A CELLULAR MODEL FOR AROMATIC L-AMINO ACID DECARBOXYLASE DEFICIENCY AND NEUROTRANSMITTER ANALYSIS OF AVAILABLE THERAPEUTICS USING A NEUROBLASTOMA CELL CULTURE

By

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Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare pediatric neurotransmitter disease. It is an autosomal recessive genetic disorder affecting to date, less than 100 children worldwide. AADC is the key enzyme required for the synthesis of neurotransmitters in the catecholaminergic and serotonergic pathways. Various mutations at position 11 on the short arm of chromosome 7 are responsible for this condition. To date, no apparent correlation can be identified to link mutations with disease outcome. Disease severity can span the spectrum from mild to complete global disability. Generally, affected children fail to achieve any developmental milestones. Movement disorder resulting from dopamine and serotonin deficiency produces characteristic dystonia and oculogyric crises that occur at regular intervals every 3 to 8 days, each episode lasting on average 6 hours. Autonomic dysregulation in the form of temperature instability and susceptibility to metabolic crisis involving sudden dramatic, life-threatening blood glucose level drop and breathing depression are also hallmarks of AADC deficiency. Current treatment intervention involves bolstering endogenous dopamine levels using MAO inhibitors with concurrent administration of dopamine agonists. Various SSRI are also used to maintain endogenous serotonin levels. Anti-cholinergic agents are also used with various outcomes. More recently, the AADC enzyme co-factor PLP and folinate supplementation has shown some promise in improving general disease outcome. However, the overall response to treatment has been dismal. Due to its rare nature, basic research into the disease is scant. Therapeutic tactics has been dependent on the

Parkinson's approach as both conditions share a dopamine deficiency. However, the dopamine deficiency of Parkinson's is due to nigrostriatal dopaminergic neuron death with a late onset while children with AADC deficiency survive entirely without developmental influences from the neurotransmitter. This key difference is believed to be the reason for poor dopamine transmission in AADC deficiency using anti-Parkinson's drugs. This study sought to create a cell model of AADC deficiency to quantify the dopamine synthesis potential of several such drugs. Building on the success of a working cell model, this study also then tried to identify competitive amino acid inhibitors of dopamine. Treatment of SH-SY5Y cells with L-DOPA coaxed dopamine production to levels detectable by HPLC. Subsequent administration of the AADC inhibitor benserazide quelled this effect. Previous efforts to transform immature neuroblastoma cells to a dopaminergic phenotype using all-trans-retinoic acid failed. No detectable level of dopamine was seen via HPLC. Using this model of L-DOPA with concomitant benserazide inhibition to model AADC deficient states, Parnate, bromocriptine, Artane and PLP were tested for their dopamine enhancing capabilities. Parnate, bromocriptine and Artane all produced detectable levels of dopamine but did so only minimally and after a long incubation time. PLP did not induce any dopamine synthesis. This suggests that Parnate, bromocriptine and Artane promotes some dopamine synthesis in this model of AADC deficiency. This mirrors the small gains accorded from their use in AADC deficiency treatment. Unfortunately it also reflects their ineffectiveness. Tryptophan, phenylalanine and tyramine were tested for competitive inhibition of dopamine. Serotonin, its precursor 5-HTP and the catecholamine noradrenaline were also tested for this possible action. 5-HTP was the only one found to inhibit dopamine production. The findings of this study are hoped to fuel research interest into a condition with minimal monetary gains potential. Most importantly, it is hoped to improve treatment outcome and quality of life for AADC deficiency sufferers towards an ultimate cure.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MODEL SEL NEUROBLASTOMA BAGI PENYAKIT KEKURANGAN ASID AROMATIK L-AMINO DEKARBOKSILASE DAN ANALISA KADAR NEUROTRANSMITER DENGAN RAWATAN UBATAN SEDIA ADA

Oleh

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Gejala kekurangan enzim asid aromatik L-amino dekarboksilase adalah suatu penyakit jarang jumpa. Keadaan yang disebabkan autosom resesif ini menyerang kurang daripada 100 orang kanak-kanak dalam dunia. AADC merupakan enzim utama dalam pembentukan neurotransmitter katekolamin dan serotonin. Pelbagai mutasi pada kedudukan 11 lengan pendek kromosom 7 berupaya menghasilkan gangguan ini. Sehingga kini, belum ada korelasi dapat mengkaitkan lokasi mutasi dengan tahap serius penyakit. Penghidap boleh tergolong dari keadaan yang tidak serius hinggalah yang kurang upaya sepenuhnya. Lazimnya, kanak-kanak sebegini langsung tidak mencapai sebarang perkembangan kebolehan. Kekurangan dopamin dan serotonin mengakibatkan penyakit gangguan pergerakan. Ia penyebab berlakunya distonia dan krisis okulogirik dengan kerap selang 3 hingga 8 hari. Keadaan ini biasanya berlarutan purata 6 jam setiap kali. Kecelaruan autonomik berupa ketidakstabilan suhu badan dan kecenderungan menjurus krisis metabolik boleh berlaku. Ia mengakibatkan paras gula dalam darah menjunam mendadak dan gangguan proses pernafasan yang amat serius. Kecenderungan terhadap kecelaruan autonomik adalah salah satu ciri utama penyakit kekurangan enzim ini. Intervensi ubatan melibatkan usaha meningkatkan kadar dopamin sedia ada dalam badan pesakit menggunakan pengekang monoamine oksidase (MAO-I) dan agonis dopamin. Beberapa jenis SSRI juga diguna bagi mengekalkan tahap serotonin sedia ada. Ubat anti-kolinergik turut diguna pakai dengan hasil yang berbeza-beza. Kajian terbaharu menunjukkan potensi penggunaan kofaktor enzim AADC iaitu PLP dan folinat sebagai zat suplemen bagi meringankan impak penyakit secara amnya.

Hakikatnya, keberkesanan ubat sedia ada sekadar hambar sahaja. Disebabkan penyakit ini amat jarang dijumpai, sukar menjana minat menjalankan kajian asas terhadapnya. Taktik mengubatnya juga banyak menumpang pendekatan penyakit Parkinson. Ini kerana kedua-duanya mengakibatkan kekurangan dopamin. Akan tetapi, kekurangan dopamin Parkinson disebabkan kematian neuron dopaminergik di nigrostriata. Ia lazimnya berlaku pada usia lanjut. Sebaliknya, kanak-kanak kekurangan enzim AADC lahir dan membesar tanpa pengaruh perkembangan yang diarus neurotransmitter tersebut. Perbezaan utama ini punca mengapa ubatan Parkinson tidak berjaya menjana dopamin dalam situasi kekurangan enzim AADC. Maka, kajian ini cuba menghasilkan model penyakit kekurangan enzim AADC dari sel agar dapat mengukur keberhasilan beberapa ubatan menjana dopamin. Dengan adanya model sel, kajian ini dapat meneliti sifat pengekang bersaing pada beberapa asid amino dalam jaluran katekolamin dan serotonergik terhadap dopamin. Proses pempotensi mengaruh sel SH-SY5Y hasilkan dopamin hingga dapat dikesan menggunakan teknik HPLC. Kemudian, apabila sel dirawat dengan penghalang enzim AADC benserazide, dopamin hilang semula. Usaha awal mentransformasi sel neuroblastoma yang dianggap tidak matang ini menggunakan asid retinoic semua-trans tidak berhasil menjana dopamin. Kaedah menggunakan $_{L}$-DOPA dan benserazide berhasil membentuk model sel penyakit kekurangan enzim AADC. Ini membolehkan kami mengukur keberkesanan ubat Parnate, bromocriptine, Artane dan PLP. Parnate, bromocriptine dan Artane berupaya menghasilkan dopamin tetapi memerlukan masa yang agak lama. PLP pula langsung tidak membuahkan hasil. Pengamatan ini menyamai hasil penggunaan ubatan tersebut dalam merawat kekurangan AADC. Ada sedikit keberkesanan tetapi tidak cukup untuk melegakan simptom. Triptophan, phenilalanin dan tiramin kemudiannya dianalisa untuk sifat penghalang bersaing terhadap dopamin. Serotonin, pemulanya 5-HTP dan noradrenalin yang juga katekolamin turut dianalisis untuk sifat pengekang bersaing. Hasil kajian ini diharap dapat menjana minat dalam bidang yang tidak menjanjikan pulangan kewangan. Yang paling utama, ia diharap dapat membantu menambahbaik kualiti hidup pesakit kekurangan AADC dan seterusnya membolehkan mereka sembuh sepenuhnya.

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Verily for every disease there is a cure.
Subhanallah.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AADC	aromatic $_{L}$-amino acid decarboxylase
AAV2	adeno-associated virus, serotype 2
AC	adenylyl cyclase
ACN	acetonitrile
APS	ammonium persulfate
ATP	adenosine triphosphate
BDNF	brain-derived neurotrophic factors
BSA	bovine serum albumin
cAMP	cyclic adenosine monophosphate
CATs	catecholamines
cDNA	complementary DNA
CNS	central nervous system
COMT	catechol-ortho-methyltransferase
CSF	cerebrospinal fluid
CT	computed tomography
DA	dopamine (3,4-dihydroxyphenylethylamine)
DBH	dopamine β-hydroxylase
DDC	dopa decarboxylase
DHBA	dihydroxybenzylamine
dH_2O	distilled water
DMSO	dimethyl sulfoxide
DOPAC	3,4-dihydroxyphenylacetic acid
$_{L}$-DOPA	$_{L}$-β-3,4-dihydroxyphenyl alanine

EPI / AD	epinephrine / adrenaline
GABA	γ-amino butyrate
GAP-43	growth-associated protein 43
GCH	GTP cyclohydrolase I
GPCR	G-protein coupled receptors
GPL	lateral globus pallidus
GPM	medial globus pallidus
5-HIAA	5-hydroxyindoleacetic acid
HIOMT	hydroxyindole-O-methyltransferase
HPLC-ED	high performance liquid chromatography with electrochemical detection
HRP	horseradish peroxidase
5-HTP	5-hydroxytryptophan
HVA	homovanillic acid / homovanillate
IC_{50}	inhibition constant at 50%
IP_3	inositol triphosphate
kDA	kilo Dalton
MAO-B	monoamine oxidase B
MAO-I	monoamine oxidase inhibitor
MAPT	microtubule-associated protein tau
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
mRNA	messenger RNA
NE / NA	norepinephrine / noradrenaline
3-O-MD	3-ortho-methyldopa

PBS	phosphate buffer saline
PI	phosphotidylinositol
PIP_2	phosphatidylinositol bisphosphate
PKA	protein kinase A
PLC	phospholipase C
PLP	pyridoxal phosphate
PND	paediatric neurotransmitter diseases
PNMT	phenylethanolamine N-methyltransferase
P-5-P	pyridoxal 5'-phosphate
RA / ATRA	retinoic acid / all-trans retinoic acid
RBFOX3	RNA binding protein, fox-1 homolog 3 (homolog of neuronal nuclei, NeuN)
ROS	reactive oxygen species
RT-PCR	reverse transcriptase polymerase chain reaction
SAM	S-adenosylmethionine
SCN	suprachiasmatic nucleus
SDS	sodium dodecyl sulphate
SMA	supplementary motor area
SNpc	substantia nigra pars compacta
SNpr	substantia nigra pars reticulata
SOS	sodium octyl sulphate
SSRI	selective serotonin reuptake inhibitor
STN	subthalamic nucleus
SYP	synaptophysin
TH	tyroxine hydroxylase

TPA	phorbol ester 12-O-tetradecanoylphorbol-13-acetate
VLN	ventral lateral nucleus
VMA	vanilmalonic acid / vanillylmandelate (3-methoxy-4-hydroxymandelate)
VTA	ventral tegmental area

CHAPTER 1

INTRODUCTION

1.1 Introduction of study

Aromatic L-amino acid decarboxylase deficiency (AADC) is a rare autosomal recessive paediatric neurotransmitter disease. It is caused by a mutation in the AADC gene resulting in a deficiency of the AADC enzyme required to convert L-DOPA to dopamine and 5-HTP to serotonin. The resultant dopamine, serotonin and all downstream metabolite deficit manifests in a debilitating disease that appears in infancy. Hyland and Clayton first characterized AADC deficiency in 1990 and to date it remains a poorly understood disease (Hyland & Clayton, 1990). Awareness of this disease is relatively low among physicians and patients are frequently initially misdiagnosed as suffering from cerebral palsy, epilepsy or gastric reflux. Worldwide, 74 patients have been identified as suffering from AADC (http://www.biopku.org/BioPKU_databasesJAKE.asp). In Malaysia, 5 children are correctly diagnosed with the disease. Taiwan appears to have the largest population of diagnosed AADC children (Lee et al., 2008). Active awareness creation, diagnosis and research in Taiwan may to a certain extent account for this prevalence but a true prevalence may also be the case. Elsewhere, AADC has been diagnosed in the United States, Europe, Israel, Japan and Singapore.

1.2 Problem Statement

AADC deficiency needs a model system in which to study its pathological ideosyncrasies. To date, relatively little research has been carried out to elucidate this disease. A mouse animal model is still being perfected (Hwu et. al., 2013). A zebrafish AADC model had been in use earlier but for obvious reasons not entirely practical (Shih et. al., 2013). This study set out to design a cell culture model for AADC. The neuroblastoma cell culture SH-SY5Y, a line frequently used to model dopaminergic systems in Parkinson's disease was chosen. Preliminary Western Blotting screening revealed it to express key proteins of the dopaminergic pathway and circadian rhythm machinery, thus validating our choice of model system. AADC deficiency symptoms fluctuate with the time of day with a worsening as the day progresses hinting at a strong circadian rhythmicity. The circadian rhythm proteins Period 1, Cryptochrome 1 and 2, CLOCK and BMAL were also detected in the cells via Western Blot. Next, L-DOPA treatment led the cells to produce detectable amounts of dopamine on HPLC-ECD before this *de novo* synthesis is inhibited with

the use of benserazide. Our cell model of AADC deficiency was thus created. The cells were shown to express markers of mature neurons via RT-PCR. Disease-specific medications are desperately needed to address the symptoms of AADC deficiency. Currently, available therapies are based on anti-Parkinson's medication with a generally poor treatment outcome. Due to the lack of alternative treatment, in the short term it is advantageous to assess the dopaminergic synthesis potential of these medications in an AADC context. Thus, this study also set out to test the effects of anti-Parkinson's medications on the cell culture AADC model. Artane, Bromocriptine and Parnate promote cells to synthesize dopamine after more than 4 hours of exposure. The catecholamine and serotonin pathways are made up of biogenic amines sharing a conserved benzene ring structure. Substances with a similar structure can compete for binding at a substrate's receptor binding site. This gives rise to our second parameter of interest involving the possibility of competitive inhibition of dopamine synthesis by another substrate. Testing on our AADC deficiency cell model revealed 5-HTP the precursor to serotonin, to compete with dopamine synthesis.

1.3 Significance of study

Findings from this study will add to the basic knowledge of a very rare pediatric neurotransmitter disease. Due to its rare nature, there is little chance for research funding by large pharmaceutica as the prospect of monetary gains are next to none. It is hoped that research interest can be generated purely on the basis of scientific and humanitarian interest.

1.4 Objectives of study

1.4.1 General objective

1. To contribute to the limited body of knowledge on the pathology of AADC deficiency.

1.4.2 Specific objectives:

1. To create a cell model of AADC deficiency

2. To test the dopamine synthesis capabilities of anti-Parkinson's drugs used for treatment of AADC deficiency.

3. To determine if any of the biogenic amines are competitively inhibiting dopamine / $_{L}$-DOPA

1.5 Study hypotheses:

1. A cellular model of AADC deficiency can be created from SH-SY5Y cells.

2. The dopamine synthesis potential of various anti-Parkinson's drugs will be represented by the level of dopamine produced in the AADC deficient cell model.

3. Biogenic amines of the catecholaminergic and serotonergic pathways competitively inhibit dopamine synthesis.

REFERENCES

Abeling, N. G. G. M., Brautigam, C., Hoffmann, G. F., Barth, P. G., Wevers, R. A., Jaeken, J., Fiumara, A., Knust, A. and van Gennip, A. H. 2000. Pathobiochemical implications of hyperdopaminuria in patients with aromatic L-amino acid decarboxylase deficiency. *Journal of Inherited Metabolic Diseases*. 23: 325-328.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Bray, D., Hopkin, K., Roberts, K. and Walter, P. (2004). Essential cell biology (2ed.). New York: Garland Science.

Allen, G. F. G., Land, J. M. & Heales, S. J. R. 2009. A new perspective on the treatment of aromatic L-amino acid decarboxylase deficiency. *Molecular Genetics and Metabolism.* 97: 6 – 14.

Anton, A. H. and Sayre, D. F. 1962. A study of the factors affecting the aluminum oxide trihydroxyindole procedure for the analysis of catecholamines. *Journal of Pharmacology and Experimental Therapeutics.* 138: 360 – 374.

Assmann, B., Surtees, R. and Hoffmann, G. F. 2003. Approach to the diagnosis of neurotransmitter diseases exemplified by the differential diagnosis of childhood-onset dystonia. *Annals of Neurology*. 54(6): S18 – S24.

Balk, J. -H., Picetti, R., Salardi, A., Thiriet, G., Dietrich, A., Depaulis M. Le Meur, A. and Borrelli, E. 1995. Parkinsonian-like locomotor impairment in mice lacking dopamine D_2 receptors. *Nature*. 377: 424 - 428.

Benowitz, L. I. and Routtenberg, A. 1997. *Trends in Neuroscience*. 20: 84 – 91.

Berry, M. D., Juorio, A. V., Li, X. M. and Boulton, A. A. 1996. Aromatic L-amino acid decarboxylase: a neglected and misunderstood enzyme. *Neurochemistry Research*. 21: 1075 – 1087.

Biedler, J. L., Helson, L. and Spengler, B. A. 1973. Morphology and growth, tumorigenicity, and cytogenetics of buman

neuroblastoma cells in continous culture. *Cancer Research*. 33: 2643 – 2652.

Biedler, J.L., Roffler-Tarlov, S., Schachner, M. and Freedman, L. S. 1978. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Research*. 38: 3751 - 3757.

Bjorklund, A. and Dunnett, S. B. 2007. Dopamine systems in the brain: an update. *Trends in Neurosciences*. 30(5): 194 - 202.

Byrson, S. E., Drew, G. M., Hall, A. S., Ball, S. G. and Balmforth, A. J. 1992. Characterization of the dopamine receptor expressed by rat glomerular mesangial cells in culture. *European Journal of Pharmacology*. 225: 1 - 5.

Cassiman, D., van Pelt, J., De Vos, R., Lommel, F. V., Desmet, V., Yap, S-H. and Roskams, T. 1999. Synaptophysin: a novel marker for human and rat hepatic stellate cells. *American Journal of Pathology*. 155(6): 1831 – 1839.

Chang, Y. T., Sharma, R., Marsh, J. L., McPherson, J. D., Bedell, J. A., Knust, A., Brautigam, C., Hoffmann, G. F. and Hyland, K. 2004. Levodopa-repsonsive aromatic L-amino acid decarboxylase deficiency. *Annals of Neurology*.55: 435 – 438.

Chen, D. F., Schneider, G. E., Martinou, J. C., and Tonegawa, S. 1997. Bcl-2 promotes regeneration of severed axons in mammalian CNS. *Nature*. 385(6615): 434 - 439.

Colamartino, M., Padua, L., Meneghini, C., Leone, S., Cornetta, T., Testa, A. and Cozzi, R. 2012. Protective effect of L-Dopa and carbidopa combined treatments on human catecholaminergic cells. *DNA and Cell Biology*. 31(11): 1572 - 1579.

Constantinescu, R., Constantinescu, A. T., Reichmann, H. and Janetsky, B. 2007. Neuronal differentiation and long-term culture of the human neuroblastoma line SH-SY5Y. *Journal of Neural Transmission*. Supplementary 72: 17 -28.

Daya, S. and Berns, K. I. 2008. Gene therapy using adeno-associated virus vectors. *Clinical Microbiology Reviews*. 21(4): 583 – 593.

Dearry, A., Gingrich, J. A., Falardeau, P., Fremeau, R. T., Bates, M. D. and Caron, M. D. 1990. Molecular cloning and expression of the gene for human D_1 dopamine receptor. *Nature*. 347: 72 - 76.

Devlin, T. M. 2011. Textbook of biochemistry with clinical correlations. *Amino acid and heme metabolism.* 19: 751 – 805. USA: John Wiley & Sons, Inc.

Dixon, M. 1953. The determination of enzyme inhibitor constants. *Biochemical Journal*. 137: 143 – 144.

Dorsam, R. T. and Gutkind, J. S. 2007. G-protein-coupled receptors and cancer. *Nature Reviews Cancer*. 7: 79 – 94.

Dunlap, J. C. 1999. Molecular bases for circadian clocks. *Cell.* 96: 271 – 290.

Encinas, M., Iglesias, M., Liu, Y., Wang, H., Muhaisen, A., and Cena, V. 2000. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor dependent, human neuron-like cells. *Journal of Neurochemistry*. 75: 991 – 1003.

Fan, D. S., Ogawa, M., Fujimoto, K. I., Ikeguchi, K., Ogasawara, Y., Urabe, M., Nishizawa, M., Nakano, I., Yoshida, M., Nagatsu, I., Ichinose, H., Nagatsu, T., Kurtzman, J. G. and Ozawa, K. (1998). Behavioural recovery in 6-hydroxydopamine-lesioned rats by cotransduction of striatum with tyrosine hydroxylase and aromatic $_L$-amino acid decarboxylase genes using two separate adeno-associated virus vectors. *Human Gene Therapy*. 9: 2527-2535.

Ferrier, D. R. 2014. Biochemistry. *Inhibition of enzyme activity*. 5: 60. Sixth edition. Baltimore: Walters Kluwer and Lippincott Williams & Wilkins.

Fitzgerald, M. J. T., Gruener, G. and Mtui, E. 2012. Clinical Neuroanatomy and Neuroscience. *Basal ganglia*. 33:342 - 351. Elsevier Saunders.

Frazer, A. and Hensler, J. G. 1999. Serotonin receptors. American Society for Neurochemistry. Bookshelf ID: NBK28234.

Fremeau, R. J., Duncan, G. E., Fornaretto, M. G., Dearry, A., Gingrich, J. A., Breese, G. R. and Caron, M. G. 1991. Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proceedings of the National Academy of Science USA*. 91: 12,564 - 12,568.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Mosma Jr., F. J. and Sibley, D. R. 1990. D_1 and D_2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*. 250: 1429 - 1432.

Gerlach, M., Double, K., Arzberger, T., Leblhuber, F., Tatschner, T. and Riederer, P. 2003. Dopamine receptor agonists in current clinical use: comparative dopamine receptor binding profiles defined in the human striatum. *Journal of Neural Transmission*. 110: 1119 - 1127.

Green, D. R., Reed, J. R. 1998. Mitochondria and apoptosis. *Science*. 281: 1309 - 1312.

Han, S. K., Mytilineou, C. and Cohen, G. 1996. L-DOPA up-regulates glutathione and protects mesencephalic cultures against oxidative stress. *Journal of Neurochemistry*. 66: 501 - 510.

Harmer, S. L., Panda, S. & Kay, S. A. 2001. Molecular bases of circadian rhythms. *Annual Review of Cell Developmental Biology.*17: 215 – 253.

Hilaire, G., Voituron, N., Menuet, C., Ichiyama, M., Subramanian, H. H. and Dutchmann, M. 2010. The role of serotonin in respiratory function and dysfunction. *Respiratory Physiological Neurobiology*. 174: 76-88.

Hinz, M., Stein, A. & Uncini, T. 2011. APRESS: apical regulatory super system, serotonin, and dopamine interaction. *Neuropsychiatric Disease and Treatment.* 7: 457 – 463.

Hwu, W. L., Lee, N. C., Chien, Y. H., Muramatsu, S. and Ichinose, H. 2013. AADC deficiency: occuring in humans, modeled in rodents. *Advanced Pharmacology*. 68: 272 -84.

Hyland, K. & Clayton, P. T. 1990. Aromatic amino acid decarboxylase deficiency in twins. *Journal Of Inherited Metabolic Diseases*. 13: 301-304.

Hyland, K. 2007. Inherited disorders affecting dopamine and serotonin: critical neurotransmitters derived from aromatic amino acids. *The Journal of Nutrition*. 137: 1568S – 1572S.

Ikeda, H., Pastuszko, A., Ikegaki, N., Kennett, R. H. and Wilson, D. F. 1994. 3,4-dihydroxyphenylalanine (DOPA) metabolism and retinoic acid induced differentiation in human neuroblastoma. *Neurochemical Research*. 19(12): 1487 - 1494.

Ishida, Y., Yokoyama, C., Inatomi, T., Yagita, K., Dong, X., Yan, L., Yamaguchi, S., Nagatsu, I., Komori, T., Kitahama, K. & Okamura, H. 2002. Circadian rhythm of aromatic L-amino acid decarboxylase in the rat suprachiasmatic nucleus: gene expression and decarboxylating activity in clock oscillating cells. *Genes to Cells*. 7: 447-459.

Jaber, M., Robinson, S. W., Missale, C., Caron, M. G. (1996). Dopamine receptors and brain function. *Neuropharmacology*. 35: 1503 - 1519.

Jahng, J. W., Wessel, T. C., Houpt, T. A., Son, J. H., Son, J. H. and Joh, T. H. 1996. Alternate promoters in the rat aromatic L-amino acid decarboxylase gene for neuronal and nonneuronal expression: an in situ hybridization study. *Journal of Neurochemistry*. 66: 14-19.

Kaeffer, B. & Pardini, L. 2005. Clock genes of mammalian cells: practical implications in tissue culture. *In Vitro Cell Developmental Biology – Animal*. 41: 311 – 320.

Kaplitt, M. G., Leone, P., Samulski, R. J., Xiao, X., Pfaff, D. W., O'Malley, K. L. and During, M. J. (1994). Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Natural Genetics*. 8: 148-154.

Kebabian, J. W. and Calne, D. B. 1979. Multiple receptors for dopamine. *Nature*. 277: 93 -96.

Khalid, M. 2011. Dopamine preservatives in cell culture. Proceeding from AACB '11: *The Australasian Association of Clinical Biochemists*. Sydney, Australia.

Kim, D. S., Froelick, G. J. and Palmiter, R. D. 2002. Dopamine-dependent desensitization of dopaminergic signaling in the

developing mouse striatum. *Journal of Neuroscience*. 22: 9841 - 9849.

Kim, K. K., Adelstein, R. S. and Kawamoto, S. 2009. Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. *The Journal of Biological Chemistry*. 284(45): 31051 – 31061.

Kitamura, Y., Kakimura, J. and Taniguchi, T. 2002. Antiparkinsonian drugs and their neuroprotective effects. *Biological Pharmacology Bulletin*. 25(3): 284 - 290.

Korenke, G. C., Christen, H. J., Hyland, K., Hunneman, D. H. &Hanefeld, F. 1997. Aromatic L-amino acid decarboxylase deficiency: an extrapyramidal movement disorder with oculogyric crises. *European Journal of Pediatric Neurology*.1: 67 – 71.

Kuoppamaki, M., Korpela, K., Marttila, R., Kaasinen, V., Hartikainen, P., Lyytinen, J., Kaakkola, S., Hassinen, J., Loyttyniemi, E., Kailajarvi, M. et. al. 2009. Comparison of pharmacokinetic profile of levodopa throughout the day between levodopa / carbidopa / entacapone and levodopa / carbidopa when administered four or five times daily. *European Journal of Clinical Pharmacology*. 65: 443 - 455.

Lyras, L., Zeng, B. Y., McKenzie, G., Pearce, R. K., Halliwell, B. and Jenner, P. (2002). Chronic high dose L-DOPA alone or in combination with the COMT inhibitor entacapone does not increase oxidative damage or impair the function of the nigrostriatal pathway in normal cynomologous monkeys. *Journal of Neural Transmission*. 109: 53 - 67.

Lee, H. F., Tsai, C. R., Chi, C. S., Chang, T. M. and Lee, H. J. 2008. Aromatic L-amino acid decarboxylase deficiency in Taiwan. *European Journal of Paediatric Neurology*. Article in press.

Levant, B., Ling, Z. D. and Carvey, P. M. 1999. Dopamine D_3 receptors. Relevence for drug treatment of Parkinson's disease. *CNS Drugs*. 12: 391 - 402.

Levey, A. I., Hersch, S. M., Rye, D. B., Sunahara, R. K., Niznik, H. B., Kitt, C. A., Price, D. L., Maggio, R., Brann, M. R. and Ciliax, B. J. 1993. Localization of D_1 and spiperone (398) and in GH_3 cells that express a D_2-like receptor with an unusual low affinity for haloperidol in response to NGF (305). D_2 dopamine receptors in

brain with subtype-specific antibodies. *Proceedings of the National Academy of Science USA*. 90: 8861 - 8865.

Lin, C. W., MIller, T. R., Witte, D. G., Bianchi, B. R., Stashko, M., Manelli, A. M. and Frail, D. E. 1995. Characterization of cloned human dopamine D_1 receptor-mediated calcium release in 293 cells. *Molecular Pharmacology*. 47: 131 - 139.

Lin, M. T. 1980. Effects of brain monoamine depletions on thermoregulation in rabbits. *American Journal of Physiology*. 238: R364 – R371.

Lovenberg, W., Weissbach, H. and Udenfriend, S. 1962. Aromatic $_L$-amino acid decarboxylase. *Journal of Biological Chemistry*. 237: 89-93.

Lyras, L., Zheng, B. Y., McKenzie, G., Pearce, R. K., Halliwell, B. and Jenner, P. 2002. Chronic high dose of L-DOPA alone or in combination with the COMT inhibitor entacapone does not increase oxidative damage or impair the function of the nigrostriatal pathway in normal cynomologus monkeys. *Journal of Neural Transmission*. 109: 53 – 67.

Marin-Valencia, I., Serrano, M., Ormazabal, A., Perez-Duenas, B., Garcia-Cazorla, A., Campistol, J. & Artuch, R. 2008. Biochemical diagnosis of cerebrospinal fluid homovanillic acid and other biogenic amines. *Clinical Biochemistry*. 41: 1306 – 1315.

Maronde, E. & Motzkus, D. 2003. Oscillation of human Period 1 (*hPER1*) reporter gene activity in human neuroblastoma cells in vivo. *Chronobiology International*. 20(4): 671-681.

Marty, H., Stein, A. and Uncini, T. 2011. APRESS: apical regulatory super system, serotonin and dopamine interaction. *Neuropsychiatric Disease and Treatment.* 7: 457 – 463.

Maruyama, W., Benedetti, M. S., Takahashi, T. and Naoi, M. 1997. A neurotoxin N-methyl(R)salsolinol induces apoptotic cell death in differentiated human dopaminergic neuroblastoma SH-SY5Y cells. Neuroscience Letters. 232: 147 – 150.

Meiser, J., Weindl, D. and Hiller, K. 2013. Complexity of dopamine metabolism. *Cell Communication and Signalling*. 11: 34 – 51.

Melino, G. Thiele, C. J., Knight, R. A. and Piacentini, M. 1997. Retinoids and the control of growth / death decisions in human neuroblastoma cell lines. *Journal of Neurooncology*. 31: 65 – 83.

Memo, M., Missale, C., Carruba, M. O. and Spano, P. F. 1986. Pharmacology and biochemistry of dopamine receptors in the central nervous system and peripheral tissue. *Journal of Neural Transmission*. 22: 19 - 32.

Mena, M. A., Davila, V. and Sulzer, D. 1997. Neurotrophic effects of L-DOPA in postnatal midbrain dopamine neuron/cortical astrocyte cocultures. *Journal of Neurochemistry*. 69: 1398 - 1408.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M. and Caron, M. G. 1986. Dopamine receptors: from structure to function. *Physiological Reviews*. 78(1): 189 - 225.

Monahan, P. E. and Samulski, R. J. (2000). AAV vectors: Is clinical success on the horizon? *Gene Therapy*. 7: 24-30.

Muramatsu, S., Fujimoto, K., Kato, S., Mizukami, H., Asari, S., Ikeguchi, K., Kawakami, T., Urabe, M., Kume, A., Sato, T., Watanabe, E., Ozawa, K and Nakano, I. 2010. A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. *Molecular Therapy*. 18(9): 1731-1735.

Mytilineou, C., Han, S. K. and Cohen, G. (1993). Toxic and protective effects of L-dopa on mesencephalic cell cultures. *Journal of Neurochemistry*. 61: 1470 - 1478.

Nichols, D. E. and Nichols, C. D. 2008. Serotonin receptors. *Chemistry Reviews*. 108: 1614 - 1641.

Nicolini, G., Miloso, M., Zoia, C., Di Silvestro, A., Cavaletti, G. and Tredici, G. 1998. Retinoic acid differentiated SH-SY5Y human neuroblastoma cells: an in vitro model to assess drug neurotoxicity. *Anticancer Research*. 18: 2477 – 2481.

Oishi, K., Sakamoto, K., Okada, T., Nagase, T. and Ishida, N. 1998. Antiphase circadian expression between BMAL1 and period homologue mRNA in the suprachiasmatic nucleus and peripheral tissues of rats. *Biochemistry and Biophysical Research Communication.*253: 199 – 203.

O'Connell, D. P., Botkin, S. J., Ramos, S. I., Sibley, D. R., Ariano, M. A., Felder, R. A. and Carey, R. M. 1995. Localization of dopamine D_{1A} receptor protein in rat kidneys. *American Journal of Physiology*. 37: F1185 - F1197.

Olanow, C. W., Gauger, L. L. and Cedarbaum, J. M. 1991. Temporal relationships between plasma and cerebrospinal fluid pharmacokinetics of levodopa and clinical effects in Parkinson's disease. *Annals of Neurology*. 29: 556 - 559.

Oli, R. G., Fazeli, G., Kuhn, W., Walitza, S., Gerlach, M. and Stopper, H. 2010. No increased chromosomal damage in L-DOPA-treated patients with Parkinson's disease: a pilot study. *Journal of Neural Transmission*. 117: 737 – 746.

Oyarce, A. M. and Fleming, P. J. 1991. Multiple forms of human dopamine beta-hydroxylase in SH-SY5Y neuroblastoma cells. *Archives of Biochemistry and Biophysiology*. 290: 503 – 510.

Pahlman, S., Ruusala, A. I., Abrahamsson, L., Mattson, M. E. and Esscher, T. 1984. Retinoic acid-induced differentiation of cultured human neuroblastoma cells: a comparison with phorbolester-induced differentiation. *Cell Differentiation*. 14: 135 – 144.

Pons, R., Ford, B., Chiriboga, C. A., Clayton, P. T., Hinton, V., Hyland, K., Sharma, R. & De Vivo, D. C. 2004. Aromatic L-amino acid decarboxylase deficiency. Clinical features, treatment and prognosis. *Neurology*. 62: 1058 – 1065.

Presgraves, S. P., Ahmed, T., Borwege, S. and Joyce, J. N. 2004. Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists. *Neurotoxicity Research*. 5: 579 – 598.

Prigione, A., Begni, B., Galbussera, A., Beretta, S., Brighina, L., Garofalo, R., Andreoni, S., Piolti, R. and Ferrarese, C. 2006. Oxidative stress in peripheral blood mononuclear cells from patients with Parkinson's disease: negative correlation with levodopa usage. *Neurobiological Diseases*. 23: 36 – 43.

Ricci, A., Rossodivita, I. and Amenta, F. 1991. Dopamine DA-2 receptor sites in the rat renal cortex: a light microscope autoradiographic study. *Naunym-Schmiedebergs Arch. Pharmacology*. 344: 259 - 261.

Rutter, J., Reick, M., Wu, L. C. & McKnight, S. L. 2001. Regulation of Clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science.*293: 510 – 514.

Saxena, P. R. 1995. Serotonin receptors: subtypes, functional responses and therapeutic relevance. *Pharmacological Therapeutics*. 66: 339 – 369.

Schapira, A. H. 2008. The clinical relevance of levodopa toxicity in the treatment of Parkinson's disease. *Movement Disorder*. 23 (Supplementary 3): S515 - S520.

Schmauss, C. 2000. Dopamine receptors: novel insights from biochemical and genetic studies. *The Neuroscientist*. 6: 127 - 138.

Seabrook, G. R., Knowles, M., Brown, M., Myers, J., Sinclair, H., Patel, S., Freedman, S. B. and McAllister, G. 1994. Pharmacology of high threshold calcium currents in GH_4C_1 pituitary cells and their regulation by activation of human D_2 and D_4 dopamine receptors. *British Journal of Pharmacology*. 112: 728 - 734.

Self, D. W., Barnhart, W. J., Lehman, D. A. and Nestler, E. J. 1996. Opposite modulation of cocaine-seeking behaviour by D_1- and D_2-like dopamine receptor agonists. *Science*. 271: 1586 - 1589.

Shen, Y., Muramatsu, S. I., Ikeguchi, K., Fujimoto, K. I., Fan, D. S., Ogawa, M., Mizukami, H., Urabe, M., Kume, A., Nagatsu, I., Urano, F., Suzuki, T., Ichinose, H., Nagatsu, T., Monahan, J., Nakano, I. and Ozawa, K. (2000). Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic $_L$- amino-acid decarboxylase and GTP cyclohydrolase I for gene therapy of Parkinson's disease. *Human Gene Therapy*. 11: 1509-1519.

Shi, Y. L., Benzie, I. F. and Buswell, J. A. 2002. L-DOPA oxidation products prevent H_2O_2-induced oxidative damage to cellular DNA. *Life Science*. 71: 3047 - 3057.

Shih, D. F., Hsiao, C. D., Min, M. Y., Lai, W. S., Yang, C. W., Lee, W. T. and Lee, S. J. 2013. Aromatic L-amino acid decarboxylase (AADC) is crucial for brain development and motor functions. *PLoS One*. 8(8): e71741.

Siegel, G. J., Agranoff, B. W. and Albers, R. W. 1999. Basic Neurochemistry: molecular, cellular and medical aspects. *Serotonin receptors*. Philadelphia: Lippincott-Raven.

Simpson, P. B., Bacha, J. I., Palfreyman, E. L., Woollacott, R. M. and Kerby, J. 2001. Retinoic acid-evoked differentiation of neuroblastoma cells predominates over growth factor stimulation: an automated image capture and quantitation approach to neuritogenesis. *Analytical Biochemistry*. 298: 163 – 169.

Snell, R. S. 2010. Clinical Neuroanatomy. *The basal nuclei (basal ganglia) and their connections.* 10: 316 – 330. Philadelphia: Wolters Kluwer Lippincott Williams & Wilkins.

Stocchi, F., Vacca L., Ruggieri, S. and Olanow, C. W. 2005. Intermitten vs. continous levodopa administration in patients with advanced Parkinson disease: a clinical and pharmacokinetic study. *Archive of Neurology*. 62: 905 - 910.

Sumi-Ichinose, C., Hasegawa, S., Ichinose, H., Sawada, H., Kobayashi, K., Sakai, M., Fujii, T., Nomura, H., Nomura, T., Nagatsu, I., Hagino, Y., Fujita, K. and Nagatsu, T. 1995. Analysis of the alternative promoters that regulate tissue-specific expression of human aromatic L-amino acid decarboxylase. *Journal of Neurochemistry*. 64: 514-524.

Surmeier, D. J., Bargas, J., Hemmings, H. C., Nairn, A. C. and Greengard, P. 1995. Modulation of calcium currents by a D_1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron*. 14: 385 - 397.

Swoboda, K.J. and Hyland, K. 2002. Diagnosis and treatment of neurotransmitter-related disorders. *Neurology Clinical.* 20(4): 1143 – 1161, viii.

Swoboda, K. J., Saul, P., McKenna, K. & Speller, N. B. 2003. Aromatic L-amino acid decarboxylase deficiency: overview of clinical features and outcomes. *Annals of Neurology*. 54(6): S49-S55.

Takahashi, T., Deng. Y., Maruyama, W., Dostert, P., Kawai, M. and Naowi, M. 1994. Uptake of a neurotoxin candidate – (R)-1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline into human dopaminergic neuroblastoma cells by dopamine

transport system. *Journal of Neural Transmission. General Section.* 98: 107 – 118.

Tay, S. K. H., Poh, K. S., Hyland, K., Ong, H. T., Low, P. S. and Goh, D. L. M. 2007. Unusually mild phenotype of AADC deficiency in 2 siblings. *Molecular Genetics and Metabolism*. 4: 374 – 378.

Thomas, S. A., Matsumoto, A. M. &Palmiter, R. D. 1995. Noradrenaline is essential for mouse fetal development. *Nature*.374: 643 – 646.

Tsuchiya, Y. & Nishida, E. 2003. Mammalian cultured cells as a model system of peripheral circadian clocks. *Journal of Biochemistry*.134: 785 – 790.

Undie, A. S. and Friedman, E. 1990. Stimulation of a dopamine D_1 receptor enhances inositol phosphates formation in rat brain. *Journal of Pharmacology and Experimental Therapies*. 253: 987 - 992.

Uitti, R. J. and Ahlskog, J. E. 1996. Comparative review of dopamine receptor agonists in Parkinson's disease. *CNS Drugs. 5:* 369 - 388.

Vallar, L., Muca, C., Magni, M., Albert, P., Buznow, J., Meldolesi, J. and Civelli, O. 1990. Defferential coupling of dopaminergic D_2 receptors expressed in different cell types. Stimulation of phosphatidylinositol 4,5-bisphosphate hydrolysis in Ltk$^-$ fibroblasts, hyperpolarization and cytosolic-free Ca^{2+} concentration decrease in GH_4C_1 cells. *Journal of Biological Chemistry*. 265: 10,320 - 10,326.

Verbeek, M. M., Geurtz, P. B. H., Willemsen, M. A. A. P. & Wevers, R. A. 2006. Aromatic L-amino acid decarboxylase enzyme activity in deficient patients and heterozygotes. *Molecular Genetics and Metabolism.* 90: 363 – 369.

Vermeulen, R. J., Drukarch, B., Wolters, E. C. and Stoof, J. C. 1999. Dopamine D_1 receptor agonists. *CNS Drugs*. 11: 83 - 91.

Voong, L. & Eriksson, T. 1992.Is rat brain content of large neutral amino acids (LNAAs) a reflection of plasma LNAA concentrations? *Journal of Neural Transmission of Genetic Selection.*87: 133 – 143.

Waddington, J. L. and Boyle, K. M. 1989. Drugs acting on brain dopamine receptors: a conceptual re-evaluation five years after the first selective D_1 agonist. *Pharmacological Therapies*. 43: 1 - 52.

Xie, H.-R., Hu, L.-S. and Li, G.-Y. 2010. SH-SY5Y human neuroblastoma cell line: *in vitro* cell model of dopaminergic neurons in Parkinson's disease. *Chinese Medical Journal*. 123 (8): 1086 – 1092.

Xu, M., Hu, X. -T., Cooper, D. C., Moratalla, R., Gray-Biel, A. M., White, F. J. and Tonegawa, S. 1994. Elimination of cocaine-induced hyperactivity and dopamine-mediated neurophysiological effects in dopamine D_1 receptor mutant mice. *Cell*. 79: 945 - 955.

Zhang, K. Z., Westberg, J. A., Holtta, E., Anderson, L. C. 1996. Bcl-2 regulates neural differentiation. *Proceedings of the National Academy of Science USA*. 93: 4504 - 4508.

Ziv. I., Zilkha-Falb, R., Offen, D., Shirvan, A., Barzilai, A. and Melamed, E. 1997. Levodopa induces apoptosis in cultured neuronal cells - a possible accelerator of nigrostriatal degeneration in Parkinson's disease? *Movement Disorder*. 12: 17 - 23.