MOLECULAR PHARMACOLOGY, 21:362-367

Characterization of Serotonin Uptake in Cultured Neuroblastoma Cells

Difference between Differentiated and Nondifferentiated Cells

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Received July 1, 1981; Accepted November 18, 1981

SUMMARY

Neuroblastoma cells clone N-2a, differentiated by serum deprivation, were found to take up tritiated serotonin ([^{3}H]5-HT) from the external medium by means of a saturable mechanism which follows Michaelis-Menten kinetics. The apparent K_m of uptake was 1.27 μM and the V_{max} 720 fmoles/min/10^6 cells. The uptake was temperature-dependent and partially sodium-dependent, and was inhibited by ouabain and by selected metabolic inhibitors (sodium azide, 2,4-dinitrophenol, and iodoacetamide). Fluoxetine and desmethylimipramine (DMI) were equally effective inhibitors of [^{3}H]5-HT uptake (IC_{50} = 13.7 μM and 13.6 μM). The uptake was structurally specific, since unlabeled 5-HT was a better inhibitor of [^{3}H]5-HT uptake than norepinephrine (NE) (IC_{50} = 0.6 μM and 9.4 μM). The neurotoxins 6-hydroxydopamine and 5,6-dihydroxytryptamine were cytotoxic to differentiated N-2a cells, causing time- and concentration-dependent inhibition of [^{3}H]thymidine incorporation into DNA. 5,7-Dihydroxytryptamine had little cytotoxic effect. Nondifferentiated N-2a cells, supplemented with 5% fetal calf serum, were also found to take up [^{3}H]5-HT by a concentration-, temperature-, and energy-dependent process. The apparent K_m of uptake was 0.96 μM and the V_{max} was 619 fmoles/min/10^6 cells. However, in nondifferentiated cells [^{3}H]5-HT uptake was not sodium-dependent, not inhibited by ouabain, less effectively inhibited by fluoxetine and DMI (IC_{50} = 148 μM and 107 μM), and not selectively inhibited by unlabeled 5-HT as compared with NE (IC_{50} = 7.9 μM and 6.0 μM).

INTRODUCTION

The C1300 mouse neuroblastoma originated as a spontaneous, transplantable tumor of the spinal cord. During adaption to tissue culture, neuroblastoma cells lose their tumorigenicity and may be induced to express many of the properties of mature neurons. These include extension of neurites, increased activity of enzymes involved in the synthesis of transmitters, electrical excitability, and expression of a specific neuronal protein and neuronal antigen (1-7). This inductive process, referred to as differentiation, is associated with agents also causing inhibition of cell division, such as serum deprivation (8, 9), treatment with dibutyryl cyclic AMP (10, 11), dimethyl sulfoxide (12), vitamin E (13), 5-bromodeoxyuridine (14), and hypertonic medium (15).

Many clonal cell lines have been derived and characterized as adrenergic or cholinergic, according to their endogenous transmitters and the activity of their synthetic enzymes (16). Adrenergic clones have been widely used as an *in vitro* model of the adrenergic neuron. The N-2a clone has been shown to contain endogenous 5-HT[2] (17), to take up tryptophan, and to possess the enzymes required for the synthesis of 5-HT (18). The aim of this investigation was to determine whether the N-2a clone is a suitable model for the serotonergic neuron, which could then be used to investigate the mechanism by which the dihydroxytryptamines, e.g., 5,6-DHT and 5,7-DHT, exert their neurotoxic effects.

5-HT transport has been characterized extensively in synaptosomes (19), brain slices (20-22), blood platelets (23-27), bovine pinealocytes (28), bovine retina (29), pancreatic islets (30), and adipose capillary endothelium (31). 5-HT transport occurs by two different routes, one saturable route with high affinity and low capacity and one nonsaturable mechanism. This dual transport mechanism causes complex uptake kinetics. Therefore it has been suggested that, for meaningful studies of the high-affinity uptake mechanism, the substrate concentration should not exceed the apparent K_m value (24). The high-

This research was supported by United States Public Health Service Research Grant NS-15695.

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[2] The abbreviations used are: 5-HT, 5-hydroxytryptamine; 5,6-DHT, 5,6-dihydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; DMI, desmethylimipramine; 6-OHDA, 6-hydroxydopamine; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; NE, norepinephrine; TCA, trichloroacetic acid.

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affinity system in serotonergic neurons is an active process, inhibited by low temperatures and metabolic inhibitors. It is concentration-dependent, sodium-dependent, structurally specific, and inhibited by tricyclic antidepressants, such as DMI and the new nontricyclic drugs such as fluoxetine (32).

This investigation showed that neuroblastoma cells clone N-2a, differentiated by serum deprivation, accumulate serotonin by an active process. The results suggest neuroblastoma cells clone N-2a might serve as a suitable model for serotonergic neurons.

MATERIALS AND METHODS

Source of chemicals. [1,2-^{3}H]5-HT creatinine sulfate (32.1 Ci/mmole) was purchased from New England Nuclear Corporation (Boston, Mass.) and diluted 1:10 in Buffer B or C. The following drugs were purchased from Sigma Chemical Company (St. Louis, MO.): DMI·HCl, 5,6-DHT, and 5,7-DHT creatinine sulfates; 2,4-dinitrophenol, 5-HT creatinine sulfate, 6-OHDA·HBr, and ouabain octahydrate. Other drugs used were fluoxetine·HCl (Lilly), iodoacetamide (Fischer Scientific), sodium azide (Aldrich Chemical), and [^{3}H]thymidine (60 Ci/mmole, Schwarz-Mann).

Cell culture. Starter cultures of neuroblastoma cells clone N-2a were kindly provided by Dr. X. O. Breakefield (Yale University School of Medicine). Cells were grown in monolayer culture and passaged by trituration. Cell doubling time was 30–36 hr. The medium used was DMEM (GIBCO), supplemented with 5% fetal calf serum (FCS) (K. C. Biologicals or M. A. Bioproducts) and gentamycin sulfate, 70 mg/liter (Sigma Chemical Company). For nondifferentiated cultures, cells were plated at a density of 5×10^5 cells per 100-mm dish (Costar or Lux). They were fed at 2- to 3-day intervals and used after 1 week, at which time the monolayer had reached confluency and most of the cells were rounded. For differentiated cultures, cells were plated at a density of 1×10^6 cells per 100-mm dish in DMEM/5% FCS. After 3 days the medium was removed, the cells were washed with fresh DMEM and fed with DMEM containing no FCS. They were used after 36–48 hr, at which time most of the cells possessed two or more neurites.

Incubation conditions and procedures. Preliminary experiments showed no significant difference in the velocity or inhibition of [^{3}H]5-HT uptake or in the cytotoxicity of neurotoxic analogues between monolayer cultures and suspensions of differentiated N-2a cells. For convenience and reproducibility, all further experiments were carried out using cell suspensions.

Buffer A was an isotonic modified Dulbecco's phosphate-buffered saline, containing 129 mM NaCl, 2.5 mM KCl, 7.4 mM Na_2HPO_4, and 1.3 mM KH_2PO_4. Buffer B was prepared fresh daily by supplementing with 0.63 mM $CaCl_2$, 0.74 mM $MgSO_4$, 5.3 mM glucose, and 0.1 mM ascorbic acid. Buffer C was prepared for studies by substituting choline chloride for NaCl in Buffer A and supplementing with 0.63 mM $CaCl_2$, 0.74 mM $MgSO_4$, 5.3 mM glucose, and 0.1 mM ascorbic acid.

Monolayer cultures were rinsed with Buffer B or C. The cells were suspended by trituration, precipitated using a Dynac centrifuge, and resuspended in an appropriate volume of uptake buffer. An aliquot (100 μl) of cell suspension was placed in each tube and these were incubated either at 37° in a water bath or at 0–4° in an ice bath. A 100-μl aliquot of cell suspension was used to determine the exact cell number per sample, using a hemocytometer. The viability of cells after this procedure was greater than 90%, as determined by trypan blue exclusion. The uptake experiments were terminated by placing the tubes in ice, diluting with 5 ml of cold Buffer A, and filtering immediately through GF/C glass-fiber filters in a Millipore manifold. Each tube was rinsed with 5 ml of cold Buffer A. The filters were rinsed with an additional 15 ml of cold Buffer A, placed in glass scintillation vials, dried in a warm oven for 15 min, then digested with 0.5 ml of NCS tissue solubilizer (Amersham) (33). To each sample were added 10 ml of toluene-based preblended dry fluors 2a70 (Research Products International Corporation) and the samples were counted, after overnight storage in the dark, using a Beckman LS-7500 scintillation counter. The amount of radioactivity bound directly to the filter in the absence of cells was determined at each concentration of [^{3}H]5-HT used and subtracted from the total counts. This nonspecific binding could not be blocked by presoaking the filters in 1 mM 5-HT.

Uptake of [^{3}H]5-HT. To determine the time course of [^{3}H]5-HT uptake, aliquots (100 μl) of cell suspension were incubated for up to 20 min with 10 μl of [^{3}H]5-HT solution and sufficient uptake Buffer B or C to give a final concentration of 0.1 μM. To determine the kinetics of uptake, 2.5–50 μl of [^{3}H]5-HT solution were added to 100 μl of cell suspension and the appropriate volume of Buffer B or C to give final concentrations of 0.025 μM–0.5 μM. Incubation was carried out for 2 min at 37° or at 0–4°.

Inhibition of [^{3}H]5-HT uptake. Aliquots (100 μl) of the cell suspension were incubated for 15 min at 37° with 1-ml solutions of the following metabolic inhibitors (final concentrations): iodoacetamide (5 mM), sodium azide (10 mM), 2,4-dinitrophenol (1 mM), and ouabain (1 mM). Cells were then separated by centrifugation and resuspended in 100 μl of Buffer B. The suspension was then incubated at 37° with [^{3}H]5-HT (0.1 μM) for 2 min.

With the uptake inhibitors (fluoxetine, DMI), aliquots (100 μl) of the cell suspension were incubated with the drugs (0.5–50 μM) for 15 min at 37°. [^{3}H]5-HT was then added to the incubation mixture to give a final concentration of 0.1 μM and the cells were incubated for an additional 2 min at 37°.

Specificity of [^{3}H]5-HT uptake. Solutions of unlabeled 5-HT, NE, 6-OHDA, 5,6-DHT, and 5,7-DHT were prepared immediately before use. Aliquots of drug solution giving final concentrations of 0.1–50 μM and 10 μl of [^{3}H]5-HT (0.1 μM) solution were added simultaneously to 100 μl of cell suspension and incubated for 2 min at 37°.

Cytotoxicity of neurotoxic analogues. Cytotoxicities of 6-OHDA, 5,6-DHT, and 5,7-DHT were determined by measuring the inhibition of incorporation of [^{3}H]thymidine into the DNA of differentiated N-2a cells (34). Solutions of 6-OHDA, 5,6-DHT, and 5,7-DHT in DMEM were prepared immediately before use. An aliquot (1 ml) of the drug solution was added to 100 μl of cell suspension.

After a 30- or 90-min incubation at 37°, the cells were separated by centrifugation, washed with 3 ml of Buffer A, and reprecipated. The cell pellets were resuspended in 500 μl of DMEM, 1 μl of [^{3}H]thymidine was added, and the cells were incubated for an additional 90 min at 37°. The incubation was terminated by placing the tubes on ice and adding 5 ml of cold 5% TCA. After 10 min the precipitated ^{3}H-labeled DNA was collected on GF/C glass-fiber filters previously wetted with 20% TCA (35) and the tubes were rinsed with 5 ml of cold 5% TCA. The filters were rinsed with 15 ml of cold 1% TCA and 3 ml of 95% EtOH, then dried in a warm oven, solubilized in 0.5 ml of NCS, and counted in 10 ml of toluene-based scintillation fluid, as described above.

Expression and analysis of results. Results were standardized by expression as uptake per 10^6 cells. Each experiment was performed three times in duplicate and the mean values ± standard error of the mean are given. Counts per minute were converted to femtomoles, using an external standard; the counting efficiency was 35%. The active uptake of [^{3}H]5-HT was calculated by subtracting the accumulation due to passive diffusion (0–4° values) from the total accumulation (37° values).

The effectiveness of inhibitors was assessed by comparing the concentrations required to give 50% inhibition of active uptake (IC_{50} values). These were determined by plotting the percentage inhibition of active uptake versus log concentration and using regression analysis to determine the slope of the best-fitting line.

RESULTS

Characteristics of [^{3}H]5-HT uptake in differentiated N-2a cells. As shown in Fig. 1, the uptake of [^{3}H]5-HT (0.1 μM) proceeded rapidly at 37°. It was linear with time for up to 2 min of incubation, and thereafter accumulated at a much slower rate. The uptake at 0–4° followed a similar pattern but represented only 5–17% of the uptake at 37°.

The velocity of uptake of [^{3}H]5-HT during a 2-min incubation was determined over the concentration range 0.025–0.5 μM at 37°, 0–4°, and in sodium-free buffer at

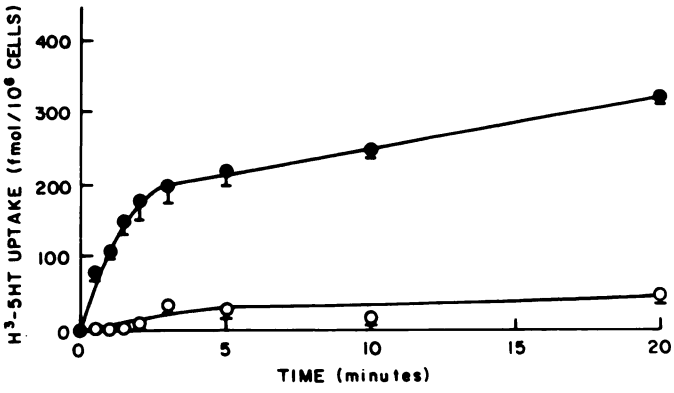

FIG. 1. *Apparent uptake of [^{3}H]5-HT in differentiated N-2a cells as a function of time*

Differentiated N-2a cells in suspension (10^6 cells/100 μl) were incubated in 0.1 μM [^{3}H]5-HT at 37° (●) and at 0–4° (○) as described under Materials and Methods. [^{3}H]5-HT uptake was terminated after a 30-sec to 20-min incubation by dilution and filtration through glass-fiber filters, as described under Materials and Methods.

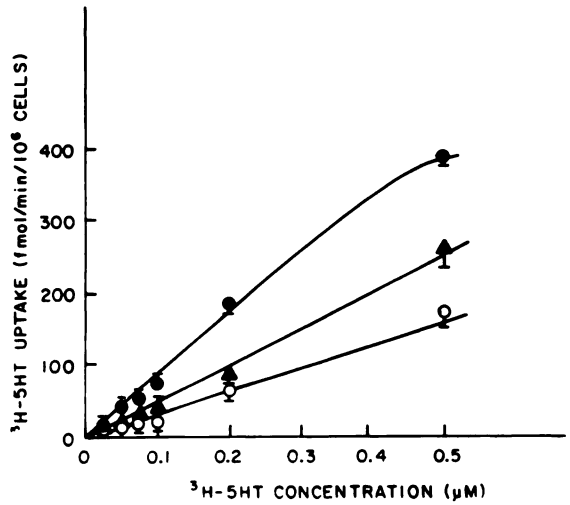

FIG. 2. *Apparent uptake of [^{3}H]5-HT in differentiated N-2a cells as a function of concentration*

Differentiated N-2a cells in suspension (10^6 cells/100 μl) were incubated for 2 min with increasing concentrations of [^{3}H]5-HT at 37° (●), 0–4° (○), or in sodium-free buffer at 37° (▲) as described under Materials and Methods.

37° (Fig. 2). The uptake at 37° was concentration-dependent, and the plot of velocity versus [^{3}H]5-HT concentration was a modified hyperbola similar to that previously described by Stahl and Meltzer (26). The uptake of [^{3}H]5-HT was greatly reduced at 0–4°. The amount of [^{3}H]5-HT accumulated from an external concentration of 0.1 μM at 0–4° was similar to that accumulated in the presence of 1 mM unlabeled serotonin (19.0 fmoles/min/10^6 cells compared with 16.0 fmoles/min/10^6 cells). The uptake at 0–4° was thus considered to be a linear, nonspecific component due to diffusion or other nonsaturable process. Therefore the values obtained at 0–4° were subtracted from the total uptake at 37° to give the active uptake. In the absence of external sodium the active uptake of [^{3}H]5-HT was inhibited by 60–74%.

Characteristics of [^{3}H]5-HT uptake in nondifferentiated N-2a cells. The velocity of uptake of [^{3}H]5-HT during the 2-min incubation was again determined over the concentration range 0.025–0.5 μM at 37°, 0–4°, and in sodium-free buffer at 37° (Fig. 3). The accumulation of [^{3}H]5-HT at 37° and at 0–4° was very similar to that in differentiated cells. However, in nondifferentiated cells the absence of external sodium had no significant effect on the uptake of [^{3}H]5-HT.

Lineweaver-Burk analysis. The velocity versus [^{3}H]5-HT concentration data shown in Figs. 2 and 3 were analyzed by constructing Lineweaver-Burk plots as shown in Fig. 4. Analysis of these plots gave an apparent K_m of 1.27 ± 0.6 μM and V_{max} of 780 fmoles/min/10^6 cells for uptake of [^{3}H]5-HT into differentiated cells and an apparent K_m of 0.96 ± 0.5 μM and V_{max} of 619 fmoles/min/10^6 cells for uptake of [^{3}H]5-HT into nondifferentiated cells.

Effect of metabolic inhibitors. The ability of iodoacetamide (5 mM), sodium azide (10 mM), and 2,4-dinitrophenol (1 mM) to block the accumulation of [^{3}H]5-HT (Table 1) indicates that the uptake is mediated by an active transport process. In differentiated cells ouabain (1 mM)

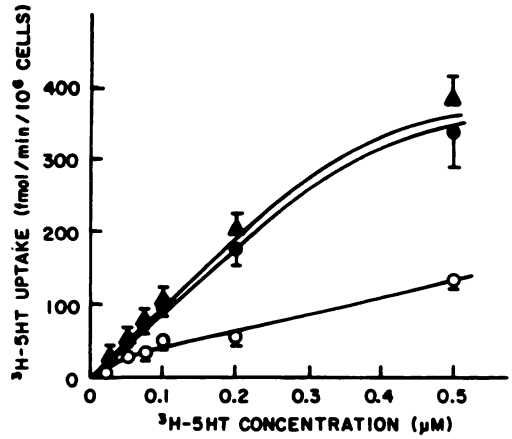

FIG. 3. *Apparent uptake of [^{3}H]5-HT in nondifferentiated N-2a cells as a function of concentration*

Nondifferentiated N-2a cells in suspension (10^6 cells/100 μl) were incubated for 2 min with increasing concentrations of [^{3}H]5-HT at 37° (●), 0–4° (○), or in sodium-free buffer at 37° (▲) as described under Materials and Methods.

also caused inhibition of uptake, but in nondifferentiated cells ouabain had no effect.

Effect of antidepressants. The ability of fluoxetine and DMI to inhibit [^{3}H]5-HT uptake was determined in differentiated and nondifferentiated N-2a cells (Table 2). The IC_{50} values of fluoxetine were comparable to those of DMI in both cell types. However, both drugs were less effective in nondifferentiated N-2a cells.

Specificity of uptake. The specificity of the 5-HT uptake system was determined by comparing the ability of unlabeled 5HT and NE to antagonize the uptake of [^{3}H] 5-HT (Table 2). In differentiated cells 5-HT was 16 times more effective than NE, whereas in nondifferentiated

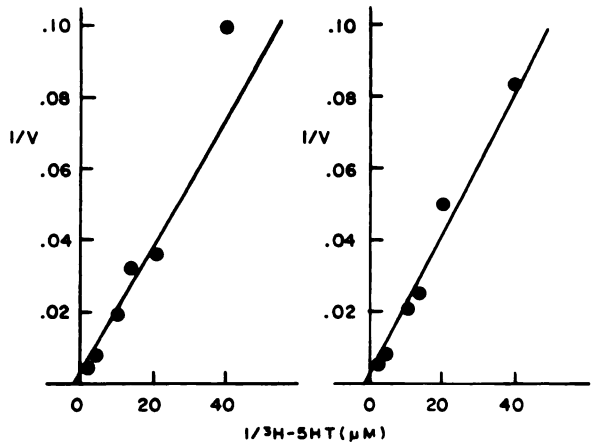

FIG. 4. *Lineweaver-Burk plots of the active transport of ^{3}H-5HT into differentiated (left) and nondifferentiated (right) N-2a cells*

Active uptake was determined by subtracting the accumulation at 0–4° from the total accumulation at 37°. The reciprocals of the velocity (V) of active uptake expressed as femtomoles of [^{3}H]5-HT per 10^6 cells per minute were plotted against the reciprocals of the external concentration of ^{3}H-5HT.

Left. Differentiated cells: K_m = 1.27 μM, V_{max} = 780 fmoles/min/10^6 cells.

Right. Nondifferentiated cells: K_m = 0.96 μM, V_{max} = 619 fmoles/min/10^6 cells.

TABLE 1

Effect of metabolic inhibitors on the uptake of [^{3}H]5-HT into differentiated and nondifferentiated N-2a cells

N-2a cells in suspension (10^6 cells/100 μl) were incubated with the metabolic inhibitors for 15 min at 37°. The cells were then washed with Buffer A and incubated with 0.1 μM[^{3}H]5-HT for 2 min. Results are expressed as percentage inhibition of active uptake.

Metabolic inhibitor	Concentration	% Inhibition of uptake	
		Differentiated	Nondifferentiated
	mM		
Iodoacetamide	5	60 ± 2	75 ± 3
Sodium azide	10	43 ± 4	50 ± 6
2,4-Dinitrophenol	1	51 ± 2	49 ± 4
Ouabain	1	29 ± 4	5 ± 8

cells they had a comparable effect. In both cells, complete inhibition was caused by 1 mM 5-HT and 1 mM NE.

Neurotoxic analogues of 5-HT and NE inhibit [^{3}H]5-HT uptake in differentiated N-2a cells but were less effective than 5-HT. Their order of potency was 5,6-DHT > 5,7-DHT > 6-OHDA.

Cytotoxicity of neurotoxic analogues. 6-OHDA was the most effective drug tested, causing considerable inhibition of [^{3}H]thymidine incorporation after 30 min and almost total inhibition after 90 min of incubation (Fig. 5). 5,6-DHT had a moderate effect after 30 min, but was equally as effective as 6-OHDA after 90 min incubation. 5,7-DHT had no significant effect after 30 min, and little effect even after 90 min incubation.

DISCUSSION

The term nondifferentiated is used to describe rapidly proliferating neuroblastoma cells, whether grown in suspension or monolayer culture. Although adaption to monolayer culture involves membrane reorganization and spontaneous morphological differentiation of a small proportion of cells, the major biochemical changes associated with differentiation occur after inhibition of cell growth (35–38). In this investigation serum deprivation was chosen as the method of inducing differentiation as

TABLE 2

Effect of antidepressants and unlabeled analogues on the uptake of [^{3}H]5-HT in differentiated and nondifferentiated N-2a cells

N-2a cells in suspension (10^6 cells/100 μl) were preincubated with either fluoxetine (0.5–50 μM) or DMI (0.5–50 μM) or unlabeled analogues added at the same time as [^{3}H]5-HT. Results are expressed as concentrations causing 50% inhibition of active uptake (IC_{50}).

Drug	IC_{50}	
	Differentiated	Nondifferentiated
	μM	*μM*
Fluoxetine	13.7	148
DMI	13.6	107
5-HT	0.6	7.9
NE	9.4	6.0
6-OHDA	53.8	
5,6-DHT	4.0	
5,7-DHT	22.6	

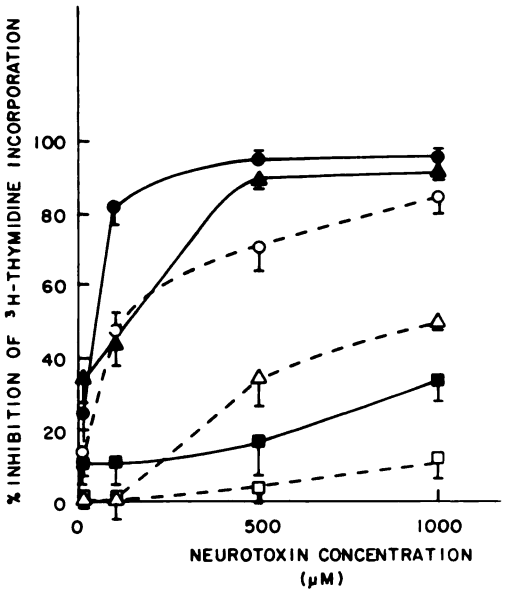

FIG. 5. *Cytotoxicity of 6-OHDA, 5,6-DHT, and 5,7-DHT toward differentiated N-2a cells*

Differentiated N-2a cells in suspension (10^6 cells/100 μl were incubated for 30 min (*open symbols*) or for 90 min (*closed symbols*) with 6-OHDA (○, ●), 5,6-DHT (△, ▲), or 5,7-DHT (□, ■). The cells were washed once with Buffer A, then incubated for an additional 90 min with [^{3}H]thymidine (0.5 μCi/500 μl). [^{3}H]Thymidine incorporation was terminated by TCA precipitation and filtration through glass-fiber filters, as described under Materials and Methods. Cytotoxicity was expressed as percentage inhibition of [^{3}H]thymidine incorporation.

it is more rapid and more effective at causing neurite extension than is dibutyryl cyclic AMP (39, 40), although the process is similar with reference to other biochemical markers of differentiation (9, 11, 41).

Differentiated neuroblastoma cells clone N-2a have been shown here to possess a mechanism for taking up serotonin which has many of the characteristics of the energy-requiring specific mechanism in serotonergic neurons (19–21). The uptake is saturable, temperature-dependent, and follows apparent Michaelis-Menten kinetics, with an apparent K_m of 1.27 μM. This K_m value was slightly higher than the values previously reported for neurons (19–22), platelets (23–27), and other tissues (28–32) (0.1–0.8 μM). The accumulation at 0–4° was considered to be due to passive diffusion or other nonsaturable, nonspecific processes. The [^{3}H]5-HT uptake was inhibited by metabolic inhibitors and ouabain. It was partially sodium-dependent, being inhibited by 60–74% in the absence of external sodium. Another characteristic of the active transport of 5-HT is its susceptibility to inhibition by low concentrations of antidepressant drugs. Fluoxetine is considered to be the most selective (IC_{50} 0.4–3 μM) (22, 31, 32), but DMI is also very effective (IC_{50} 0.7–50 μM) (20, 22, 24). In differentiated N-2a cells, both drugs were equally effective in inhibiting [^{3}H]5-HT uptake. [^{3}H]5-HT uptake in differentiated N-2a cells also showed structural specificity, since 5-HT analogues NE and 6-OHDA had some affinity for the 5-HT uptake mechanism, causing competitive inhibition of [^{3}H]5-HT uptake. 5-HT itself was the most effective inhibitor of [^{3}H]5-HT uptake into differentiated N-2a cells.

Nondifferentiated N-2a cells were also shown to accumulate [^{3}H]5-HT. The process was concentration-, temperature-, and energy-dependent, and the apparent K_m of uptake was 0.96 μM. However, the V_{max} for uptake into the nondifferentiated N-2a cells was less than that observed with the differentiated cells, and there were several striking differences in the characteristics and selectivity of the uptake process. The uptake into nondifferentiated cells was not sodium-dependent, not inhibited by ouabain, and less effectively inhibited by antidepressant drugs. The uptake process was not selectively inhibited by unlabeled 5-HT when compared with NE.

The 5-HT carrier in neuronal tissue is proposed to have two binding sites (23, 28). Binding of sodium to one site is believed to increase the affinity of the other site for 5-HT. The relatively low intracellular sodium concentration causes displacement of the sodium by potassium, leading to decreased affinity for the serotonin which then dissociates. An essential feature of this process is maintenance of the sodium pump. Thus in the presence of ouabain or the absence of external sodium this carrier becomes nonfunctional. The results of this investigation suggest that this sodium-dependent carrier is present in N-2a cells only after differentiation. This could be related to the higher levels of Na^+,K^+-ATPase in differentiated cells (37) or the appearance of a group of glycoproteins, which may be associated with the Na^+ channel (42).

Dihydroxytryptamines are widely used for the chemical lesioning of central serotonergic neurons, in a manner similar to 6-OHDA and noradrenergic neurons (43). Their relatively selective action is due to uptake by specific neuronal pumps. Their toxicity is nonselective and probably related to their rapid autoxidation. There are two proposed mechanisms of neurotoxicity, one involving production of oxidative radicals and hydrogen peroxide and the other the covalent reaction of the quinoid-like reaction product with cell constituents (34). For technical reasons the effect of prolonged treatment with 6-OHDA and 5,6-DHT on the serotonin uptake system in N-2a cells could not be determined. [^{3}H]5-HT was found to bind to the insoluble, colored reaction products generated by oxidation of 6-OHDA and 5,6-DHT, and it was not possible to determine how much of the radioactivity in the samples was due to this nonspecific binding. Preincubation with 5,7-DHT for 90 min caused inhibition of [^{3}H]5-HT uptake, but this was not an early event as it paralleled the cytotoxic effect (results not shown). 6-OHDA and 5,6-DHT were similarly effective at causing loss of cell viability, although 6-OHDA was more rapidly effective. This more rapid onset of toxicity with 6-OHDA is probably due to the fact that, although 5,6-DHT has a greater affinity for the uptake receptor, 6-OHDA oxidizes more rapidly (4.4 nmoles of O_2 per minute compared with 2.7) (44). Despite the fact that 5,7-DHT has a higher affinity for the 5-HT receptor than 6-OHDA and oxidizes more rapidly than 5,6-DHT (44) (33 nmoles of O_2 per minute), it had very little cytotoxic effect. These observations further confirm the idea that 5,7-DHT acts by a cytotoxic mechanism different from that of 6-OHDA and 5,6-DHT. The mechanism of cytotoxicity for 5,7-DHT is not clearly understood, as it does not generate H_2O_2 or cause protein cross-linking (44, 45).

There is considerable evidence that the 5-HT and NE

uptake systems are not completely specific, so that 5-HT and its analogues can inhibit NE uptake (43, 45) and vice versa (22,). The noradrenergic transport system is also capable of taking up 5-HT by a low-affinity process with an apparent K_m of 8 μM (21). This may explain the inhibitory effect of DMI and the cytotoxic effect of 6-OHDA. However, N-2a cells have been reported to be a mixed population containing both adrenergic and serotonergic cells (17). Therefore, in differentiated N-2a cells, we may be seeing [^{3}H]5-HT accumulation by two active processes, a high-affinity serotonin carrier and a low-affinity noradrenergic carrier. This may explain why the observed uptake has an apparent K_m higher than usually reported and why 6-OHDA has such potent cytotoxic action.

From the results of this investigation it is clear that differentiated neuroblastoma cells clone N-2a may be used as a model of the serotonergic neuron for the study of 5-HT neurotoxins.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support provided by the Center for Biomedical Research, University of Kansas.

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