6

Neurology of Myalgic Encephalomyelitis

Introduction

Several theories have been put forward with respect to chronic fatigue syndrome or myalgic encephalomyelitis. Persistent viral infections especially the Epstein barr virus and enteroviruses have been described in ME. Several immune system abnormalities have been described. Deficiencies in the amounts of IgG_1 and IgG_3 and decreased amounts of IgA have been noticed. Elevated levels of alpha interferon in the spinal fluid and increased levels of interleukin-2 have been reported by some groups. T_4 cells have been reported to not function as effectively as normal when stimulated with phytohaemagglutinin in ME. T_8 suppressor cell changes have also been reported. F delayed type of hypersensitivity skin testing is abnormal in 80% of patients. Physical and mental stress have been reported to predispose one to ME. Muscle fatigue, myalgia and muscle twitching are noticed in ME. Mitochondrial abnormalities have also been reported in the muscle in ME. Altered brain function has been reported in ME including loss of concentration and loss of recent memory.

Global warming can lead to osmotic stress consequent to dehydration. The increase in actinidic archaeal growth leads to cholesterol catabolism and digoxin synthesis. Digoxin produces membrane sodium potassium ATPase inhibition and increase in intracellular calcium producing mitochondrial dysfunction. This results in oxidative stress. The oxidative stress and osmotic stress can induce the enzyme aldose reductase which converts glucose to fructose. Fructose has got a low km value for ketokinase as compared to glucose. Therefore fructose gets phosphorylated more to fructose phosphate and the cell is depleted of ATP. The cell depletion of ATP leads to oxidative stress and chronic inflammation consequent to induction of NFKB. The fructose phosphate can enter the pentose phosphate pathway synthesizing ribose and nucleic acid. The depletion of cellular ATP results in generation of AMP and ADP which are acted upon by

deaminases causing hyperuricemia. Uric acid can also produce mitochondrial dysfunction. The fructose phosphate can enter the glucosamine pathway synthesizing GAG and producing mucopolysaccharide accumulation. Fructose can fructosylate proteins making them antigenic and producing an autoimmune response. This can lead to global warming related autoimmune disease.

The isoprenoid pathway is an important pathway crucial in cellular regulation. It produces important metabolites (digoxin, dolichol, ubiquinone and cholesterol). Digoxin is an endogenous membrane Na^+-K^+ ATPase inhibitor secreted by the human hypothalamus. Dolichol is important in N-glycosylation of proteins and protein processing. Ubiquinone is an important component of the mitochondrial electron transport chain. Cholesterol is an important component of cellular membranes. It was therefore considered pertinent to assess the isoprenoid pathway in ME. Since endogenous digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of hemispheric dominance. The pathway was also assessed in individuals of differing hemispheric dominance to find out the role of hemispheric dominance in the predisposition to ME.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in ME. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na^+-K^+ ATPase and serum magnesium were decreased.

(2) The concentration of serum tryptophan, quinolinic acid and serotonin was increased in the plasma while that of tyrosine, dopamine and noradrenaline was decreased in ME.

(3) Nicotine (1.07 ug/l00 ml) and strychnine (9.54 ug/dL) were detected in the plasma of patients with ME but were not detectable in the control serum. Morphine was not detected in the plasma of ME patients.

(4) The concentration of total glycosaminoglycan increased in the serum of ME patients. The concentration of heparan sulphate (HS), heparin (H), dematan sulphate (DS), chondroitin sulphate (ChS) and hyaluronic acid (HA) was increased. The concentration of total hexose, fucose and sialic acid was increased in the glycoproteins of the serum in these patients. The concentration of gangliosides, glycosyl diglycerides, cerebroside and sulphatide showed significant increase in the serum of these patients.

(5) The activity of glycosaminoglycan (GAG) degrading enzymes (beta glucuronidase, beta N-acetyl hexoseaminidase, hyaluronidase and cathepsin-D) was increased in ME when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase increased in ME.

(6) The concentration of total GAG and hexose and fucose residues of glycoproteins in the RBC membrane decreased significantly in ME. The concentration of RBC membrane cholesterol increased in ME while that of phospholipid decreased. The ratio of RBC membrane cholesterol against phospholipids increased in ME.

(7) The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes decreased significantly in ME. In ME the concentration of MDA, hydroperoxides, conjugated dienes and NO increased significantly. The concentration of glutathione and alpha tocopherol decreased in ME. Iron binding capacity, ceruloplasmin and albumin decreased significantly in ME.

(8) The results showed that HMG CoA reductase activity, serum digoxin and dolichol were increased and ubiquinone reduced in left handed / right hemispheric dominant individuals. The results also showed that HMG CoA reductase activity, serum digoxin and dolichol were decreased and ubiquinone increased in right handed / left hemispheric dominant individuals. The results showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be higher in the plasma of left handed / right hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was lower. The results also showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was higher.

Discussion

Archaeal Digoxin and Membrane Na^+-K^+ ATPase Inhibition in Relation to Myalgic Encephalomyelitis

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The increase in the activity of HMG CoA reductase in ME suggests an upregulation of the isoprenoid pathway. There is a marked increase in plasma digoxin and dolichol and this increase may be a consequence of increased channeling of intermediates of the isoprenoid pathway for their biosynthesis. In this connection, incorporation of ^{14}C-acetate into digoxin in rat brain has been shown by us indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. In ME there was significant inhibition of the RBC membrane Na^+-K^+ ATPase activity. The inhibition of Na^+-K^+ ATPase by

digoxin is known to cause an increase in intracellular calcium resulting from increased Na^{+}-Ca^{++} exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. This increase in intracellular calcium by displacing magnesium from its binding sites causes a decrease in the functional availability of magnesium. This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation which along with low magnesium can cause further inhibition of Na^{+}-K^{+} ATPase, since ATP-magnesium complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms: ATP dependent calcium extrusion from cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus a progressive inhibition of Na^{+}-K^{+} ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to Na^{+}-K^{+} ATPase inhibition appears to be crucial to the pathophysiology of ME. Serum magnesium was assessed in ME and was found to be reduced.

Archaeal Digoxin and Immune Activation in Myalgic Encephalomyelitis

Increased intracellular calcium activates the calcium dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin-3,4, 5, 6 and TNF alpha. TNF alpha binds to its receptor TNFRI and activates the transcription factors NFkB and AP-l leading to the induction of proinflammatory and immunomodulatory genes. This can explain the immune activation in ME. Polyclonal B cell activation and proliferation have been described in ME. Membrane Na^{+}-K^{+} ATPase inhibition can produce

immune activation and is reported to increase CD_4/CD_8 ratios as exemplified by the action of lithium.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Myalgic Encephalomyelitis

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters and neuroactive alkaloids. Digoxin apart from affecting cation transport is also reported to influence the transport of various metabolites across cellular membranes, including amino acids and various neurotransmitters. Two of the amino acids in this respect are important, tryptophan, a precursor for strychnine, and nicotine and tyrosine, a precursor for morphine. We have already shown the presence of endogenous morphine in the brain of rats loaded with tyrosine and endogenous strychnine and nicotine in the brain of rats loaded with tryptophan. The results showed that the concentration of tryptophan, quinolinic acid, nicotine, strychnine and serotonin was found to be higher in the plasma of patients with ME while that of tyrosine, morphine, dopamine and norepinephrine was lower. Thus there is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the patient's serum. This could be due to the fact that digoxin can regulate neutral amino acid transport systems with preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^+-K^+ ATPase activity in ME could be due to the fact that the hyperpolarising neurotransmitters (dopamine, morphine and noradrenaline) are reduced and the depolarising neuroactive compounds (serotonin, strychnine, nicotine and quinolinic acid) are increased.

The schizoid neurotransmitter pattern of reduced dopamine, noradrenaline and morphine and increased serotonin, strychnine and nicotine is common to ME and schizoid state. This could be the basis of the schizophreniform

psychosis described in ME. Quinolinic acid, an NMDA agonist, can contribute to NMDA excitotoxicity reported in schizoid state. Strychnine by blocking glycinergic transmission can contribute to the decreased inhibitory transmission in schizoid state. Recent data suggest that the initial abnormality in schizoid State involves a hypodopaminergic state and the low dopamine levels now observed agree with this. Nicotine by interacting with nicotinic receptors can facilitate the release of dopamine, promoting the dopaminergic transmission in the brain. This can explain the increased dopaminergic transmission in the brain in the setting of decreased dopamine synthesis. The increased serotoninergic activity and reduced noradrenergic outflow from locus coreuleus reported earlier in the schizoid state agrees with our finding of elevated serotonin and reduced noradrenaline levels in ME and schizophrenia. Quinolinic acid has been implicated in immune activation in other autoimmune diseases like SLE and could contribute to the same in ME. Serotonin, dopamine and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation serotonin is increased with a corresponding reduction in dopamine and noradrenaline in the brainstem monoaminergic nuclei. Thus elevated serotonin and reduced noradrenaline and dopamine could contribute to the immune activation in ME. Endogenous morphine deficiency is noticed in patients with ME. Morphine has an immunosuppressive effect and its deficiency can contribute to immune activation in ME. Quinolinic acid as well as neurotransmitter induced immune activation can promote ME. Thus a schizoid neurotransmitter pattern can predispose to ME.

In the presence of hypomagnesemia, the Mg^{++} block on the NMDA receptor is removed leading to NMDA excitotoxicity. The increased presynaptic neuronal Ca^{++} can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling. Increased intracellular Ca^{++} in the post synaptic neuron can

also activate the Ca^{++} dependent NMDA signal transduction. The plasma membrane neurotransmitter (on the surface of the glial cell and presynaptic neuron) is coupled to a Na^{+} gradient which is disrupted by the inhibition of Na^{+}-K^{+} ATPase, resulting in decreased clearance of glutamate by presynaptic and glial uptake at the end of synaptic transmission. By these mechanisms, inhibition of Na^{+}-K^{+} ATPase can promote glutamatergic transmission. The elevated levels of quinolinic acid and serotonin can also contribute to NMDA excitotoxicity. Quinolinic acid and serotonin are positive modulators of the NMDA receptor. Strychnine can also contribute to NMDA excitotoxicity. Strychnine displaces glycine from its binding sites 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 excitatory transmission. NMDA excitotoxicity has been implicated in neuronal degeneration and could contribute to altered brain function including loss of concentration and memory in ME.

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Myalgic Encephalomyelitis

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The Mg^{++} depletion can affect the metabolism of glycosaminoglycans, glycoproteins and glycolipids. The elevation in the level of dolichol may suggest its increased availability for N-glycosylation of proteins. Magnesium deficiency can lead to increased cerebroside and ganglioside synthesis. In Mg^{++} deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more of glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results show an increase in the concentration of serum total and differential GAG fractions, glycolipids and carbohydrate components of

glycoproteins in ME. The increase in the carbohydrate components total hexose, fucose and sialic acid in ME was not to the same extent suggesting qualitative change in glycoprotein structure. The activity of GAG degrading enzymes and that of glycohydrolases showed significant increase in the serum of ME patients. Intracellular Mg^{++} deficiency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires Mg^{++} for its funetin. The increase in the activity of glycohydrolases and GAG degrading enzymes could be due to reduced lysosomal stability and consequent leakage of lysosomal enzymes into the serum. The increase in the concentration of carbohydrate components of glycoproteins and GAG inspite of increased activity of glycohydrolases may be due to their possible resistance to cleavage by glycohydrolases consequent to qualitative change in their structure. Proteoglycan complexes formed in the presence of altered calcium/magnesium ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate.

The protein processing defect can result in defective glycosylation of exogenous viral glycoprotein antigens with consequent defective formation of MHC-glycoprotein antigen complex. The MHC linked peptide transporter, a P-glycoprotein which transports MHC antigen complex to the antigen presenting cell surface, has an ATP binding site. The peptide transporter is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class-1 (viral glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD_4 or CD_8 cell). Defective presentation of exogenous viral antigens can produce immune evasion by the virus in ME and viral persistence. This could be the reason for the persistence of enterovirus and EB virus in ME. A number of fucose and sialic acids containing natural ligands are involved in adhesion of the lymphocyte, producing leukocyte

trafficking and extravasation into the perivascular space and the same phenomena could contribute to the pathology of ME.

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Myalgic Encephalomyelitis

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. In the alteration in the isoprenoid pathway specifically, cholesterol as well as changes in glycoproteins and GAG can affect cellular membranes. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to an increase in intracellular calcium activating phospholipases A_2 and D. The cholesterol phospholipid ratio of the RBC membrane was increased in ME. The concentration of total GAG, hexose and fucose of glycoprotein decreased in the RBC membrane and increased in the serum suggesting their reduced incorporation into the membrane and defective membrane formation. The glycoproteins, GAG and glycolipids of cellular membrane are formed in the endoplasmic reticulum, which is then budded off as a vesicle which fuses with the golgi complex. The glycoconjugates are then transported via the golgi channel and the golgi vesicle fuses with the cell membrane. This trafficking depends upon GTPases and lipid kinases which are crucially dependent on magnesium and are defective in magnesium deficiency. The change in membrane structure produced by alteration in glycoconjugates and cholesterol: phospholipid ratio can produce changes in the conformation of sodium-potassium ATPase resulting in further membrane Na^+-K^+ ATPase inhibition. The same changes can affect the structure of organelle membrane. This results in defective lysosomal stability and leakage of glycohydrolases and

GAG degrading enzymes into the serum. Defective peroxisomal membranes lead to catalase dysfunction which has been documented in ME.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Myalgic Encephalomyelitis

The archaeaon vitaminocyte contributes to the synthesis of ubiquinone and mitochondrial electron transport chain function. The mitochondrial function related free radical generation is regulated by the archaeaon vitaminocyte synthesized tocopherol and ascorbic acid. The concentration of ubiquinone decreased significantly in ME which may be the result of low tyrosine levels, consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from tyrosine. Ubiquinone, which is an important component of the mitochondrial electron transport chain, is a membrane antioxidant and contributes to free radical scavenging. The increase in intracellular calcium can open up the mitochondrial PT pore causing a collapse of the hydrogen gradient across the inner membrane and an uncoupling of the respiratory chain. Intracellular magnesium deficiency can leads to a defect in the function of ATP synthase. All these lead to defects in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of a superoxide ion which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free radical scavenging. The increase in intracellular calcium may lead to increased generation of NO by inducing the enzyme nitric oxide synthase which combines with superoxide radical to form peroxynitrite. Increased calcium can also activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Increased generation of free radicals like the superoxide ion and hydroxyl radical can produce lipid peroxidation and cell membrane damage which can further inactivate Na^+-K^+ ATPase, triggering the

cycle of free radical generation once again. Magnesium deficiency can affect glutathione synthase and glutathione reductase function. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with an increased intracellular calcium-related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective owing to membrane Na^+-K^+ ATPase inhibition-related defect in membrane formation and leads to reduced catalase activity. There was an increase in lipid peroxidation as evidenced from the increase in the concentration of MDA, conjugated dienes, hydroperoxides and NO with decreased antioxidant protection as indicated by a decrease in ubiquinone and reduced glutathione in ME. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase is decreased in ME suggesting reduced free radical scavenging. Mitochondrial dysfunction related free radical generation could be implicated in the pathogenesis of ME. The mitochondrial dysfunction could account for the muscle pain and fatigability described in ME.

The increased intracellular calcium and ceramide-related opening of the mitochondrial PT pore also leads to volume dysregulation of the mitochondria causing hyperosmolallty of the matrix and expansion of the matrix space. The outer membrane of the mitochondria ruptures and releases an apoptosis inducing factor and cytochrome C into the cytoplasm. This results in activation of caspase-9 and caspase-3. Caspase-9 can produce apoptosis of the cell. Increased apoptosis could also contribute to pathogenesis of ME.

Archaea, RNA Viroids and Retroviruses in Myalgic Encephalomyelitis

Retroviruses have been related to the parthogenesis of ME. The retroviral genome is probably integrated into the genome of mammals including humans as vertically transmitted endogenous proviruses. These retroviral sequences are

transposable. The retroviral transposons are kept silent by DNA methylation. Increased secretion of hypothalamic archaeal digoxin contributes to an intracellular magnesium deficiency, which leads to a DNA methylation defect. DNA methylation requires abundant supply of S-adenosyl methionine which requires magnesium for its generation. In the presence of hyperdigoxinemia, DNA methylation is defective and the retroviral transposons are activated and expressed. This leads to transcription of retroviral proteins and assembly of the virus and on to retroviral persistence and ME.

Archaeal Digoxin and Hemispheric Dominance in Relation to Myalgic Encephalomyelitis

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus the isoprenoid pathway and endogenous Na^+-K^+ ATPase inhibition can play a role in the genesis of the ME. The neurotransmitter patterns of reduced dopamine, morphine and noradrenaline and increased serotonin, strychnine and nicotine is associated with right hemispheric dominance. The digoxin and dolichol synthesis is also increased and ubiquinone levels are low in right hemispheric dominant individuals. The membrane Na^+-K^+ ATPase activity is inhibited and serum magnesium depleted in right hemispheric dominance. Right hemispheric dominant individuals may have an increased predilection for ME. Left hemispheric dominant individuals have reduced digoxin and dolichol levels, increased ubiquinone levels, upregulated RBC membrane Na^+-K^+ ATPase activity, serum hypermagnesemia, increased levels of serum dopamine, noradrenaline and morphine and reduced levels of serum strychnine, nicotine and serotonin. These neurotransmitter patterns and hypodigoxinemia could protect against ME. Thus, myalgic encephalomyelitis may be a reflection of

right hemispheric dominance and the neurotransmitter and immune changes related to it.

(1) NMDA excitotoxicity due to: (a) membrane Na^+-K^+ ATPase inhibition related hypomagnesemia, (b) presence of NMDA agonists like quinolinic acid, strychnine and serotonin.

(2) Digoxin induced hypomagnesemia and elevated dolichol related protein processing defects and defective presentation of viral glycoprotein antigen leading to an immune evasion by the virus and viral persistence.

(3) Mitochondrial dysfunction due to low ubiquinone levels, digoxin induced alteration in intracellular calcium/magnesium ratios and increased ceramide levels leading on to: (a) apoptosis (b) free radical generation.

(4) DNA methylation defect due to digoxin induced hypomagnesemia and retroviral transposon expression.

(5) ME occurs in the right hemispheric dominant state.

References

[1] Kurup RK, Kurup PA. *Hypothalamic Digoxin, Cerebral Dominance and Brain Function in Health and Diseases*. New York: Nova Medical Books, 2009.