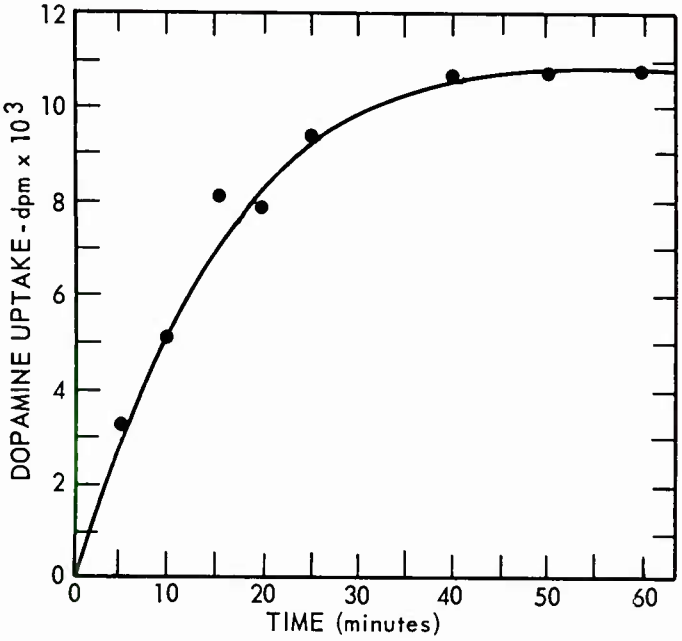

Figure 1. Dopamine uptake as a function of time. Exponential growth phase NX31 were incubated in 5 μM ^{3}H-dopamine for varying periods of time to determine if uptake were saturable.

Figure 2 (open circles) shows dopamine uptake velocity as a function of dopamine concentration. There are two distinct accumulation mechanisms operating in NX31 in exponential growth phase. For dopamine incubation medium concentrations less than 60 μM, a high affinity transport system is predominant. At concentrations greater than 100 μM, a low affinity uptake system is apparent. Dopamine concentrations between 0.01 μM and 5 mM were investigated; however, for purposes of presentation, results for the range of 1 μM to 150 μM are shown. Uptake velocities at dopamine concentrations less than 1 μM were linear with respect to the high affinity component.

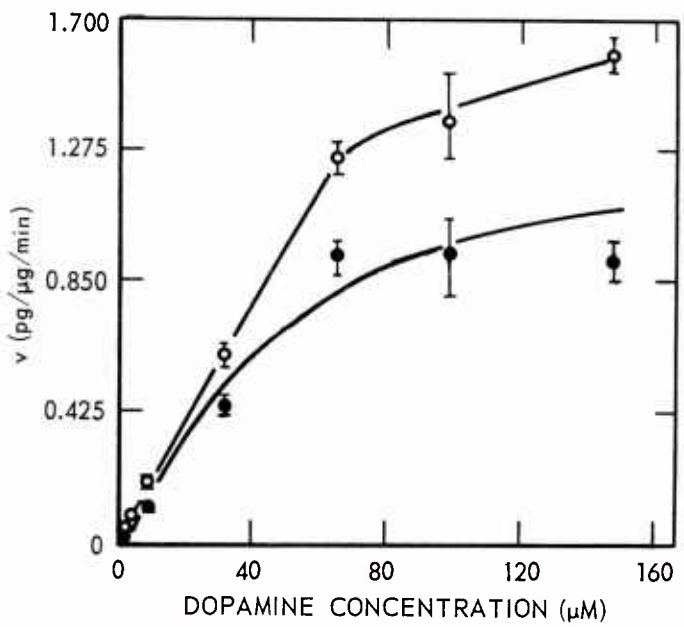

Figure 2. Dopamine uptake velocity versus medium dopamine concentration. Open circles represent data uncorrected for the nonsaturable, low affinity uptake component. Closed circles represent data with the nonsaturable dopamine uptake contribution subtracted, resulting in a plot that demonstrates a high affinity, saturable uptake in exponential growth phase NX31.

Uptake velocities at dopamine concentrations greater than 0.5 mM were linear with respect to the low affinity component. Since it can be assumed that the low affinity system is also operable at low dopamine concentrations, the component of the low affinity system contributing to the velocity at low dopamine concentrations can be subtracted. This was done by subtracting from every ordinate value (velocity) the product of the slope of the low affinity uptake and the corresponding abscissa value (dopamine concentration). The resultant plot (Figure 2, closed circles) indicates a saturable, high affinity uptake system for dopamine in NX31 in exponential growth phase.

A double reciprocal plot of the data for the high affinity dopamine uptake from Figure 2 (open circles) is shown in Figure 3. The plot is nonlinear with an upward curvature, possibly suggesting cooperativity and kinetics other than first order. The determination of an apparent Michaelis constant (K_m) was therefore not possible. However, a K_m determined from Figure 2, where velocity is half maximal, is 31.6 μM with a V_{max} of 1.4 pg/μg per min (9.2 nmoles/g per min). Figure 4 is a Hill plot to determine the degree of cooperativity, if any, suggested by the double reciprocal plot. A useful "n" value was determined where the quantity $v/(V_{max}-v)$ equals one. This value (n = 1.32) suggests little or no cooperativity.

Figure 5 shows the results of velocity versus substrate studies when NX31 is grown in the presence of dibutyryl cyclic adenosine monophosphate. This compound is known to slow down the rate of cell division and cause modulation of both morphological and biochemical characteristics of neuroblastoma cells in culture. The cells appear to take on the properties and appearance of a differentiated cell.[14-16,25] For cells grown in dibutyryl cyclic adenosine monophosphate the plot becomes more hyperbolic with a

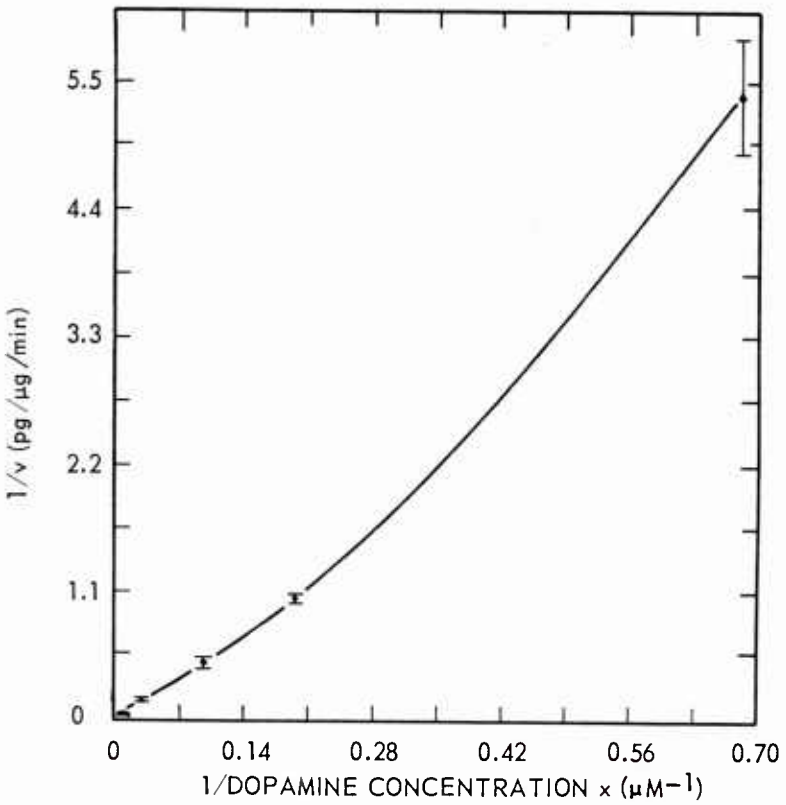

Figure 3. Double reciprocal plot of dopamine uptake in exponential growth phase NX31. Upward curvature of plot would continue if smaller concentrations were shown; however, for graphic purposes, the reciprocal values for 1 μM through 60 μM are shown. Each point represents the average of at least eight determinations (± S.E.).

higher V_{max}, suggesting a modulation of the carrier or an increased number of transport sites. A low affinity uptake does not appear to be significant. A double reciprocal plot of the data (Figure 6) is linear with a K_m of 40.2 μM and a V_{max} of 3.9 pg/μg per min (25.8 nmoles/g per min). A Hill plot of the data (Figure 7) is linear and has a slope (n = 1.37) that indicates the stoichiometry of the dopamine carrier is the same for cells in exponential growth phase and cells exposed to dibutyryl cyclic adenosine monophosphate.

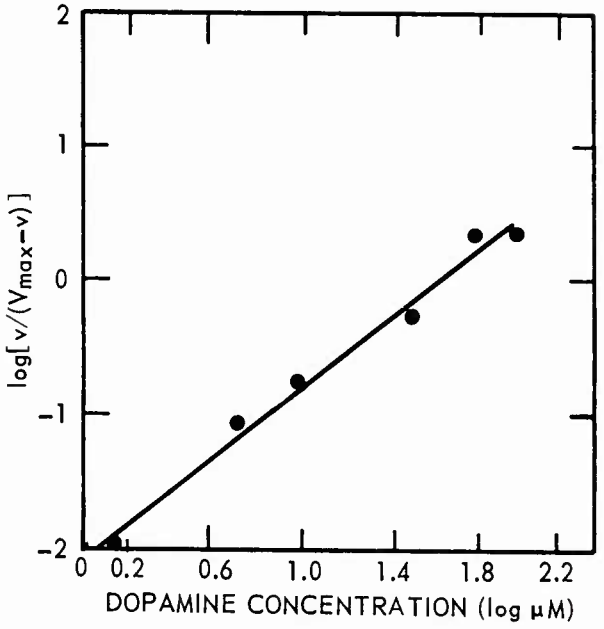

Figure 4. Hill plot of uptake data for exponential growth phase NX31

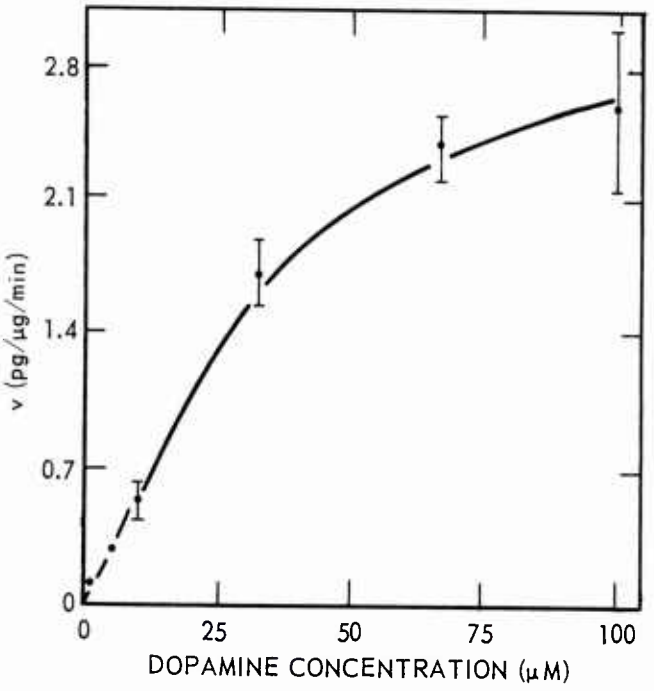

Figure 5. Dopamine uptake velocity versus medium dopamine concentration for NX31 grown in dibutyryl cyclic adenosine monophosphate. Each point represents the average of four to eight determinations (± S. E.).

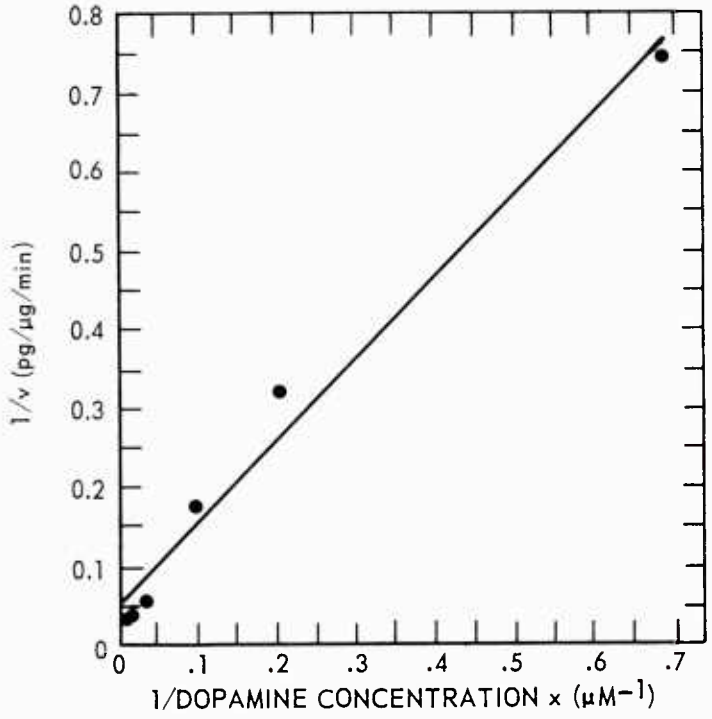

Figure 6. Double reciprocal plot of uptake data for NX31 grown in dibutyryl cyclic adenosine monophosphate

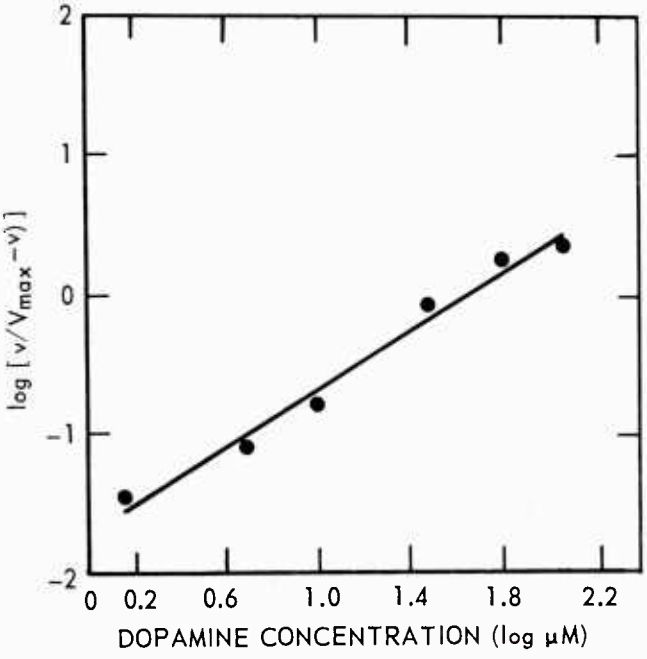

Figure 7. Hill plot of dopamine uptake data for NX31 grown in dibutyryl cyclic adenosine monophosphate

Figure 8 is a comparison of velocity versus dopamine concentration between the parent neuroblastoma N18TG2 (open circles) and NX31 (closed circles) in exponential growth phase. The left panel of the figure is a graphic expansion of the results from low dopamine concentrations. In N18TG2, uptake is linear and is not saturable. This indicates that NX31, as a result of hybridization, acquires a high affinity carrier which the parent neuroblastoma lacks.

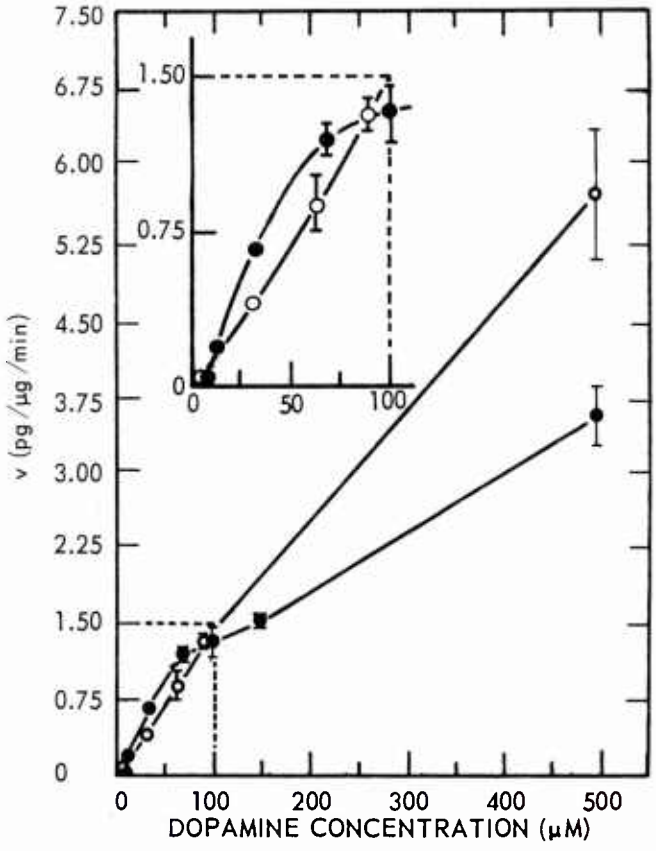

Figure 8. Dopamine uptake velocity as a function of medium concentration for exponential growth phase N18TG2 and NX31. Open circles represent uptake for the parent neuroblastoma N18TG2. Closed circles represent uptake for the sympathetic ganglion cell X neuroblastoma hybrid NX31.

Effects of drugs, temperature, and sodium on high affinity dopamine uptake.

Table I shows that a number of drugs known to be catecholamine uptake inhibitors effectively inhibit dopamine uptake in exponential growth phase NX31. Reserpine, benztropine, and d-amphetamine are the most potent inhibitors of NX31 dopamine uptake. Benztropine has been shown to be a competitive dopamine uptake inhibitor in rat brain.[8] Reserpine is known to inhibit neurotransmitter uptake into vesicles while amphetamine acts at the neuron membrane uptake site.

Table I. Effect of Drugs on Dopamine Uptake in NX31*

Drug	Percent inhibition
d-amphetamine	45.6
benztropine	51.8
reserpine	55.5
amantadine	28.7
imipramine	35.0
6-hydroxydopamine	33.6
noradrenaline	31.4
ouabain	27.3

* The effects of known uptake inhibitors on dopamine uptake in exponential phase NX31. Preincubation times were 2 minutes, except for ouabain which was 30 minutes. Results are expressed as percent inhibition relative to control uptake levels in the absence of the drug. Dopamine concentration was 0.62 μM. Drug concentration was 10 μM, except for ouabain which was 0.1 mM. Each value is the average of four to eight determinations.

Figure 9 shows that temperature significantly affects dopamine transport with a Q_{10} of 4.7 between 27° and 37°C. Experiments on temperature effects using high dopamine concentrations (greater than 100 μM) indicated that the low affinity uptake

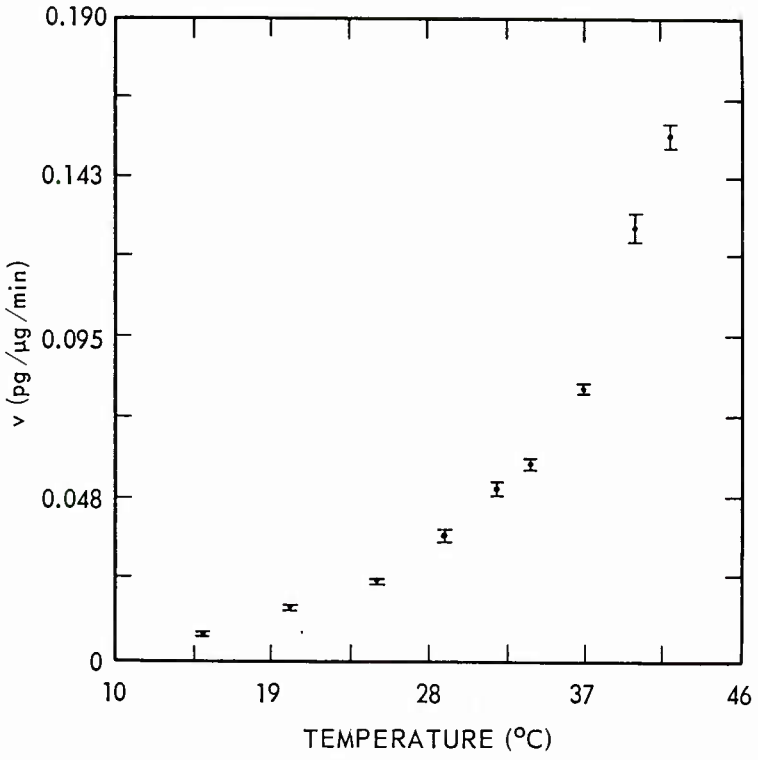

Figure 9. Dopamine uptake velocity as a function of temperature. Dishes of exponential growth phase NX31 were placed on a brass plate which formed the top of a container with water circulating through it at constant temperatures. Dishes were allowed to equilibrate 3 minutes prior to initiation of incubations. Each point represents the average of four to eight determinations (± S.E.).

velocity was also decreased by low temperature. The possibility that dopamine uptake in NX31 was an amino acid or noradrenaline carrier was explored by adding tyrosine or noradrenaline to the incubation medium. Tyrosine, the precursor to catecholamines, was added in increasing concentrations to the medium 2 minutes prior to the addition of ^{3}H-dopamine. Cells preincubated in tyrosine concentrations, ranging from 50 percent normal medium levels (30 µM) to five times normal tyrosine (300 µM), showed no significant change in rate of dopamine uptake. Table I illustrates that the transport system studied here has more affinity for dopamine than noradrenaline. A concentration

of noradrenaline in the incubation medium 20 times greater than the dopamine concentration inhibited uptake by 31.4 percent.

Experiments to determine sodium dependence of uptake showed that the absence of sodium in the medium did not lower uptake velocity. This was true for both cells in exponential growth phase and cells grown in the presence of dibutyryl cyclic adenosine monophosphate. Choline chloride was the best substitute for sodium chloride. Sucrose and magnesium chloride were inadequate for sodium chloride substitution since cells rounded up and detached from the dish.

Metabolism of accumulated dopamine in NX31. The metabolism of newly accumulated ^{3}H-dopamine was investigated primarily by using thin-layer chromatographic separation of metabolites. Column chromatography with Dowex 50W-X8 was used to confirm these results. Dopamine and its metabolites were completely separated using thin-layer chromatography, while there was a tendency toward overlap of compounds into adjacent fractions when using column chromatography. Of the total activity in the spots for dopamine and its metabolites, most remained as dopamine (Table II). This result suggests that newly accumulated dopamine is not rapidly metabolized in NX31. There was some indication of monoamine oxidase activity, since significant radioactivity appeared consistently in the 3,4-dihydroxyphenylacetic acid spot. Results from the column chromatography confirmed the finding that most of the accumulated dopamine remains unmetabolized.

Autoradiography. To confirm that dopamine entered the cells, autoradiography on the light microscope level was carried out with NX31 in exponential growth phase after incubation in ^{3}H-dopamine. Grains appeared over the cytoplasm, but not the

Table II. Dopamine Metabolism in NX31*

Compound	Percent total dpm
dopamine	61.8 (2.5)
3,4-dihydroxyphenylacetic acid	16.6 (3.2)
3-methoxytyramine	6.0 (0.3)
noradrenaline	5.9 (1.3)
homovanillic acid	4.6 (0.4)
origin	5.2 (1.7)

* Metabolism of newly accumulated ^{3}H-dopamine by exponential growth phase NX31. See text for methods. Results are expressed as percent of total radioactivity (± S. E.) that ran in the spots after subtraction of background.

nucleus, indicating that dopamine was taken up into the cell body and was not localized on the cell membrane exclusively.

IV. DISCUSSION

This study demonstrates for the first time that a cultured cell line is capable of transporting the putative neurotransmitter dopamine by a saturable, high affinity process. The parent neuroblastoma N18TG2 lacks a high affinity uptake and accumulates neurotransmitter by an apparently nonsaturable process. The fusion of N18TG2 with sympathetic ganglion cells has resulted in the expression of a new transport system, or possibly the inheritance of a transport system from a sympathetic ganglion cell.

The most significant difference between dopamine uptake in NX31 in exponential growth phase and NX31 grown in dibutyryl cyclic adenosine monophosphate is a difference in maximal velocities attained at higher dopamine concentrations. The apparent K_m's determined for cells grown under the two conditions are not significantly different.

The increased velocity of uptake may be due to appearance of more carrier sites, since it has been shown by others[14-16,25] that dibutyryl cyclic adenosine monophosphate causes increases in protein synthesis, enzyme activity and neurite extension.

The upward curvature of the double reciprocal plot for NX31 exponential growth phase (Figure 3) raised the possibility that the carrier for dopamine displayed cooperativity. Enzymes with multiple binding sites are known to give this type of plot.[3] A similar plot (Figure 6) for NX31 grown in dibutyryl cyclic adenosine monophosphate was linear. Similarly, a Hanes plot (dopamine concentration/velocity versus dopamine concentration; plot not shown) for NX31 in exponential growth phase was nonlinear, while it was linear for NX31 grown in dibutyryl cyclic adenosine monophosphate. However, Hill plots of the data from cells grown under the two conditions (Figures 4 and 7) resulted in essentially identical slopes (n = 1.32-1.37). Although "n" is somewhat larger than one, the binding coefficients for exponential growth phase and differentiated NX31 are the same and the carrier displays little cooperativity. Even though differences exist in the double reciprocal and Hanes plots for cells in exponential growth phase and cells treated with dibutyryl cyclic adenosine monophosphate, the data demonstrate identical stoichiometry for the transport sites in the nondifferentiated and differentiated condition and an increased V_{max} as a result of the dibutyryl cyclic adenosine monophosphate treatment.

The low affinity uptake system occurring at nonphysiological dopamine concentrations in exponential growth phase NX31 could possibly be due to diffusion; however, no experiments have been done to further investigate this aspect of uptake. Such low

affinity uptake systems have also been found in cultured cells for gamma-aminobutyric acid and glutamate.[7,18]

The pharmacology of dopamine uptake in NX31 is similar to that of most catecholamine uptake systems. Drugs known to inhibit dopamine uptake and also low temperature were both effective inhibitors of NX31 uptake. Uptake velocity was not lowered in the absence of sodium. Most catecholamine uptake systems display a sodium requirement.[19] This may be due to the necessity for a sodium gradient across the membrane or a cationic site on the carrier. Since it is very difficult to remove all the sodium from a monolayer of cells, sufficient levels of sodium may have remained for dopamine transport to occur. Alternatively, dopamine transport in NX31 simply may not have a sodium requirement.

Table I indicates that ouabain partially inhibits dopamine uptake. This could possibly be in disagreement with the finding that dopamine uptake in NX31 does not require a sodium gradient, since ouabain inhibits the sodium-potassium transport by adenosine triphosphatase. However, the results of Tissari et al.[22] and White and Keen[26] suggest that ouabain may inhibit amine transport by a means not involving sodium-potassium activated adenosine triphosphatase.

Dopamine uptake described for NX31 has some characteristics similar to uptake systems reviewed by Iversen.[10] It is saturable, temperature sensitive, and inhibited by known uptake blockers. Results using other tissues have demonstrated that they have a higher affinity and larger maximal velocity for dopamine. Hellmann et al.[6] reported a K_m of 0.68 μM and a V_{max} of 1.45 nmoles/g per min for a high affinity dopamine uptake system in rat atrium. The NX31 high affinity uptake for cells grown

in the presence of dibutyryl cyclic adenosine monophosphate has a K_m of 29.4 μM and a V_{max} of 0.18 nmoles/g per min. Quantitative dopamine uptake studies have not been reported. Since there exist quantitative differences in dopamine transport characteristics and the apparent lack of a sodium requirement, further work is required to ascertain whether the uptake system in NX31 is the same as dopamine uptake systems studied in vivo.

The results from experiments on the metabolism of newly accumulated dopamine showed that the amine is not rapidly metabolized. Monoamine oxidase activity appeared consistently since radioactivity cochromatographed with 3,4-dihydroxyphenylacetic acid. Catechol-O-methyl transferase activity was minimal if present at all. Radioactivity that cochromatographed with 3-methoxytyramine and homovanillic acid was not significantly above background. These observations may indicate that the newly accumulated dopamine is taken up and stored in vesicles, thus metabolism remained at low levels because of the unavailability of dopamine to the soluble catabolic enzymes. Autoradiography confirmed that dopamine had entered the cells. Vesicular storage may explain why reserpine, known to block uptake into vesicles, was effective as an uptake inhibitor in NX31. Breakefield (personal communication) found strong evidence for vesicular storage of accumulated noradrenaline in a neuroblastoma cell line, N115.

Collectively these results demonstrate an active accumulation of dopamine by NX31. The process is saturable and sensitive to drugs and temperature. Low levels of catabolism of newly accumulated dopamine further suggest active accumulation and possibly storage. The use of homogeneous populations of cells in culture known to

have an active uptake system may prove of value in determining the membrane basis of neurotransmitter uptake.

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13. ABSTRACT

The transport, metabolism and pharmacology of the putative neurotransmitter dopamine by a somatic cell hybrid were studied. Dopamine uptake was investigated using NX31 and N18TG2 in exponential growth phase, and NX31 grown in the presence of dibutyryl cyclic adenosine monophosphate. The cell line N18TG2 is a clonal line of the mouse neuroblastoma C1300, and NX31 is a somatic cell hybrid between N18TG2 and mouse sympathetic ganglion cells. NX31 in exponential growth phase displayed a saturable high affinity uptake system and an apparently nonsaturable low affinity uptake system. The K_m for the saturable component was 31.6 μM with a V_{max} of 1.4 pg/μg per min (9.2 nmoles/g per min). Dopamine uptake in NX31 grown in dibutyryl cyclic adenosine monophosphate has a K_m of 40.2 μM and a V_{max} of 3.9 pg/μg per min (25.8 nmoles/g per min). The parent N18TG2 accumulates dopamine by a nonsaturable linear process. Dopamine transport in NX31 is sensitive to temperature and drugs known to inhibit dopamine uptake in vivo. The uptake in NX31 in exponential growth phase and NX31 grown in dibutyryl cyclic adenosine monophosphate is not dependent on sodium. The data demonstrate for the first time the presence of a saturable catecholamine uptake system in a cultured cell line. The K_m of the dopamine carrier is not significantly different after cells are treated with dibutyryl cyclic adenosine monophosphate; however, the maximal velocity is increased.

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