Chapter 3

Archaeal Digoxin and Amino Acid Metabolism - The Tryptophan / Tyrosine Catabolic Pattern

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 kunurenic 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. Very few reports are available on tyrosine metabolism in these disorders. Morphine, an alkaloidal neurotransmitter, is synthesized from tyrosine. Recently the presence of endogenous strychnine and nicotine has been reported in the brain of rats loaded with tryptophan.

It is known that 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 the transport of various substances including neurotransmitters and amino acids and therefore the levels of digoxin can influence the concentration of these substances in the brain. 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 the mitochondrial electron transport chain) is also product of the isoprenoid pathway and the tyrosine in the precursor of its aromatic ring portion. Deficiency of ubiquinone has been reported in some neurological disorders.

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, CNS 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. The results are discussed in this paper.

Results

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

Concentration of tryptophan in the plasma was significantly more in patients of all the disorders studied, when compared to that in the control subject. 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 an increase in free fatly acid and a decrease in albumin in the plasma. The level of quinolinic acid and kynurenic acid was higher in the plasma of all patients, the increase in the kynurenic acid being smaller than that in quinolinic acid.

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

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 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 in the plasma

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 with 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, 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 channeled for the synthesis of the side chain of ubiquinone. It may also be due to a 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 in this study are: (a) increase in archaeal digoxin in these disorders, (b) increase in tryptophan levels with increase in all its catabolites (namely serotonin, 5-hydroxyindoleacetic acid, quinolinic acid, kynurenic acid, strychnine and nicotine) in all the disorders studied 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 a consequent increase in free tryptophan. Digoxin is reported to increase catecholaminergic transmission and catecholamines promote lipolysis with resultant increase in free fatty acid with a consequent increase in free tryptophan and its transport. Decrease in albumin consequent to membrane Na^{+}-K^{+} ATPase inhibition related hypomagnesemia induced blockade of protein synthesis may cause a 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 probably also 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 thyroxine and increased levels of serotonin, nicotine, strychnine and quinolinic acid. It is known that inhibition of this enzyme leads to increase in intracellular calcium due to increase in Na^{+}-Ca^{++} exchange, increased entry of calcium via voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. The increase in intracellular calcium by displacing magnesium from its binding sites leads to a decrease in the functional availability of magnesium. Decrease in magnesium inhibits Na^{+}-K^{+} ATPase further, as the 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 the calcium dependent NMDA signal transduction system. The plasma membrane

neurotransmitter transporter of glutamate in the glial cell and presynaptic neuron is coupled to a sodium gradient 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. 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. As discussed above, this is mediated by increase in the intraneuronal calcium load. The low levels of tyrosine can result in decreased dopamine synthesis which can lead to a defect in nigrostriatal dopaminergic transmission, as observed in Parkinson's disease. Nicotine can lead on 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 depolarisation shift and epileptogenesis. Dopamine and noradrenaline deficiency contributing to the epileptogenesis consequent to loss of their hyperpolarising action has been reported before.

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 the 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. But the level of kynurenic acid is far lower than that of quinolinic acid for the former to exert its neuroprotective effect and 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 TNF alpha (Tumour necrosis factor alpha). TNF alpha can activate the transcription factors NFkB and AP-l leading on to the induction of proinflammatory and immunomodulatory genes. This can explain the immune activation described in MS. TNF alpha can also bring about apoptosis of the cell by activating caspase-9 and ICE protease which converts the interleukin-1 beta precursor to interleukin-1 beta. lnterleukin-1 beta produces apoptosis of oligodendrocytes, the myelin forming cell in MS. Interleukin-1 beta can also produce apoptosis of the neuron 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 increased intraneuronal calcium 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 AIF (apoptosis inducing factor) and cyto C (Cytochrome C) which activates the caspase cascade producing cell death.

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 on to defective glycoconjugate synthesis. Defective glycosylation of the endogenous myelin glycoprotein antigen can lead to defective formation of MHC-antigen complex. Defective presentation of myelin glycoprotein antigen to the CD_8 cell can explain the immune dysregulation in MS. Defective glycoproteins can lead to altered contact inhibition and oncogenesis. Defectively processed glycoproteins can also resist lysosomal digestion and accumulate, leading on to neuronal degeneration as in the case of an amyloid. Defective glycoproteins can also result in disordered synaptic connectivity and functional disorders like epilepsy and schizophrenia.

Increased intracellular calcium via activation of phospholipase C-beta produces DAG (diacyl glycerol) activating 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 ras oncogene activation. The major tumour suppressor gene is P_{53} is impaired owing to intracellular magnesium deficiency producing phosphorylation defects. All these lead to oncogenesis.

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. 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. The consequent increase in calcium within the cell, especially the beta cell, can lead to an increased release of insulin from the beta cell. A cellular magnesium deficiency and increase in a calcium overloaded state can have the following consequences: (1) Intracellular cellular magnesium deficiency can lead to protein tyrosine kinase dysfunction, an insulin receptor defect. (2) Increased intracellular calcium can lead to increased G-protein coupled signal transduction of the contra insulin hormones - glucagon, the growth hormone and adrenaline. Increased intra cellular calcium can open up the mitochondrial PT pore producing a 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. Increased intracellular calcium can increase the signal transduction of the G-protein coupled platelet activating factor receptor and thrombin receptor producing thrombosis. Intra cellular magnesium deficiency can also produce vasospasm as described in syndrome X. 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. Nicotine administration has also been reported to produce significant changes in lipid metabolism.

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 a multiple lacunar state. The interrelationship between neuronal degeneration, immune mediated neuronal disorders and functional neuropsychiatric disorders has been documented in literature. We have described a family with a coexistence of these disorders. Auto

antibodies have been demonstrated in MS, motor neuron disease, paraneoplastic syndrome X, schizophrenia and epilepsy. Psychosis has been described in MS. Parkinson's disease, epilepsy and cancer syndrome. The relationship between Hodgkin's lymphoma and MS, lymphoma and MND, and lymphomatous transfusion in autoimmune diseases like neuro lupus has been described.

References

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