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UPTAKE AND BINDING OF DOPAMINE AND 6-HYDROXYDOPAMINE IN MURINE NEUROBLASTOMA AND FIBROBLAST CELLS

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Abstract—The uptake and binding of radioactive 6-hydroxydopamine (6-OH-DA) and radioactive dopamine (DA) were investigated in an adrenergic clonal line of neuroblastoma, N1E-115, derived from the murine tumor C-1300. Monolayer cultures of N1E-115 cells accumulated four times more 6-OH-DA than DA. Reserpine inhibited the uptake of DA, but not that of 6-OH-DA. When uptake was followed by a release period, these cells retained a much greater proportion of 6-OH-DA than DA. Determination of protein binding showed that 1300 pmoles/mg of protein of 6-OH-DA or its oxidation products was bound, compared to 120 pmoles/mg of protein of DA. Disc gel electrophoresis of the cellular proteins revealed an increase in the proportion of high molecular weight protein in cells incubated with 6-OH-DA when compared to proteins from cells treated with DA. The high molecular weight proteins contained bound 6-OH-DA. In a murine L-cell, B82, the uptake of both 6-OH-DA and DA was considerably less than in the neuroblastoma cell N1E-115. The percentage of radioactivity bound to protein in 6-OH-DA-treated cells was similar in both cell types and represented about 20 per cent of the total radioactivity. The results suggest that the cytotoxicity of 6-OH-DA results from irreversible covalent binding of one of the oxidation products of 6-OH-DA to cellular proteins with concomitant protein-crosslinking.

6-Hydroxydopamine (6-OH-DA) exerts a selective cytotoxic effect on adrenergic neurons in both the peripheral sympathetic and central nervous systems. Cytotoxicity apparently results when 6-OH-DA is accumulated in high concentrations within these cells, and undergoes oxidation and covalent binding to cellular proteins (for reviews, see Refs. 1–3). In the present studies, an adrenergic clonal line of neuroblastoma, N1E-115 [4], derived from the murine tumor C1300 [5], was used as a model system to investigate the interaction of 6-OH-DA and/or its oxidation products with neuronal proteins. The C1300 tumor is presumed to originate from a sympathicoblast cell, and the cell lines derived from it might be expected to exhibit a sensitivity to 6-OH-DA similar to that expressed by developing adrenergic neurons. The N1E-115 clone and certain other cell lines obtained from the C1300 tumor possess properties typical of adrenergic neurons (for reviews, see Refs. 6 and 7). These include electrical excitability [8], extensive neurite formation [4], high activities of tyrosine hydroxylase [4] and dopamine β-hydroxylase [9], and large dense core vesicles (M. Daniels and M. Nirenberg, unpublished data). Uptake of dopamine (DA), the conversion of DA to norepinephrine and catecholamine storage in N1E-115 cells are inhibited by reserpine, and thus probably associated with storage vesicles [10]. If binding of 6-OH-DA and/or its oxidation products occurs preferentially to particular protein species in adrenergic neuronal cells, it is probable that these proteins will also be found in N1E-115 cells.

Previous studies have indicated that 6-OH-DA may have a differential cytotoxic effect on murine neuroblastoma versus other cell types. Thus, Angeletti and Levi-Montalcini [11] showed that exposure to a 1×10^{-4} M concentration of 6-OH-DA for 24 hr caused extensive death of neuroblastoma cells, but had no apparent effect on HeLa or murine sarcoma cells. Prasad [12] observed a 50 per cent inhibition of increases in neuroblastoma cell numbers after 48 hr of exposure to 5×10^{-5} M 6-OH-DA; similar inhibition of Chinese hamster ovary (CHO-K) and baby hamster kidney (BHK-21) cells occurred only with at least 2-fold higher concentrations of amine. DA under similar conditions at 10-fold higher concentrations inhibited neuroblastoma growth by 50 per cent, CHO-K growth by only 20 per cent and BHK-21 growth not at all. In the present report, we present evidence that 6-OH-DA accumulates in cultures of N1E-115 and undergoes such extensive covalent binding to cellular proteins that the distribution and character of the proteins of this cell line are actually altered.

METHODS AND MATERIALS

Cell culture. The adrenergic neuroblastoma clone N1E-115 [4] used in these studies was kindly provided by Dr. M. W. Nirenberg. This cell line was originally obtained as a single cell clone from an uncloned line of the murine neuroblastoma C1300. Murine L-cells B82 [13] were also used in some experiments. Cells were maintained routinely in monolayer culture in the Dulbecco–Vogt modification of Eagle's

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medium (DMEM) free from contamination by mycoplasma, as previously described [14].

For uptake experiments cells were suspended by trituration and plated at a density of 10^5 cells/35-mm well (7 cm^2) in six well plates (Linbro Chemical Co.) using DMEM medium containing 3% fetal calf serum (Colorado Serum Co.). Cells were fed at 2- to 3-day intervals and used after approximately 1 week of culture. At this time, the monolayer was approximately 80 per cent confluent and the cells had formed neurites several hundred microns in length.

Isotopic measurements of uptake and release. Immediately upon removal from the incubator, monolayers were rinsed three times with an isotonic, modified Dulbecco's phosphate-buffered saline containing 129 mM NaCl, 2·5 mM KCl, 7·4 mM Na_2HPO_4, 1·3 mM KH_2PO_4, 0·63 mM $CaCl_2$, 0·74 mM $MgSO_4$, 5·3 mM glucose, 46 mM sucrose, 0·1 mM ascorbic acid and 0·2 mM nialamide (Sigma), pH 7·2, 330 mOsm. This buffer was prepared fresh daily by adding the last four components (stored frozen) to the salt solutions. Cells were incubated in 0·3 to 0·5 ml of this buffer per well. In some cases, reserpine (Sigma) was added at a final concentration of 2×10^{-5} M. All experiments were performed in air at 36°. [^{3}H(G)]3,4-dihydroxyphenethylamine (DA) (New England Nuclear) was presented to the cells at final concentrations of $1{\cdot}4 \times 10^{-4}$ M, and specific activities of 14–100 μCi/μmole. [^{3}H]6-OH-DA was presented at 1 to $5{\cdot}5 \times 10^{-4}$ M, 10–30 μCi/μmole. After various intervals, monolayers were washed rapidly four times with 2-ml aliquots of ice-cold buffer containing no calcium. Uptake was determined by drying the monolayers at 50° for 20 min, dissolving the cells in a volume of 0·2 N NaOH equivalent to the incubation buffer and counting 100-μl aliquots of the cell digests in 1 ml of 1 M Tris HCl, pH 7·4 and 10 ml of a scintillation mixture consisting of 166 ml RPI Scintillator (New England Nuclear), 834 ml toluene and 1000 ml Triton X-100. This procedure was modified from Catterall and Nirenberg [15]. For release studies, 1-ml aliquots of fresh buffer were added to each well after washing. Release incubations were continued at 36° or as otherwise indicated for additional intervals as specified in the text. The buffer was then removed and the radioactivity retained by the cells was determined without further washing. Protein determinations were performed on cell digests by the method of Lowry *et al.* [16] using bovine serum albumin as a standard.

Preparation of [^{3}*H*]*2,4,5-trihydroxyphenethylamine (6-OH-DA).* [^{3}H]6-OH-DA was prepared by reduction of 2,4,5-tribenzyloxynitrostyrene [17] with [^{3}H]$NaBH_4$ (New England Nuclear; 3·8 mCi/mg) followed by simultaneous reduction of the nitro group to an amine and removal of the protecting benzyl groups by catalytic hydrogenation. Small amounts of the product were purified prior to use by ascending chromatography on Whatman No. 1 paper in *n*-butanol–2 N HCl (1:1). The radioactivity migrating with authentic 6-OH-DA was eluted with methanol and the solvent removed under nitrogen [18].

Subcellular distribution of radioactivity. After uptake of either 6-OH-DA or DA for 60 min, cells were washed with isotonic saline buffered at pH 7·2 with 0·01 M sodium phosphate buffer, transferred to a small (2 ml) conical glass homogenizer and sedimented by centrifugation. The sedimented cells were then homogenized with a motor-driven glass pestle in a final volume of 0·5 ml buffered saline, and this homogenate was centrifuged at 100,000 *g* for 60 min, yielding a supernatant and a sediment fraction. The sediment fraction was subjected to repeated homogenizations in 12% trichloroacetic acid (TCA), 0·5 ml, and centrifugation at 20,000 *g* for 30 min, until no further radioactivity could be extracted. The supernatant fraction was brought to a final concentration of 12% TCA and the precipitated protein was treated in the same manner as the sediment fraction. In both cases, the radioactivity remaining in the TCA-insoluble sediment was considered to be "protein-bound." The TCA-insoluble proteins were digested with 0·2 ml of a mixture of two parts H_2O_2 (30%) and one part perchloric acid (60%) in a counting vial at 70° for 4 hr and cooled to −20°. Radioactivity was determined in 10 ml of scintillation fluid (Aquasol: New England Nuclear) and corrected for counting efficiency by the addition of internal standards.

The distribution of protein and radioactivity in the supernatant fraction was also examined by gel chromatography on a column of Sephadex G-200 (superfine, 1 × 50 cm) in buffered saline. The column was calibrated with protein standards with molecular weights ranging from 24,000 to 153,000 and the void volume with Dextran blue 2000 (Pharmacia) with a mean molecular weight of 200,000. Protein was estimated by continuous monitoring of the absorption at 280 μm of the effluent, which was collected in 0·5-ml fractions controlled by a drop-counter. The fraction of the radioactivity which eluted from the column at a volume of eluent equal to or greater than proteins with a molecular weight of 24,000 was considered to be protein-bound. 6-OH-DA or DA alone, or with bovine serum albumin added as carrier (200 μg), migrated on the column exclusively with the small molecule fraction equivalent to a molecular weight of 2000 or less.

Electrophoresis of cellular proteins on acrylamide gel. After uptake of either 6-OH-DA or DA, cells were washed and transferred with buffered saline to a plastic centrifuge tube (5 ml) and sedimented by centrifugation. The cells were suspended in 1·0 ml of 0·01 M phosphate buffer, pH 7·0, containing 1% sodium dodecysulfate (SDS) and sonicated with a narrow probe for three successive intervals of 60 sec (Ultrasonic Inc. model 185, equipped with a microtip). Liquid temperatures were maintained at 1–5° during sonication. The sonicated material was then centrifuged at 100,000 *g* for 60 min. Aliquots of this supernatant were chromatographed on 5 and 7% polyacrylamide gels with a 3% acrylamide stacking gel according to the procedure of Neville [19]. The gels were stained with Coomassie blue and destained against activated charcoal for 5 days with a mixture of glacial acetic acid (40 ml)–methanol (150 ml) and water (1810 ml). The linear density of stain distribution was determined with a gel scanner attached to a Beckman Acta V spectrophotometer. Gels were cut into 1- to 2-mm cross sections, digested in H_2O_2–perchloric acid (see above) and the tritium content was measured. Control experiments were performed by adding equivalent amounts of 6-OH-DA to suspensions of untreated

cells just prior to the sonication procedure. Such additions did not alter the appearance of the protein bands on the gel.

RESULTS

Uptake of radioactive DA and 6-OH-DA by N1E-115 cells was compared over a 2-hr incubation period (Fig. 1). The amount of 6-OH-DA uptake was substantially higher than that of DA over the entire time period. In the depicted experiment the rate of uptake for 6-OH-DA was 8-fold higher than the rate of uptake for DA during the first 15 min. Accumulation of 6-OH-DA continued throughout the 2-hr incubation, whereas the uptake of DA plateaued after 60–90 min. In nine separate experiments the uptake of 6-OH-DA was an average of 4·1-fold ± 2·0 (S. D.) higher than that of DA after 60 min of uptake when these compounds were present at 10^{-4} M.

Neuroblastoma cells exposed to either DA or 6-OH-DA for 2 hr appeared by phase microscopic examination to be morphologically identical to control cells. Experiments on uptake of 6-OH-DA at varying substrate concentrations suggested that uptake and/or binding occurs by diffusion rather than by action of a high affinity system (Fig. 2). The effect of reserpine, a blocker of vesicular amine uptake, on 6-OH-DA and DA uptake was assessed in four separate experiments. Uptake of 6-OH-DA at 10^{-4} M for 60 min was not inhibited by 2×10^{-5} M reserpine, whereas DA uptake under these conditions was depressed 36 ± 8·0 per cent (S. D.). Reduction of the incubation temperature from 36° to 2° reduced the accumulation of DA over a 60-min period by approximately 50 per cent, while that of 6-OH-DA was reduced only 15 per cent. In one competition experiment the accumulation of radioactive 6-OH-DA ($2{\cdot}3 \times 10^{-4}$ M) over a 15-min period was inhibited 45 per cent by 10^{-3} M DA, while accumulation of radioactive DA ($2{\cdot}3 \times 10^{-4}$ M) was not inhibited by 10^{-3} M 6-OH-DA.

Murine L-cells, B82, accumulated only 40 per cent as much DA and 50 per cent as much 6-OH-DA as N1E-115 cells during a 60-min uptake period with

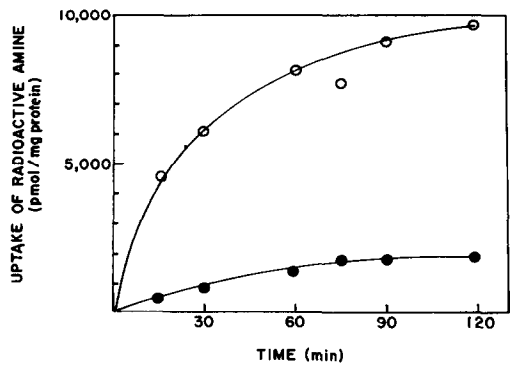

Fig. 1. Uptake of radioactive amines into N1E-115 cells. Monolayer cultures were exposed to 6-OH-DA, 22 μCi/μmole, 10^{-4} M (○——○), and DA, 100 μCi/μmole, 10^{-4} M (●——●) for 15–120 min in 0·6 ml buffer/well. Zero time incubations for both compounds under similar conditions gave values of < 100 pmoles/mg of protein. The values are from individual wells run in parallel in a single experiment, similar findings were observed in two other experiments. For further details, see Methods and Materials.

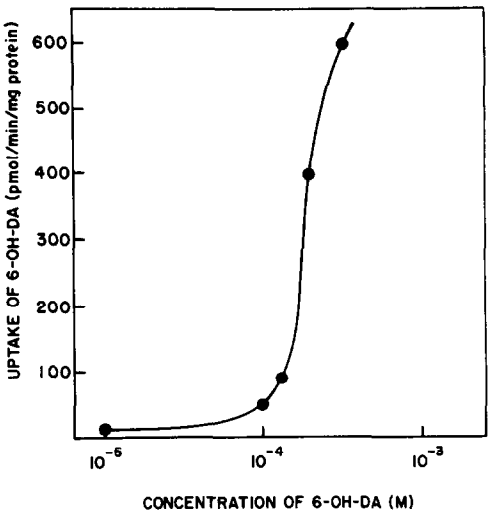

Fig. 2. Uptake of 6-OH-DA by N1E-115 cells as a function of substrate concentration. Rates were determined after 15-min periods of uptake; each circle represents a determination from an individual well in separate experiments. For further details, see Methods and Materials.

the amines present at 10^{-4} M (Table 1). These L-cells, like the neuroblastoma cells, accumulated about four times as much 6-OH-DA as DA. In another experiment, the L-cells accumulated only one-sixth as much 6-OH-DA as neuroblastoma cells, and accumulation of DA was minimal (Table 2).

The ability of cells to retain radioactive 6-OH-DA and DA after uptake was assessed by exposing them for equal time periods to buffers lacking these compounds. In six separate experiments N1E-115 cells, exposed to sequential 60-min uptake and release periods, retained an average of 72 ± 8·2 per cent (S. D.) of the radioactivity derived from 6-OH-DA, and only 37 ± 8·8 per cent (S. D.) of the radioactivity derived from DA (Table 1). The rate of release of radioactivity was similar for both compounds, being most rapid over the first 20 min and then slowing considerably. When cells were exposed to only 5 min of uptake and then 60 min of release, approximately 37 per cent of label associated with both 6-OH-DA and DA was retained, indicating that the cumulative "tight" binding of 6-OH-DA is a time-dependent process. When reserpine was present during the 60-min uptake and the subsequent 60-min release period, the final retention of 6-OH-DA was not altered; in contrast, more than 90 per cent of the radioactivity derived from DA was released during incubations with reserpine, compared to only about 60 per cent (see above) during incubations without reserpine. When cells were labeled with radioactive 6-OH-DA at 4×10^{-4} M for 60 min and then incubated in the presence of a 4×10^{-3} M concentration of either nonradioactive 6-OH-DA or DA for a subsequent 60-min period, they retained only 40–50 per cent as much radioactivity as that retained in controls post incubated in buffer alone. However, cells, labeled with 4×10^{-4} M radioactive DA did not release additional radioactivity due to the presence of 4×10^{-3} M 6-OH-DA during the release period and released only an additional 22 per cent in the presence of 4×10^{-3} M DA. The B82 cells retained 61 per cent

Table 1. Uptake and retention of 6-OH-dopamine and dopamine in N1E-115 and B82 cells

Cell line	Uptake (pmoles/mg protein)*		Retention (% retained)†	
	Dopamine	6-OH-Dopamine	Dopamine	6-OH-dopamine
N1E-115	940 (4)‡	3500 (4)	37 (6)	72 (6)
B82§	390 (1)	1800 (1)	28 (1)	61 (1)

* Total uptake was determined after 60 min of incubation with 10^{-4} M radioactive DA or 6-OH-DA.

† Percent of radioactivity retained per mg of protein was calculated from values after 60 min of uptake, washing and 60 min of release, as compared to values after 60 min of uptake.

‡ Values given are an average of the number of experiments given in parentheses.

§ Cells from six wells were used for each amine.

Table 2. Irreversible binding of radioactivity in 6-hydroxydopamine- and dopamine-treated N1E-115 and B82 cells

Cell line	Total uptake* (pmoles/mg protein)		% Bound†	
	Dopamine	6-Hydroxydopamine	Dopamine	6-Hydroxydopamine
N1E-115	3588	6052	3	21
B82	51	940	10‡	17

* Total uptake determined after 60 min of incubation with 10^{-4} M radioactive 6-OH-DA or DA.

† TCA-insoluble material (see Methods and Materials).

‡ In view of very low levels of radioactivity in TCA-insoluble material, this number is subject to considerable error.

of 6-OH-DA and 28 per cent of DA after 60 min of uptake and 60 min of release. These values are similar to those observed in the neuroblastoma cells.

The extent of covalent binding of radioactivity in N1E-115 and B82 cells was examined 60 min after presenting the cell cultures with radioactive 6-OH-DA or DA. Results presented in Table 2 are based on the distribution of radioactivity into TCA-soluble and -insoluble fractions. The amount of covalently bound 6-OH-DA or oxidation products derived from 6-OH-DA was 10-fold higher in the N1E-115 than in the B82 cell line, but the percentage bound was similar in both cell types. The percentage of covalently bound DA was much less than that of 6-OH-DA in both cell lines.

The subcellular distribution of free and bound radioactivity was examined in homogenates of N1E-115 cells labeled with 6-OH-DA or DA (Table 3). In the DA-labeled cells virtually all (> 90 per cent) of the radioactivity was present in the 100,000 *g* supernatant fraction. Nearly none of the radioactivity in fractions from DA-labeled cells was protein bound. In contrast, with cells labeled with an equivalent amount of 6-OH-DA, only one-half was present in the 100,000 *g* supernatant fraction. With 6-OH-DA both the supernatant and sediment fractions retained a significant portion of the radioactivity in a protein-bound form (Fig. 3 and Table 3).

Table 3. Distribution of protein-bound and free radioactivity in 6-hydroxydopamine- and dopamine-treated N1E-115 neuroblastoma cells*

Amine	Fraction†	Free (%)	Bound (%)
6-Hydroxydopamine	Supernatant	47·2	7·6
	Sediment	31·8	13·3
Dopamine	Supernatant	89·2	2·0
	Sediment	7·4	1·4

† The supernatant fraction represents the supernatant from the 100,000 *g* centrifugation, while the sediment fraction represents the pellet from this centrifugation (see Methods and Materials).

* "Free" radioactivity refers to that percentage of the total which is not precipitated by TCA, while "bound" radioactivity represents that associated with TCA-precipitated proteins.

Examination of proteins from 6-OH-DA- and DA-labeled N1E-115 cells by SDS-acrylamide gel electrophoresis revealed significant differences in the electrophoretic profile (Fig. 4). In the SDS-soluble proteins from 6-OH-DA-labeled cells a large increase in high molecular weight proteins was readily apparent. Greater than 90 per cent of the "bound" radioactivity derived from 6-OH-DA was associated with these high molecular weight proteins. Electrophoresis of suspensions of the sediment fraction from SDS-sonicated cells revealed a protein and radioactivity profile similar to that of the soluble fraction except that the higher molecular weight components remaining in the stacking gel represented a much greater proportion of the total protein (results not shown). With the exception of the high molecular weight proteins, the profiles on gel electrophoresis were quite similar with proteins from either 6-OH-DA-treated, DA-treated or untreated cells. These proteins, with molecular weights ranging from about 20,000 to 150,000, were associated with low, but significant amounts of radioactivity in 6-OH-DA-labeled cells, while with DA-treated cells no radioactivity was detected. A narrow

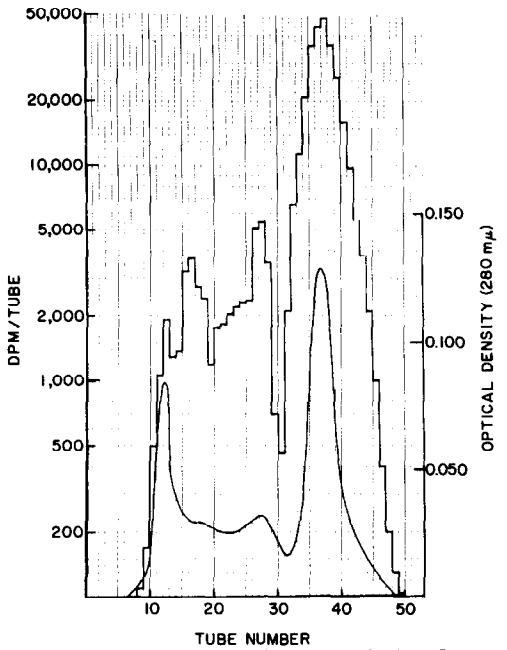

Fig. 3. Column chromatography on Sephadex G-200 of the soluble protein fraction from N1E-115 cells treated with radioactive 6-OH-DA. Protein concentration is shown by the continuous line indicating the absorption of 280 μm. Distribution of tritium is shown by the histogram, with quantitation shown on the log scale. For further details, see Methods and Materials.

band of radioactivity was present at the electrophoretic front with preparations from both 6-OH-DA- and DA-treated cells. Control gel electrophoresis of 6-OH-DA or DA alone or in the presence of proteins from untreated cells gave rise to a similar band of radioactivity at the front. Electrophoresis of larger amounts of radioactive 6-OH-DA or DA gave rise to a sharp, brown-colored band with an estimated molecular weight of 2000.

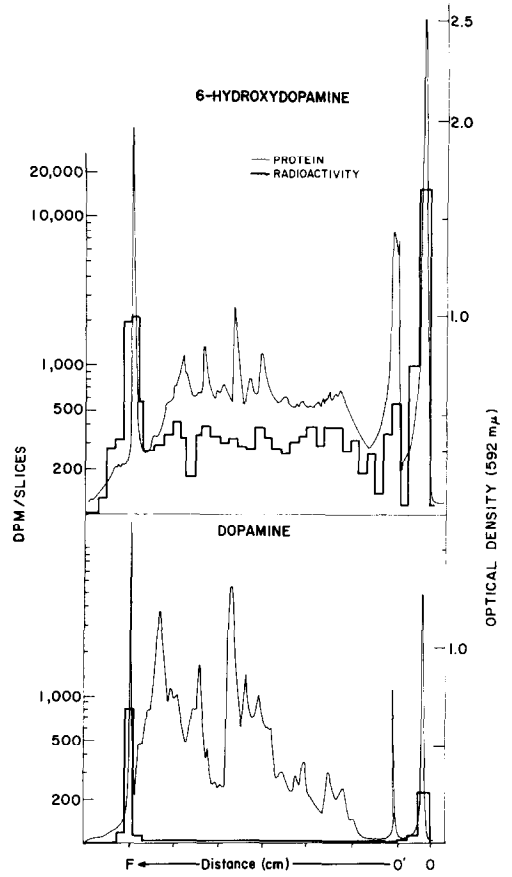

Fig. 4. Representative electrophoretic patterns for the soluble protein fraction from N1E-115 cells treated with radioactive 6-OH-DA or DA. Proteins were pretreated with SDS and chromatographed on 5% polyacrylamide gel, 0'–F, with a 3% stacking gel 0–0' and stained with Coomassie blue. The continuous line indicates absorption at 592 μm and the distribution of tritium is shown by the histogram. For details, see Methods and Materials.

DISCUSSION

The uptake and binding of radioactive 6-OH-DA and DA have now been evaluated in monolayer cultures of the adrenergic neuroblastoma clone, N1E-115, and in the L-cell line, B82. N1E-115 cells accumulated nearly 4-fold more 6-OH-DA than DA during 15-min to 2-hr incubations. Both 6-OH-DA and DA were presented to the cells at a relatively high concentration of 10^{-4} M, as previous studies indicated that N1E-115 lacks a high affinity uptake system for catecholamines (unpublished observations). The accumulation of DA in N1E-115 cells plateaued after about 90 min of uptake, whereas that of 6-OH-DA continued for at least 120 min. Uptake of DA was inhibited by reserpine, whereas uptake of 6-OH-DA was not. Furthermore, when uptake was followed by a release period, N1E-115 cells retained a much greater proportion of the 6-OH-DA radioactivity compared to that of the DA. However, radioactive 6-OH-DA was more readily displaced by high concentrations of either 6-OH-DA or DA during the release period than was radioactive DA.

Preliminary studies using B82 cultures showed that they also accumulated at least four times as much 6-OH-DA as DA over a 60-min period and retained a greater proportion of the former substance over a 60-min release period. However, these L-cells accumulated much less of either 6-OH-DA or DA than did the neuroblastoma cells.

It is apparent from these studies that N1E-115 cells accumulate and/or bind substantially more 6-OH-DA than DA. The lack of effect by reserpine on the accumulation or retention of 6-OH-DA suggests either that storage of 6-OH-DA in the dense core vesicles is not necessary for the retention of 6-OH-DA or that reserpine does not block the uptake or retention of 6-OH-DA by these vesicles. Certainly the reduction in the cellular accumulation or retention of DA in the presence of reserpine suggests a role for these dense core vesicles in the retention of DA. The nature of the transport of these amines into this neuroblastoma cell line is unknown. However, the lack of significant reduction in the ability of cells to accumulate 6-OH-DA at low temperatures and the only partial reduction in accumulations of DA at low temperatures strongly suggest that the accumulation of catecholamines occurs mainly by passive diffusion through the membrane. The partial reduction in DA

uptake at low temperatures may be due to effects on vesicular uptake or storage. The lack of apparent saturation of uptake of 6-OH-DA (Fig. 2) and of DA (unpublished results) is conconant with a passive diffusion process. The uptake of 6-OH-DA was partially inhibited by the presence of DA, while the uptake of DA was not affected by 6-OH-DA. From the present investigations, it would appear that the accumulation of 6-OH-DA is less temperature dependent, but more readily inhibited by the presence of another amine than is the accumulation of DA. Irreversible binding of 6-OH-DA or its oxidation products to cellular proteins should, however, in these cells result in a form of facilitative transport. In a similar manner, the retention of DA by dense core vesicles should result in facilitative transport of DA. Radioactive 6-OH-DA itself appeared to be more readily displaced from the cell by a catecholamine than was the radioactive DA.

Since N1E-115 cells do not possess a high affinity uptake process for DA or norepinephrine, it was necessary to use high concentrations (10^{-4} M) of DA and 6-OH-DA to study incorporation into these cells. The uptake of 6-OH-DA at 10^{-4} M into N1E-115 cells was in all experiments at least twice that obtained with B82 cells. It is noteworthy that a 2-fold increase in the concentration of 6-OH-DA was also found necessary to achieve the same level of cytotoxicity in fibroblast cell lines as compared to the more sensitive neuroblastoma lines [11, 12]. However, any comparison of the present observations on the binding of 6-OH-DA with such cytotoxicity studies should be made with caution since "nonadrenergic" neuroblastoma lines were used in the earlier studies [11, 12] and the fibroblast lines were, in all cases, from different sources. A recent paper provides further data on the greater sensitivity of neuroblastoma cells to 6-OH-DA as compared to L-cells [20].

In the present studies, it is clearly evident that although both neuroblastoma and fibroblast cell lines accumulate 6-OH-DA, the total amount of radioactivity accumulated within the cell and the total amount present in a protein-bound form are much higher in the neuroblastoma line. However, the percentage of bound radioactivity is similar in both cell types. The small amount of irreversibly bound DA in the two cell lines is probably due to binding of oxidation products of this catecholamine. Maguire *et al.* [21] have recently reported the irreversible binding of oxidation products of norepinephrine to cell particulates from glioma cells. Covalent binding of 6-OH-DA in N1E-115 cells was manifest both in soluble proteins of the 100,000 *g* supernatant fraction and in the 100,000 *g* sediment fraction. Presumably the former fraction contains mainly cytoplasmic proteins, while the latter contains mainly membrane proteins. In both fractions, radioactivity from 6-OH-DA is primarily bound to high molecular weight proteins. Indeed, a significant increase in the proportion of high molecular weight proteins is evident in the soluble fraction from 6-OH-DA-treated cells, sonicated in SDS-containing buffer (Fig. 4). Aerobic reaction of 6-OH-DA with bovine serum albumin and other model proteins has recently been found to result in covalent binding of 6-OH-DA and extensive polymerization of the model proteins to form high molecular weight complexes [22]. Thus, it is tempting to speculate that the mechanism of cytotoxicity of 6-OH-DA and related compounds [23] in sensitive cells results from the ability of such compounds to cross-link proteins. Further studies on the intracellular localization of radioactive 6-OH-DA and correlation of covalent binding to cytotoxicity in various cultured cell lines and atrial tissue are in progress.

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