

# Are SH-SY5Y and MN9D Cell Lines Truly Dopaminergic?

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**Abstract.** SH-SY5Y and MN9D cell lines are commonly used dopaminergic models in studies related to neurotoxicity, oxidative stress, and neurodegenerative diseases. Early studies suggested that SH-SY5Y cells do not convert intracellular DA to NE even though high levels of D $\beta$ M are present in these cells. In contrast, our studies show that these cells do not store substantial levels of DA or NE, but extracellular DA is taken up and converted to NE efficiently. The efficiency of DA uptake and its conversion to NE increase with the number of cell passages. Kinetic studies show that NE is a better substrate for plasma membrane transporters than DA. In contrast to undifferentiated cells, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) differentiated SH-SY5Y cells store substantially higher levels of NE. These cells take up DA and NE more efficiently than undifferentiated cells. Therefore, differentiated and undifferentiated high passage SH-SY5Y cells could be used as a noradrenergic, but not as a dopaminergic model. MN9D cells store high levels of DA under normal growth conditions, but do not convert DA to NE. They show poor catecholamine uptake characteristics compared to undifferentiated SH-SY5Y cells, however *n*-butyric acid differentiated MN9D cells show efficient DA uptake kinetics similar to undifferentiated SH-SY5Y, suggesting that they could be used as a reasonable dopaminergic model.

## 1. Introduction

The human neuroblastoma cell line, SH-SY5Y, is a subclone of the parent cell line SK-N-SH which was originally established from a bone marrow biopsy of a neuroblastoma patient. Original studies by Biedler, *et al.* have reported that these cells do not convert intracellular dopamine (DA) to norepinephrine (NE) at a detectable rate, although substantial levels of dopamine- $\beta$ -methyltransferase (D $\beta$ M) are present [1]. Based on this information, undifferentiated SH-SY5Y cells have been extensively used as an *in vitro* model for central nervous system (CNS) dopaminergic neurons in numerous studies. However, several recent studies have also used these same cells as a noradrenergic model to examine the molecular details of NE release from noradrenergic neurons [2]. Therefore, its characterization as a model of CNS dopaminergic neurons remains highly questionable.

The MN9D cell line derived from the fusion of mice embryonic ventral mesencephalic and neuroblastoma cells has recently gained popularity as a model of CNS dopaminergic neurons. This cell line is also shown to express high levels of the catecholaminergic enzyme tyrosine hydroxylase and synthesizes stores and releases DA [3]. However, it has also been poorly characterized with respect to uptake, storage, and metabolism of DA and other catecholamines.

To justify the continued use of SH-SY5Y and MN9D cell lines as reasonable CNS models, their characteristics with respect to catecholamine metabolism must be clearly defined. In the present study, we have carried out a systematic study of these two cell lines with respect to catecholamine uptake, storage, and metabolism under a variety of conditions using both undifferentiated and differentiated cells.

## 2. Experiments, Results, Discussion and Significance

**Cell Culture:** Human SH-SY5Y neuroblastoma cells (CRL-2266) and MN9D cells were grown to 80%-90% confluence in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose (4500 mg/L) supplemented with 10% fetal calf serum, 50  $\mu$ g/ml streptomycin and 50 IU/ml penicillin (Gibco) at 37°C and 5% CO<sub>2</sub>.

**Differentiation of SH-SY5Y and MN9D Cells:** The neuroblastoma SH-SY5Y cells were differentiated using 16 nM 12-O-tetradecanoyl-phorbol-13-acetate (TPA) according to the established literature procedures [2]. MN9D cells were differentiated using 1 mM *n*-butyric acid. Media was changed on the 3<sup>rd</sup> day. All the differentiated cells were used in appropriate experiments after the 6<sup>th</sup> day of differentiation.

**Intracellular Catecholamine Analysis:** The intracellular catecholamines NE, DA, DOPAC and HVA were quantified using high-performance liquid chromatography with electrochemical detection (HPLC-EC). Concentrations of individual

catecholamines were determined using standard curves constructed with authentic samples based on peak heights under same came experimental conditions. All catecholamine concentrations were normalized to the protein content of each sample.

*DA/NE Uptake Kinetics and Time Courses of Uptake and Conversion:* SH-SY5Y and MN9D were incubated with warm Krebs Ringer Buffer (KRB) containing desired concentrations of DA or NE and (+)-sodium L-ascorbate (Asc) for either 10 min (for SH-SY5Y) or 30 min (for MN9D) at 37°C. All time course experiments were carried out using similar procedures, except that incubations were carried out with constant DA or NE concentration with variable incubation times.

*Effect of DAT and NET inhibitors on DA and NE Uptake into SH-SY5Y or MN9D Cells:* Cells were pre-incubated with 1µM NET inhibitor, Desipramine·HCl or 1µM DAT inhibitor, GBR 12909·2HCl 10 minutes prior to the DA or NE addition as described above. *Results:* Undifferentiated SH-SY5Y cells contain relatively low levels of catecholamines under normal growth conditions. In contrast to the previous reports, these cells were found to convert intracellular DA to NE with high efficiency. Furthermore, TPA-differentiated cells were found to contain significantly higher levels of NE in comparison to undifferentiated cells. Although both undifferentiated and differentiated SH-SY5Y cells efficiently take up extracellular DA, the differentiated SH-SY5Y cells were found to be much more efficient.

Incubation of undifferentiated low (3), medium (10-12) and high passage (18) SH-SY5Y cells with DA and Asc resulted in the increase of intracellular DA levels in a time dependent manner. However, low passage cells were found to poorly convert DA to NE, while in the high passage cells conversion was high. The high passage cells also showed the most efficient DA uptake followed by medium passage cells, but low passage cells showed poor uptake. On the other hand, NE uptake appears to be less dependent on the cell passage numbers unlike DA.

Kinetic studies (Table 1) show that both DA & NE (10 min uptake) are substrates for the plasma membrane amine transporter of SH-SY5Y cells. The  $K_m$  for NE is about 10 times lower than that of DA and the  $V_{max}$  values for two substrates are similar, suggesting that NE is kinetically a better substrate for the transporter. In agreement, the specific NET inhibitor reduces both DA and NE uptake by about 80%, whereas the same concentration of specific DAT inhibitor reduces uptake only by about 10-15%.

The efficiencies of NE uptake by undifferentiated and TPA-differentiated cells are similar as indicated by the corresponding  $V_{max}/K_m$  parameters. However, TPA-

differentiated cells appear to have a more efficient DA uptake system in comparison to the undifferentiated cells based on the magnitudes of the corresponding  $V_{max}/K_m$  parameters.

**Table 1**

**Kinetic data for SH-SY5Y and MN9D cells**

	$V_{max}$ (pmol/mg/min)	$K_m$ (µM)	$V_{max}/K_m$
DA uptake by undifferentiated SH-SY5Y	0.91±0.07	19.0 ±3.7	0.048
DA uptake by differentiated SH-SY5Y	3.0 ± 0.5	27.3 ±8.4	0.110
NE uptake by undifferentiated SH-SY5Y	1.0 ± 0.1	2.1 ± 0.9	0.476
NE uptake by differentiated SH-SY5Y	1.8 ± 0.2	3.7 ± 1.1	0.486
DA uptake by differentiated MN9D	0.77±0.07	38.6±6.6	0.020

In contrast to SH-SY5Y cells, undifferentiated MN9D cells store large baseline levels of DA and significant, but lesser amounts of NE. Similarly, the baseline catecholamine levels are not significantly different in undifferentiated and differentiated MN9D cells. Undifferentiated MN9D cells do not take up extracellular DA or NE at a detectable rate. In addition, both differentiated and undifferentiated MN9D cells convert intracellular DA to NE, but only very slowly. However, differentiated MN9D cells take up extracellular DA (Table 1), but rates are slower than those of SH-SY5Y cells.

**3. Conclusions:** These studies show that both undifferentiated and differentiated high passage SH-SY5Y cells closely mimic noradrenergic neurons with respect to the catecholamine metabolism, but not dopaminergic neurons. Therefore, they could only be effectively used as noradrenergic models. The inability of undifferentiated low passage SH-SY5Y cells to convert intracellular DA to NE appears to be related to the improper granular accumulation and/or storage of cytosolic DA. Therefore, low passage SH-SY5Y cells may not be valuable as either a dopamine or noradrenergic model. Although the intracellular catecholamine profiles of undifferentiated MN9D cells mimic the properties of dopaminergic neurons, they lack an efficient catecholamine re-uptake system which is distinct from CNS dopaminergic neurons. On the other hand, 1 mM n-butyric acid-differentiated cells appear to have improved uptake properties and may be a better but not an ideal dopaminergic model.

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