5

Archaeal Digoxin Mediated Model for Epilepsy

Changes involving the isoprenoid pathway have been described in primary generalised epilepsy. The isoprenoid pathway produces four key metabolites - ubiquinone (membrane antioxidant and component of the mitochondrial electron transport chain), dolichol (involved in N-glycosylation of proteins), digoxin, (an endogenous inhibitor of membrane Na^+-K^+ ATPase) and cholesterol, (1) Involvement of endogenous digoxin like activity (EDLA) has been reported in the epileptic cortex and injection of digoxin into the spinal lymphaticus sac in frog with an epileptogenic focus resulted in sharp increase in epileptiform discharges, (2) Rapport et al. measured Na^+-K^+ ATPase activity in four human epileptic cortices and found a 60% reduction suggesting a role for endogenous digoxin, (3) Digoxin can regulate the transport of neutral amino acids tyrosine and tryptophan, (4) The tryptophan metabolite, quinolinic acid has recently been implicated in the etiology of temporal lobe epilepsy. Altered dolichol and glycoproteins can also contribute to functional disorders like epilepsy. Disordered synaptic connectivity has been described in these disorders. Increased beta amyloid precursor protein expression and increased levels of alpha acid glycoprotein in the serum have been described in epilepsy. RBC membrane changes have also been described in primary generalised epilepsy. There is increased osmotic fragility of the RBC in epilepsy and the RBC membrane glycoproteins have been reported to be abnormal. Similar changes have been postulated to occur in the neuronal membrane.[1-9]

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. Oxidative stress can open the mitochondrial PT pore producing release of cyto C and activation of the caspase cascade of cell death. 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 produce endothelial dysfunction and vascular disease. 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 neurological disease.

This study was undertaken to assess, (1) the isoprenoid pathway, (2) the tryptophan / tyrosine catabolic pattern, (3) Glycoconjugate metabolism, and (4) RBC membrane changes as a reflection of neuronal and glial membrane change. A hypothesis implicating neuronal and glial membrane Na^+-K^+ ATPase inhibition as pivotal to all these changes is presented.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in primary generalised epilepsy. 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 primary generalised epilepsy. Nicotine and strychnine were detected in the plasma of patients with primary

generalised epilepsy but were not detectable in control serum. Morphine was not detected in the plasma of these patients.

(3) The concentration of total GAG increased in the serum of primary generalized epilepsy patients. The concentration of hyaluronic acid (HA), heparan sulphate (HS) heparin (H) and chondroitin sulphates (ChS) was increased, while that of dermatan sulphate (DS) was decreased. The carbohydrate residues of glycoprotein and glycolipids showed significant increase in the serum of these patients.

(4) The activity of GAG degrading enzymes was increased in primary generalised epilepsy when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase was also increased.

(5) The concentration of total GAG in the RBC membrane was not significantly altered in epilepsy. The concentration of hexose and fucose in the RBC membrane decreased significantly. The concentration cholesterol and phospholipids was significantly decreased in the RBC membrane but the cholesterol: phospholipid ratio in the RBC membrane was not significantly altered.

(6) Concentration of total serum cholesterol and LDL cholesterol increased significantly while HDL cholesterol showed no significant alteration in the plasma in primary generalised epilepsy. Serum triglycerides were unaltered while free fatty acids (FFA) increased.

Discussion

Archaeal Digoxin and Lipid Metabolism - Membrane Na^+-K^+ ATPase Inhibition in Relation to Epileptogenesis

The archaeaon steroidelle contributes to lipid synthesis and metabolism. The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The results showed that HMG reductase activity, serum digoxin and dolichol were increased and serum ubiquinone was reduced in primary generalised epilepsy. Previous studies in this laboratory have demonstrated incorporation of ^{14}C-acetate into digoxin in the rat brain indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The elevated HMG CoA reductase activity correlates well with elevated digoxin levels and reduced RBC membrane Na^+-K^+ ATPase activity. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium and a reduction in intracellular magnesium. Serum magnesium was found to be reduced in primary generalised epilepsy. Membrane Na^+-K^+ ATPase inhibition can produce defective neuronal membrane repolarisation and a paroxysmal depolarisation shift resulting in epileptogenesis.[1-9]

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Epileptogenesis

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters and neuroactive alkaloids. The present study showed that the concentration of tryptophan, quinolinic acid, serotonin, strychnine and nicotine was higher in the plasma of epilepsy patients while that of tyrosine, dopamine, morphine, 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. Endogenous nicotine and strychnine are synthesized from tryptophan and endogenous morphine from tyrosine. This could be due to the fact that digoxin can regulate neutral amino acid transport system, with a preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^+-K^+ ATPase activity in primary generalised epilepsy 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. Dopamine deficiency in primary generalised epilepsy and dopamine receptor blockade producing epileptogenesis have been documented in literature. Low dopamine levels can contribute to the hyperpolactinemia described in epilepsy. The increase in serotonin levels documented here is also significant, as serotonin is a positive modulator of the excitotoxic NMDA receptor. The decrease in noradrenaline observed can also contribute to epileptogenesis, since this catacholamine has been reported to have an antiepileptic action due to its hyperpolarising effect on the neuronal membrane. The neurotransmitter pattern of reduced dopamine, noradrenaline and morphine and increased serotonin, strychnine and nicotine could contribute to epilepsy related psychosis. Quinolinic acid, an NMDA agonist can contribute to NMDA excitotoxicity reported in epilepsy. Strychnine by blocking glycinergic transmission contributes to the decreased inhibitory transmission important in epileptogenesis. Strychnine displaces glycine from its binding sites and the glycine is free to bind to the strychnine insensitive site of the NMDA receptor and promote excitatory NMDA transmission. Nicotine acts as a CNS stimulant and has been reported to promote epileptogenesis.

In the presence of hypomagnesemia, the magnesium block on the NMDA receptor is removed leading to NMDA excitotoxicity. The increased levels of FFA can contribute to epileptogenesis by binding magnesium. This results in

the formation of magnesium soaps in the blood and hypomagnesemia. The increased presynaptic neuronal calcium can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased glutamate release into the synaptic junction and vesicular recycling. Increased intracellular calcium in the post synaptic neuron can also activate the NMDA signal transduction in the postsynaptic neuron. The membrane glutamate transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a sodium 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 and excitotoxicity contributing to epileptogenesis.[1-9]

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Epileptogenesis - The Glycosaminoglycoid

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The increased availability of dolichol for N-glycosylation of proteins and intracellular Mg^{++} deficiency can upregulate the synthesis of glycosaminoglycans, glycolproteins and glycolipids. The increase in the carbohydrate components-total hexose, fucose and sialic acid was not to the same extent suggesting a qualitative change in glycoprotein structure. Proteoglycan complexes formed in the presence of altered intracellular calcium/magnesium ratios may be structurally abnormal and resistant to lysosomal enzymes and may accumulate. The activity of GAG degrading enzymes and that of glycohydrolases showed significant increase in the serum consequent to reduced lysosomal stability resulting from an alteration in lysosomal membranes. Altered glycoconjugates of the neuronal membrane can lead to disordered synaptic connectivity and the altered sulphated proteoglycan

matrix of synaptic vesicles can modulate neurotransmission leading on to epileptogenesis.[1-9]

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Epileptogenesis

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. The concentration of cholesterol and phospholipids as well as carbohydrate residues of glycoproteins decreased in the RBC membrane in epilepsy suggesting that their incorporation into the RBC membrane is defective consequent to inhibition of membrane trafficking enzymes - lipid kinases and GTPases in the presence of magnesium deficiency. Altered membrane structure can contribute to membrane Na^+-K^+ ATPase inhibition and also defective lysosomal stability. This can contribute to epileptogenesis.[1-9]

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Epileptogenesis - The Vitaminocyte

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. In primary generalised epilepsy there is a mitochondrial dysfunction and increased generation of free radicals consequent to, (i) Digoxin induced decreased tyrosine availability which leads to inhibition of ubiquinone synthesis, (ii) Increased intracellular calcium opening up the mitochondrial PT pore and reduced intracellular magnesium inhibiting ATP synthase, and (iii) Increased intracellular calcium inducing NO synthase and liberating NO. There is reduced free radical scavenging owing to ubiquinone

deficiency. A mitochondrial dysfunction can remove the magnesium block of the NMDA receptor contributing to glutamate excitotoxicity and epileptogenesis. The opening of the mitochondrial PT pore leads to rupture of the outer membrane, and release of cytochrome C with consequent activation of caspase-9 and the apoptotic cascade. Disordered apoptosis can produce defective synaptogenesis and synaptic connectivity contributing to epileptogenesis.[1-9]

Archaeal Digoxin and Immunoregulation in Relation to Epileptogenesis - The Fructosoid, Steroidelle and Viroidelle

The archaeaon fructosoid contributes to fructolysis and immune activation. Fructose can contribute to induction of NFKB and immune activation. The archaeaon steroidelle synthesized digoxin induces NFKB producing immune activation. The archaeaon viroidelle secreted RNA viroids can produce immune activation by blocking mRNA function. In primary generalised epilepsy increased intracellular calcium activates the calcium dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin-1 and TNF alpha (Tumour necrosis factor alpha). TNF alpha can bind to its receptor TNFR1 and activates the transcription factors NFkB and AP-l leading to the induction of proinflammatory and immunomodulatory genes. This leads to immune activation documented in primary generalised epilepsy.[1-9]

Archaeal Digoxin, Oncogene Activation and Epileptogenesis

The archaeaon secreting RNA viroids is called the viroidelle. The primitive archaeal DNA is integrated along with RNA viroids which are converted to their corresponding DNA by the action of redox stress induced HERV reverse transcriptase into the human genome by the redox stress induced HERV integrase. The archaeal DNA sequences that are integrated into the human genome forms endogenous archaeal human genomic sequences akin to HERV

sequences and can function as jumping genes regulating genomic DNA flexibility. The integrated endogenous genomic archaeal sequences can get expressed in the presence of redox stress forming endosymbiotic archaeal particles which can function as a new organelle called the archaeaons. In primary generalised epilepsy there is an oncogenic tendency owing to, (i) increased intracellular calcium activating phospholipase C beta which results in increased production of diacyglycerol (DAG) with consequent activation of protein kinase C and the MAP kinase cascade, (ii) The decreased intracellular magnesium can produce dysfunction of GTPase activity of the alpha-subunit of G-protein and ras oncogene activation, as more of the ras is bound to GTP rather than GDP, (iii) The activation of P_{53} is impaired owing to intracellular magnesium deficiency producing a phosphorylation defect. This can lead on to development of benign neural tumours described in histopathological sections of surgically resected temporal lobe tissues in epilepsy.[1-9]

Archaeal Digoxin and Hemispheric Dominance in Relation to Epileptogenesis

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Primary generalised epilepsy could thus be considered as a syndrome of paroxysmal digoxin hypersecretion consequent to an upregulated isoprenoid pathway. The upregulated isoprenoid pathway and hyperdigoxinemia is suggestive of right hemispheric dominance. Right hemispheric dominance can contribute to epileptogenesis.[1-9]

References

[1] Kurup RK and Kurup PA. Hypothalamic digoxin-mediated model for epileptogenesis *Acta Neuropsychiatrica*. 2003; 15(3): 115-121.

[2] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and lipid metabolism. *Int. J Neurosci*. 2003 Jan; 113 (1): 107-15.

[3] Kurup RK, Kurup PA. Hypothalamic digoxin, hemispheric chemical dominance, and endocrine / metabolic / cellular regulation. *Int. J. Neurosci*. 2002 Dec; 112(12): 1421-38.

[4] Kurup RK, Kurup PA. Hypothalamic digoxin, hemispheric chemical dominance, and Alzheinmer's disease. *Int. J. Neurosci*. 2003 Mar; 113(3): 361-81.

[5] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and mitochondrial function / free radical metabolism. *Int. J Neurosci*. 2002 Dec; 112 (12): 1409-20.

[6] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral dominance, and Golgi body / lysosomal function. *Int. J Neurosci*. 2002 Dec; 112 (12): 1449-59.

[7] Kurup RK, Kurup PA. Hypothalamic digoxin cerebral dominance and membrane biochemistry. *Int. J Neurosci*. 2002 Dec: 112(12): 1439-47.

[8] Kurup RK, Kurup PA. Isoprenoid pathway - related membrane dysfunction to neuropsychiatric disorders. *Int. J Neurosci*. 2003; 113(11): 1579-1591.

[9] Kurup RK, Kurup PA. Hypothalamic digoxin, cerebral chemical dominance, and calcium/magnesium metabolism. *Int. J. Neurosci*. 2003 Jul; 113(7): 999-1004.

[10] Kurup RK, Kurup PA. Hypothalamic digoxin-mediated model for Parkinson's disease. *Int J Neurosci*. 2003; 113(4): 515-36.

[11] Kurup RK and Kurup PA. Hypothalamic digoxin and hypomagnesemia in relation to the pathogenesis of multiple sclerosis. *The Journal of Trace Elements in Experimental Medicine*. 2002; 15(4): 211-220.

[12] Kurup RK, Kurup PA. Isoprenoid pathway - related membrane dysfunction to neuropsychiatric disorders. *Int. J Neurosci*. 2003; 113(11): 1579-1591.

[13] Kurup RK, Kurup PA. Hypothalamic digoxin, hemispheric dominance, and neuroimmune integration. *Int J Neurosci*. 2002 Apr; 112(4): 441-62.

[14] Ravikumar A, Arun P, Devi KV, Augustine J, Kurup PA. Isoprenoid pathway and free radical generation and damage in neuropsychiatric disorders. *Indian J Exp Biol*. 2000 May; 38(5): 438-46.

[15] Ravikumar A, Deepadevi KV, Arun P, Manojkumar V, Kurup PA. Tryptophan and tyrosine catabolic pattern in neuropsychiatric disorders. *Neurol India*. 2000 Sep; 48(3): 231-8.

[16] 16. A. Ravikumar, P. Arun, K. V. Deepadevi and P. A. Kurup; Endogenous strychinine, nicotine and morphine - description of hypo and hyper - stychninergic, nicotinergic and morphinergic state in relation to neuropsychiatric disorders. *Indian Journal of Experimental Biology*, 2000; 38, 559-566.