Chapter 4

Tryptophan and Tyrosine Catabolic Patterns - Immune Escape and Parthenogenesis

Introduction

Amino acids are known to be precursors for a variety of biologically important substances, including many neuroactive compounds. The aromatic amino acids, L-tryptophan and L-tyrosine, are the most important in this respect. L-tryptophan is the precursor of not only serotonin, a well-known neurotransmitter, but also of two other neuroactive substances, quinolinic acid and kynurenic acid. L-tyrosine is the precursor of dopamine and other catecholamines. Alteration in tryptophan catabolism has been reported in neurodegenerative disorders like Huntington's disease.[1] Very few reports are available on tyrosine metabolism in these disorders. Morphine, an alkaloidal neurotransmitter, is synthesized from tyrosine.[2] Recently the presence of endogenous strychnine and nicotine has been reported in the brains of rats loaded with tryptophan.[3]

It is known that the level of free tryptophan in the blood can influence the transport of tyrosine across the blood brain barrier into the brain and vice versa, since both these amino acids share the same transport systems and compete with each other. It is also known that endogenous digoxin synthesized by the hypothalamus and other organs influences transport of various substances including neurotransmitters and amino acids. Therefore, the levels of digoxin can influence the concentration of these substances in the brain.[4, 5] This steroidal glycoside is a product of the isoprenoid pathway and the functioning of this pathway can influence digoxin levels. Ubiquinone (a membrane antioxidant and component of mitochondrial electron transport chain) is also a product of the isoprenoid pathway and tyrosine is the precursor of its aromatic ring portion. Deficiency of ubiquinone has been reported in some neurological disorders.[6]

In view of this, a study was undertaken on the catabolism of tryptophan and tyrosine in relation to the isoprenoid pathway in some neurological and

psychiatric disorders, with particular reference to the neurotransmitter and other neuroactive substances. The disorders studied included primary generalized epilepsy, schizophrenia, multiple sclerosis, glioma, Parkinson's disease and syndrome X with multiple lacunar state. A familial group (a family with familial coexistence of schizophrenia, Parkinson's disease, primary generalized epilepsy, malignant neoplasia, rheumatoid arthritis and syndrome X over three generations) was also included in this study.[7]

Material and Methods

Freshly diagnosed cases of glioma, multiple sclerosis (MS), primary generalized epilepsy, Parkinson's disease (PD), schizophrenia and syndrome-X with multiple lacunar state were selected as and when they were admitted to the medicine and neurology wards of Medical College Hospital, Trivandrum, over a period of two years. The diagnosis in each case was confined as follows.

1. Glioma: Histopathologically proved after surgery for mass lesion in brain.
2. Multiple sclerosis: Diagnosed according to Poser's criteria.
3. Primary generalised epilepsy: EEG evidence of generalized epileptiform activity; age of onset below 30 yrs; MRI scan negative.
4. Parkinson's disease: Age of onset above 50 with bradykinesia, rigidity and tremor.
5. Schizophrenia: DSM III R criteria.
6. Syndrome X: Non-insulin dependent diabetes mellitus, increased insulin levels, hyper triglyceridemia, hypertension- multiple lacunar state on CT scan.

None of the subjects studied was under medication at the time of collection of blood sample. An equal number of age and sex matched healthy subjects served

as controls. Both patients and controls were non-smokers. Fasting blood samples were collected. RBCs were separated within one hour of collection of blood for the estimation of membrane Na^+-K^+ ATPase. Plasma was used for the estimation of various others parameters.

Analytical Procedures

HMG CoA reductase activity was assayed by the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate.[8] For estimation of Na^+-K^+ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used.[9] Protein in the RBC membrane preparation was determined by Lowry's procedures.[10] Digoxin in the plasma was determined by the procedure described by Arun et al.[11] The method involved extraction of plasma with 90% ethanol, followed by purification of digoxin by TLC, and estimation by HPLC.

Magnesium in the plasma was estimated by atomic absorption spectrophotometry.[12] Tryptophan was estimated by the method of David and William,[13] and tyrosine by the method of Wong et al.[14] Serotonin and 5-hydroxyindoleacetic acid were estimated by the method of Curzon et al.[15] Estimation of catecholamines was carried out by the method of Well-Malherbe.[16] Quinolinic acid and kynurenic acid content of plasma were estimated by HPLC [C_{18} column (micro Bondapak™ 4.6 x 150 mm), solvent system 0.01 M acetate buffer (pH 3.0) and methanol (6:4), flow rate 1.0 ml/minute and detection UV 250 nm]. Morphine, strychnine and nicotine were estimated by the method described by Arun et al.[3] Albumin was estimated by the method of Spencer and Price.[17] Free fatty acids were estimated by the method described by Falhot and Land.[18] Statistical analysis was carried out by using student 't' test.

Results

1. Concentration of tryptophan, tyrosine, neurotransmitters, quinolinic acid, kynurenic acid, free fatty acid and albumin in the plasma (Table 1).

Table 1. *Concentration of tryptophan, tyrosine, quinolinic acid, kynurenic acid, neurotransmitters, free fatty acids and albumin in the plasma.*

	Control	CNS Glioma	MS	Epilepsy
Tryptophan (mg/dl)	1.11 ±0.08	2.05 ±0.07[a]	1.99 ±0.07[a]	1.96 ±0.09[a]
Tyrosine (mg/dl)	1.14 ±0.09	1.01 ±0.07[a]	0.929 ±0.06[a]	0.883 ±0.05[a]
Serotonin (µg/dl)	20.9 ±1.9	48.9 ±3.9[a]	92.1 ±7.1[a]	59.5 ±4.6[a]
5-HIAA (µg/dl)	3.67 ±0.34	10.98 ±0.53[a]	7.94 ±0.35[a]	19.04 ±1.7[a]
Dopamine (ng/dl)	12.89 ±0.67	8.43 ±0.44[a]	8.68 ±0.52[a]	8.53 ±0.53[a]
Epinephrine (ng/dl)	9.98 ±0.31	5.65 ±0.28[a]	5.92 ±0.27[a]	6.84 ±0.27[a]
Norepinephrine (ng/dl)	45.15 ±2.35	33.54 ±1.78[a]	31.52 ±1.38[a]	34.18 ±1.11[a]
Quinolinic acid (ng/dl)	370.60 ±21.07	655.73 ±48.8[a]	646.92 ±52.93[a]	549.34 ±41.21[a]
Kynurenic acid (ng/dl)	172.60 ±16.46	245.02 ±23.22[a]	263.39 ±25.93[a]	299.47 ±27.14[a]
Free fatty acid (mg/dl)	79.23 ±3.25	103.68 ±7.66[a]	88.43 ±4.04[a]	92.86 ±5.12[a]
Albumin (g/dl)	4.78 ±0.05	3.24 ±0.03[a]	3.09 ±0.04[a]	3.38 ±0.08[a]

Table 1. *Continue.*

	PD	Schizo-phrenia	Syndrome X	Familial Case
Tryptophan (mg/dl)	1.66 ±0.06[a]	2.10 ±0.09[a]	1.80 ±0.06[a]	2.02 ±0.08[a]
Tyrosine (mg/dl)	0.901 ±0.06[a]	0.862 ±0.05[a]	0.935 ±0.06[a]	0.825 ±0.03[a]
Serotonin (µg/dl)	77.8 ±5.6[a]	46.6 ±3.7[a]	52.8 ±4.8[a]	41.8 ±4.2[a]
5-HIAA (µg/dl)	7.91 ±0.42[a]	18.07 ±1.7[a]	18.48 ±1.35[a]	19.82 ±1.4[a]
Dopamine (ng/dl)	8.45 ±0.44[a]	8.44 ±0.45[a]	8.92 ±0.51[a]	8.01 ±0.62[a]
Epinephrine (ng/dl)	6.82 ±0.20[a]	5.98 ±0.21[a]	6.78 ±0.31[a]	6.92 ±0.41[a]
Norepinephrine (ng/dl)	34.85 ±1.17[a]	37.52 ±0.83[a]	35.26 ±1.85[a]	36.41 ±1.74[a]
Quinolinic acid (ng/dl)	645.53 ±51.48[a]	599.28 ±52.64[a]	655.15 ±44.93[a]	589 ±50.64[a]
Kynurenic acid (ng/dl)	240.52 ±20.26[a]	271.21 ±22.44[a]	245.87 ±20.45[a]	280.22 ±28.21[a]
Free fatty acid (mg/dl)	89.64 ±3.48[a]	91.15 ±3.92[a]	112.76 ±8.4[a]	98.61 ±3.8[a]
Albumin (g/dl)	3.36 ±0.05[a]	3.14 ±0.06[a]	3.04 ±0.04[a]	3.16 ±0.05[a]

Values are mean ±SD of 15 cases in each group. All groups have been compared with control.
a= p <0.01, MS = multiple sclerosis, PD = Parkinson's disease

Concentration of tryptophan in the plasma was significantly more in patients of all the disorders studied, when compared to that in the control subjects. On the other hand, concentration of tyrosine was significantly lower. Concentration of serotonin and 5-hydroxyindoleacetic acid in the plasma was higher while that of catecholamines (dopamine, epinephrine and norepinephrine) was lower. There was increase in free fatty acid and decrease in albumin in the plasma. Level of quinolinic acid and kynurenic acid was higher in the plasma of all patients, the increase in the kynurenic acid being lesser than that of quinolinic acid.

2. Activity of HMG CoA reductase and RBC Na^+-K^+ ATPase concentration of ubiquinone, digoxin and magnesium (Table 2).

Table 2. *Concentration of digoxin, activity of HMG CoA reductase, RBC membrane Na^+-K^+ ATPase, ubiquinone and Mg^{2+}.*

	Digoxin (ng/dl)	**Activity of HMG CoA reductase (Ratio of HMG CoA to mevalonate)**	**Membrane Na^+-K^+ ATPase (µgPi/mg protein)**	**Ubiquinone (µg/dl)**	**Plasma Mg^{2+} (mg/dl)**
Control	12.80 ±0.74	1.15 ±0.12	5.04 ±0.22	144.20 ±8.65	2.40 ±0.24
Glioma	14.60 ±0.62[a]	0.74 ±0.06[a]	1.94 ±0.18[a]	103.80 ±7.13[a]	2.16 ±0.22[a]
MS	29.15 ±2.19[a]	1.04 ±0.08[a]	1.31 ±0.12[a]	82.85 ±4.89[a]	2.11 ±0.15[a]
Epilepsy	23.50 ±1.76[a]	0.88 ±0.07[a]	1.48 ±0.14[a]	82.97 ±6.64[a]	2.08 ±0.11[a]
PD	20.90 ±1.41[a]	0.81 ±0.07[a]	1.51 ±0.14[a]	65.83 ±5.92[a]	2.13 ±0.12[a]
Schizophrenia	15.13 ±1.13[a]	0.75 ±0.04[a]	1.24 ±0.13[a]	89.33 ±5.36[a]	1.81 ±0.11[a]
Syndrome X	29.95 ±2.36[a]	0.82 ±0.06[a]	1.50 ±0.12[a]	101.6 ±6.21[a]	1.53 ±0.11[a]
Familial case	24.80 ±1.68[a]	0.89 ±0.04[a]	1.64 ±0.16[a]	88.56 ±5.34[a]	2.04 ±0.08[a]

Values are mean ±SD of 15 cases in each group. All groups have been compared with control.
a= $p < 0.01$, MS = multiple sclerosis, PD = Parkinson's disease

An elevation of the activity of HMG CoA reductase and increase in digoxin in the plasma were observed in the patients of all these disorders when compared to the control subjects. Activity of RBC membrane Na^+-K^+ ATPase

showed a significant decrease in all these patients. Concentration of ubiquinone and magnesium in the plasma was significantly lower in all these patients.

3. Level of morphine, strychnine and nicotine (Table 3) in the plasma.

Table 3. *Level of morphine, strychnine and nicotine in the plasma.*

	Morphine (µg/dl)	Strychnine (µg/dl)	Nicotine (µg/dl)
Control	ND	ND	ND
CNS glioma	ND	ND	4.56 ±0.20
MS	9.92 ±1.21	1.02 ±0.84	ND
Epilepsy	ND	11.44 ±0.46	1.25 ±0.04
PD	ND	9.54 ±0.38	1.07 ±0.03
Schizophrenia	ND	0.60	5.28 ±0.21
Syndrome X	ND	2.92 ±0.12	9.72 ±0.84
Familial case	ND	1.08 ±0.12	1.24 ±0.02

Values are mean ±SD of 15 cases in each group.
ND = not detectable

No morphine, strychnine or nicotine could be detected in the serum of control subjects. Morphine was also not detectable in the plasma of patients of primary generalised epilepsy, schizophrenia, glioma, PD, syndrome X and the familial group but was detectable in the plasma of patients with MS. Strychnine was detectable in the plasma of patients of epilepsy, schizophrenia, MS, syndrome X, familial group and PD, while it was not detectable in patients with CNS glioma. Nicotine was detected in the plasma of patients of epilepsy, schizophrenia, glioma, Parkinson's disease, familial group and syndrome X but not in MS.

Discussion

The increase in the activity of HMG CoA reductase, a key enzyme in the isoprenoid pathway in all these disorders, suggests an upregulation of this pathway which agrees with the increase in the level of digoxin, a product of this pathway. On the other hand, the level of ubiquinone, which is also a product of

this pathway, is decreased. This probably may be due to the fact that less of the concerned precursor (farnesyl pyrophosphate) is channelled for the synthesis of the side chain of ubiquinone. It may also be due to decrease in the synthesis of the aromatic ring portion of ubiquinone which is derived from the aromatic amino acid, tyrosine. The decrease in tyrosine observed in these disorders supports this view.

The important observations made in all the disorders, in this study are: (a) increase in endogenous digoxin in these disorders, (b) increase in tryptophan levels along with all its catabolites (namely serotonin, 5-hydroxyindoleacetic acid, quinolinic acid, kynurenic acid, strychnine and nicotine), and (c) decrease in levels of tyrosine and its catabolites, namely dopamine, epinephrine and norepinephrine. Morphine, which is derived from tyrosine, was not detectable in any of these disorders except in MS.

Free fatty acids compete with tryptophan for albumin binding, and the increase in the plasma free fatty acid observed in these disorders may result in less tryptophan binding with consequent increase in free tryptophan. Digoxin is reported to increase catecholaminergic transmission and catecholamines promote lipolysis with resultant increase in free fatty acid and consequent increase in free tryptophan and its transport. Decrease in albumin, consequent to membrane Na^+-K^+ ATPase inhibition related hypomagnesaemia induced blockade of protein synthesis, may cause decrease in its binding to tryptophan. The net effect of all these factors is that more free tryptophan is available to cross the blood brain barrier. The decrease in the plasma level of tyrosine in these patients may be the result of competition between it and tryptophan, for the same transport system and also probably of the differential effect of digoxin in promoting tryptophan transport.

Na^+-K^+ ATPase inhibition can also result from decreased levels of dopamine, noradrenaline, morphine and thyroxin and increased levels of serotonin, nicotine, strychnine and quinolinic acid.[19] It is known that inhibition of this enzyme leads to increase in intracellular calcium due to increase in Na^+-K^+ exchange, increased entry of calcium via voltage gated calcium channel, and increased release of calcium from intracellular endoplasmic reticulum calcium stores.[20] The increase in intracellular calcium by displacing magnesium from its binding sites leads to a decrease in functional availability of magnesium. Decrease in magnesium inhibits Na^+-K^+ ATPase further, as ATP-magnesium complex is the actual substrate for the reaction. Thus, there is a progressive inhibition of Na^+-K^+ ATPase, triggered by an initial insult.

Increased intracellular calcium in the postsynaptic neuron can activate calcium dependent NMDA signal transduction system.[21] The plasma membrane neurotransmitter transporter of glutamate in the glial cell and presynaptic neuron is coupled to a sodium gradient,[22] which is disrupted by inhibition of Na^+-K^+ ATPase resulting in decreased clearance of glutamate, by presynaptic and glial uptake at the end of synaptic transmission. By this mechanism, membrane Na^+-K^+ ATPase inhibition can promote glutamatergic transmission. Strychnine displaces glycine from its binding site and inhibits glycinergic inhibitory transmission in the brain.[23] The glycine is free to bind to the strychnine insensitive site of the NMDA receptor and promote NMDA transmission. Thus, hypercatabolism of tryptophan can result in glutamate excitotoxicity. NMDA excitotoxicity has been implicated in neuronal degeneration like Parkinson's disease.[24] As discussed above, this is mediated by increase in intraneuronal calcium load. The low levels of tyrosine can result in decreased dopamine synthesis, which can lead to defect in nigrostriatal dopaminergic transmission, observed in Parkinson's disease.[25] Nicotine can lead to increase in cholinergic transmission and the tremor of Parkinson's disease. NMDA excitotoxicity has also been implicated in

epileptogenesis. Membrane Na^+-K^+ ATPase inhibition can lead on to a paroxysmal depolarization shift and epileptogenesis.[26] Dopamine and noradrenaline deficiency, contributing to the epileptogenesis consequent to loss of their hyperpolarising action, has been reported before.[26]

Thus, both tryptophan hypercatabolism and tyrosine hypocatabolism can lead to intraneuronal calcium overloaded state and functional magnesium deficiency due to membrane Na^+-K^+ ATPase inhibition. Hypercatabolism of tryptophan can lead to increased availability of acetyl CoA, and upregulation of isoprenoid pathway resulting in increased endogenous digoxin biosynthesis. Tryptophan catabolism, apart from quinolinic acid, also leads to kynurenic acid synthesis, which is reported to be neuroprotective.[1] But the level of kynurenic acid, is far lower than that of quinolinic acid, for the former to exert its neuroprotective effect. Thus, the neurotoxic effect of quinolinic acid predominates.

Increased neuronal calcium can activate the calcium dependent calcineurin signal transduction pathway, which can produce T cell activation and secretion of tumour necrosis factor alpha (TNF alpha).[27] TNF alpha can activate the transcription factors NF-KB and AP-l leading to the induction of proinflammatory and immunomodulatory genes.[27] This can explain the immune activation described in MS.[27] TNF alpha can also bring about apoptosis of the cell by activating caspase-9 and ICE protease which converts interleukin 1 beta precursor to interleukin 1 beta.[27] Interleukin 1 beta produces apoptosis of oligodendrocytes, the myelin forming cell in MS. It can also produce apoptosis of the neurons in neuronal degeneration. Disordered apoptosis can also bring about defective synaptic connectivity contributing to schizophrenia and epilepsy. Apoptosis is mediated in another way also, by increasing intraneuronal calcium,[28] which can open the mitochondrial PT pore. This also leads to volume dysregulation of mitochondria, causing hyperosmolality of matrix and expansion of matrix space. The outer membrane of the mitochondria ruptures and releases apoptosis inducing

factor (AIF) and cytochrome C (cyto C), which activate the caspase cascade producing cell death.[28]

The uncoupling of oxidative phosphorylation due to mitochondrial PT pore opening and disruption of the proton gradient mentioned above, with decrease in ubiquinone consequent to tyrosine deficiency, may contribute to mitochondrial dysfunction. The uncoupling of oxidative phosphorylation also leads to free radical generation. Ubiquinone is also a free radical scavenger and its reduced level can lead to decreased free radical scavenging. Increase in intracellular calcium can contribute to increased free radical generation, activating nitric oxide synthase, leading on to increase in nitric oxide formation, and activation of phospholipase A_2, leading to stimulation of arachidonic acid metabolism generating free radicals. Free radicals have been implicated in neuronal degeneration and oncogenesis. Mitochondrial dysfunction has been implicated in neuronal degeneration.

Intracellular magnesium deficiency can lead to decreased ATP synthesis and defective formation of dolichol phosphate required for N-glycosylation and also decreased formation of nucleoside diphosphate sugars for O-glycosylation. This leads to defective glycoconjugate synthesis. Defective glycosylation of endogenous myelin glycoprotein antigen can lead to defective formation of MHC-antigen complex.[29] Defective presentation of myelin glycoprotein antigen to the CD_8^+ cell can explain the immune dysregulation in MS.[30] Defective glycoproteins can lead to altered contact inhibition and oncogenesis. Defectively processed glycoproteins accumulate as they resist lysosomal digestion, leading to neuronal degeneration, as in the case of amyloid.[31] Defective glycoproteins can also result in disordered synaptic connectivity and functional disorders like epilepsy and schizophrenia.

Increase in intracellular calcium via activation of phospholipase C-beta produces diacyl glycerol (DAG). This activates protein kinase C and the MAP kinase cascade, stimulating cell proliferation. The decrease in intracellular magnesium can further produce dysfunction of GTPase activity of the alpha subunit of G-protein and oncogene activation. The major tumour suppressor gene P_{53} is impaired owing to intracellular magnesium deficiency producing phosphorylation defects. All these lead to oncogenesis.[32]

NMDA excitotoxicity due to membrane sodium potassium ATPase inhibition can contribute to schizophrenia. Strychnine, by blocking glycinergic transmission, can contribute to the decreased inhibitory transmission in schizophrenia.[33] Nicotine, by interacting with nicotinic receptors, can facilitate the release of dopamine, promoting the dopaminergic transmission in the brain, even in the presence of low dopamine levels. The low levels of noradrenaline and increased levels of serotonin agree with previous reports. Cancer related psychosis and psychotic manifestations of MS could also be explained on this basis.

Low RBC sodium potassium ATPase activity has been previously described in syndrome X.[5] The consequent increase in calcium within the cell, especially the beta cell, can lead to increased release of insulin from the beta cell. A cellular magnesium deficiency and increase in calcium overload can have the following consequences: (1) Intracellular magnesium deficiency can lead to protein tyrosine kinase dysfunction, an insulin receptor defect,[34] (2) Increased intracellular calcium can lead to increased G-protein coupled signal transduction of the contra insulin hormones - glucagon, growth hormone and adrenaline, (3) Increased intracellular calcium can open up the mitochondrial PT pore producing mitochondrial dysfunction; and reduction in intracellular magnesium can inhibit ATP synthase. Decreased intra cellular magnesium can also lead to inhibition of glycolysis and citric acid cycle. Thus glucose utilization as a whole is decreased, (4) Increased intracellular calcium can increase the signal

transduction of the G-protein coupled platelet activating factor receptor and thrombin receptor, producing thrombosis. Intracellular magnesium deficiency can also produce vasospasm described in syndrome X, (5) Nicotine is known to produce vasospasm. It can also produce autonomic ganglionic stimulation, adrenal medullary stimulation and carotid and aortic body stimulation leading to hypertension,[23] and (6) Nicotine administration has also been reported to produce significant changes in lipid metabolism.[35]

Thus, patterns of tryptophan hypercatabolism and tyrosine hypocatabolism can be noticed in schizophrenia, primary generalized epilepsy, Parkinson's disease, multiple sclerosis, CNS glioma and syndrome X with multiple lacunar state. The interrelationship between neuronal degeneration, immune mediated neuronal disorders and functional neuropsychiatric disorders has been documented in literature.[36] A family with coexistence of these disorders has been described by Ravi Kumar et al.[7] Auto antibodies have been demonstrated in MS, motor neuron disease, paraneoplastic syndrome X, schizophrenia and epilepsy.[36]

Psychosis has been described in MS, Parkinson's disease, epilepsy and cancer syndrome.[36] The relationship between Hodgkin's lymphoma and MS, lymphoma and MND, and lymphomatous transfusion in autoimmune diseases like neurolupus has been described.[36]

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