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Archaeal Digoxin and Alzheimer's Disease

Introduction

Changes involving the isoprenoid pathway have been described in neuronal degeneration. Mitochondrial dysfunction particularly involving ubiquinone has been reported in platelet mitochondria in Alzheimer's disease (AD). Altered levels of dolichol in the brain and altered glycoproteins like beta amyloid have been reported in Alzheimer's disease. Dolichol and ubiquinone are both products of the isoprenoid pathway. Sodium potassium ATPase inhibition has been reported to lead to neuronal degeneration. An endogenous inhibitor of Na^{+}-K^{+} ATPase archaeal digoxin is produced by the isoprenoid pathway. It was therefore considered pertinent to study the isoprenoid pathway in Alzheimer's disease.

Archaeal digoxin induced membrane Na^{+}-K^{+} ATPase inhibition can produce Mg^{++} depletion leading on to altered glycoconjugate metabolism. The dolichol pathway can regulate N-glycosylation of glycoproteins. Alteration in glycoconjugate metabolism has also been described in neuronal degeneration. Amyloid and hyperphosphorylated tau protein in Alzheimer's disease are defectively processed proteins resistant to the action of proteases. Accumulation of heparan sulphate proteoglycan (HS-PG) and its interaction with beta amyloid precursor protein (beta APP) have been suggested as a possible mechanism for amyloid plaque formation in Alzheimer's disease. Glycolipids like ceramide can produce cell death by opening up the mitochondrial PT pore and contribute to neuronal degeneration and aging.

Archaeal digoxin can alter intracellular Ca^{++}/Mg^{++} ratios in the cell leading on to free radical generation. Alteration in the ubiquinone pathway can also lead to mitochondrial dysfunction and free radical generation. Free radical mechanisms have been implicated in the pathogenesis of the major neurodegenerative disorders like Alzheimer's disease. An altered free radical defence mechanism like decreased levels of antioxidant enzymes and

antioxidants has been suggested for the free radical damage in Alzheimer's disease. Increased formation of NO which forms peroxynitrite with superoxide promoting lipid peroxidation has also been reported in neurodegeneration.

Archaeal digoxin has been reported to regulate the transport of amino acids especially the neutral amino acids. Abnormalities in tryptophan catabolism and the kynurenine pathway have been described in neuronal degeneration. Of the kynurenines, quinolinic acid is most relevant to neurodegenerative disease. Quinolinic acid toxicity in the brain appears to be mediated through its action as an agonist of the NMDA receptor. Neurotoxicity induced by quinolinic acid occurs preferentially in the neocortex, striatum and hippocampus in the brain. The neurotoxic effects of quinolinic acid are mediated via the magnesium sensitive NMDA receptor.

Membrane abnormalities have been described in neurodegenerative disorders. In Alzheimer's disease alteration in membrane fluidity especially of the platelet membrane has been reported. The isoprenoid pathway produces four metabolites important in maintaining cell membrane structure and function - digoxin (a membrane Na^+-K^+ ATPase inhibitor), dolichol (involve in N-glycosylation of proteins), ubiquinone (a membrane antioxidant) and cholesterol.

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 patterns, (3) glycoconjugate metabolism, and (4) RBC membrane changes as a reflection of neuronal membrane change in Alzheimer's disease. A hypothesis implicating neuronal membrane Na^+-K^+ ATPase inhibition as pivotal to all these changes in Alzheimer's disease is also presented. Since digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of cerebral dominance. The isoprenoid pathway and digoxin status was studied in individuals of differing hemispheric dominance in order to elucidate the role of cerebral dominance in the pathogenesis of Alzheimer's disease.[1-8]

Methods

Fifteen cases of Alzheimer's disease were chosen randomly for the study from the medicine and neurology wards of Medical College, Trivandrum. The lCD-10 criterion was chosen for the purpose of the study. The age of the patients ranged from 50 to 70 years. None of the subjects studied was under

medication at the time of removal of blood. All the patients included in the study were non-smokers (passive and active). They were free from other systemic diseases like hypertension, diabetes, renal and hepatic disease. An equal number of age and sex matched healthy subjects served as controls.

15 normal male healthy individuals (50-70 years of age) each of the left handed/right hemispheric dominant, right handed/left hemispheric dominant and amphidextrous / bihemispheric dominant individuals diagnosed by the dichotic listening test were chosen for the study. This group was chosen at random from the general population of Trivandrum city. These individuals were not on any drugs like digoxin and were free from any systemic disease. All individuals in this group also were non-smokers (passive or active).

Fasting blood was removed from each of the patients/individuals for various estimations. RBCs were separated within 1 hour of collection of blood for the estimation of membrane Na^{+}-K^{+} ATPase. Serum was used for the estimation of HMG CoA reductase activity. Plasma/serum was used for the estimation of the other parameters. All biochemicals used in this study were obtained from M/s Sigma Chemicals, USA. Activity of HMG CoA reductase of the plasma was determined using the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate. For the determination of the Na^{+}-K^{+} ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used. Digoxin in the plasma was determined by the procedure described by Arun et al. For estimation of ubiquinone and dolichol in the plasma, the procedure described by Palmer et al. was used. Mg^{++} in the plasma was estimated by atomic absorption spectrophotometry. Tryptophan was estimated by the method of Bloxam and Warren and tyrosine by the method of Wong et al. Serotonin was estimated by the method of Curzon et al. and catecholamines by the method of Well-Malherbe et al. Quinolinic acid content of plasma was 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. Details of the procedures used for the estimation of total and individual GAG, carbohydrate components of glycoproteins, activity of enzymes involved in the degradation of GAG (beta glucuronidase, beta N-acetyl hexosaminadase, hyaluronidase and cathepsin-D) and activity of glycohydrolases (beta galactosidase, beta fucosidase and beta lucosidase) have been described before by Kurup et al. Serum glycolipids (gangliosides, glycosyl diglycerides, cerebrosides and sulphatides) were estimated as described in methods in enzymology. Cholesterol was estimated by using commercial kits supplied by Sigma chemicals, USA. SOD was assayed by the method of Nishikimi et al. as modified by Kakkar et al. Catalase activity was estimated by the method of Maehly and Chance, glutathione peroxidase by the method of Paglia and Valentine as modified by Lawrence and Burk and glutathione reductase by the method of Horn and Burns. MDA was estimated by the method of Wills et al. and conjugated dienes and hydroperoxides by the procedure of Brien et al. Reduced glutathione was estimated by the method of Beutler et al. Extraction of erythrocytes for vitamin E was carried out according to the procedure described by Cohn et al. and vitamin E estimated in the extract by HPLC (Waters HPLC, Nova-Pak C_8 column 4.6 x 150 mm). Solvent-acetonitrile: methanol: water (63:33:4), flow rate - 2 ml/min, detection UV (280 nm). For vitamin E, retention time was 3.5 mm under these conditions. Nitric oxide was estimated in the plasma by the method of Gabor and Allon. Iron binding capacity in the plasma was estimated by the method of Wootton and ceruloplasmin by the method of Henry et al. Free fatty acid were estimated by the method of Falholt et al. Statistical analysis was done by ANOVA'.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in AD. 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 AD.

(3) Nicotine and strychnine were detected in the plasma of patients with AD but were not detectable in control serum. Morphine was not detected in the plasma of these patients.

(4) The concentration of total glycosaminoglycans (GAG) and different GAG fractions increased in the serum of AD patients. The concentration total hexose, fucose and sialic acid were 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 in these patients.

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

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

(7) Concentration of total serum cholesterol and LDL cholesterol was not significantly altered while HDL cholesterol showed a significant decrease in the plasma in AD. Serum triglycerides and free fatty acids (FFA) increased in AD.

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

(9) 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 Alzheimer's Disease

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 CoA reductase activity, serum digoxin and dolichol were increased and serum ubiquinone was reduced in AD. 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 Ca^{++} resulting from increased Na^+-Ca^{++} exchange, increased entry of Ca^{++} via the voltage gated Ca^{++} channel and increased release of Ca^{++} from intracellular endoplasmic reticulum Ca^{++} stores. This increase in intracellular Ca^{++} by displacing Mg^{++} from its binding sites causes a decrease in the functional availability of Mg^{++}. This decrease in the availability of Mg^{++} can cause decreased mitochondrial ATP formation which along with low Mg^{++} can cause further inhibition of Na^+-K^+ ATPase, since the ATP-Mg^{++} complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms, ATP dependent calcium extrusion from the cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The Mg^{++} 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 Mg^{++} and

high intracellular Ca^{++} consequent to Na^{+}-K^{+} ATPase inhibition appear to be crucial to the pathogenesis of AD. Serum Mg^{++} was found to be reduced in AD.[1-8]

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Alzheimer's Disease

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 had 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 present study shows that the concentration of tryptophan, quinolinic acid, and serotonin was higher in the plasma of AD patients while that of tyrosine, dopamine and norepinephrine was lower. Serum of the patients with AD showed the presence of nicotine and strychnine. Morphine was absent in the serum of these patients. Thus there is an increase in tryptophan and its catabolites (serotonin, nicotine, strychnine and quinolinic acid) and a reduction in tyrosine and its catabolites (dopamine, norepinephrine and morphine) in the patient's serum. This could be due to the fact that digoxin can regulate the neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. Increased neuronal tryptophan load and reduced neuronal tyrosine load can upregulate tryptophan catabolism and down regulate the catabolism of tyrosine. The increase in serotonin levels and decrease in dopamine and noradrenaline could contribute to the psychiatric manifestations and cognitive dysfunction described in AD. Nicotine acts as a CNS stimulant

and can bind to the central nicotinic receptors producing a biphasic effect with initial stimulation followed by inhibition. Prolonged nicotinic action may produce degeneration of the nicotinic cholinergic receptors in a phenomenon similar to glutamate excitotoxicity. Membrane Na^{+}-K^{+} ATPase inhibition can lead on to increased glutamatergic excitatory transmission important in the pathogenesis of AD. The decrease in membrane Na^{+}-K^{+} ATPase activity in AD 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 as also to increased digoxin levels. In the presence of hypomagnesemia, consequent to Na^{+}-K^{+} ATPase inhibition, the Mg^{++} block on the NMDA receptor is removed leading to NMDA excitotoxicity. The increased levels of FFA can contribute to NMDA excitotoxicity by binding Mg^{++}. This results in the formation of Mg^{++} soaps in the blood and hypomagnesemia. The increased presynaptic neuronal Ca^{++} can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased glutamate 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 glutamate transporter (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 excitatory glutamatergic transmission. Serotonin and quinolinic acid are NMDA agonist and positive modulators and could contribute to increased NMDA transmission. Strychnine by blocking glycinergic transmission contributes to the decreased inhibitory transmission in the brain. 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. NMDA excitotoxicity contributes to neuronal degeneration in AD by increasing the intracellular Ca^{++} levels.[1-8]

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Alzheimer's Disease - The Glycosaminoglycoid

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The membrane Na^{+}-K^{+} ATPase inhibition related Mg^{++} depletion and elevated dolichol levels 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 on to defective metabolism of sphinganine producing its accumulation, which may lead to increased cerebroside and ganglioside synthesis. In Mg^{++} deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results show an increase in the concentration of serum total GAG, glycolipids and carbohydrate components of glycoproteins in AD. The increase in the carbohydrate components - total hexose, fucose and sialic acid - in AD was not to the same extent suggesting qualitative change in glycoprotein structure. The activity of GAG degrading enzymes and glycohydrolases was increased in AD when compared to the controls. Intracellular Mg^{++} deficiency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires Mg^{++} for its function. Defective ubiquitin dependent proteolytic processing of proteins has been described in AD. 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 in spite of increased activity of many 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 Ca^{++}/Mg^{++} ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate.

Previous reports of alteration in glycoproteins in this connection include the beta amyloid in AD. Structurally abnormal glycoproteins resist catabolism by lysosomal enzymes and accumulate in neuronal degeneration. Interaction between HS-proteoglycan and ChS proteoglycan with proteins like beta amyloid and tau protein and reduced proteolytic digestion of these complexes can lead on to their accumulation in the neurons. Alteration in the sulphated proteoglycan matrix of the synaptic vescicles can alter acetyl choline release into the synapse and contribute to the pathogenesis of AD. Altered glycoproteins, glycolipids and GAG of the neuronal membrane can also contribute to AD by producing disordered synaptic connectivity in the central cholinergic pathways. The protein processing defect can result in defective glycosylation of endogenous neuronal glycoprotein antigens and exogenous viral glycoproteins antigens with consequent defective formation of the MHC antigen complex. The MHC linked peptide transporter, a P-glycoprotein which transports the MHC-antigen complex to the antigen presenting cell surface, has an ATP binding site which is dysfunctional in the presence of Mg^{++} deficiency. This results in defective transport of the MHC class-1 glycoprotein antigen complex to the antigen presenting cell surface for recognition by the CD_4 or CD_8 cell. Defective presentation of the endogenous neuronal glycoprotein antigen can explain the immune dysregulation and autoimmunity described in neuronal degenerations like Alzheimer's disease. Defective presentation of exogenous viral antigens can produce immune evasion by the virus as in viral persistence leading on to neuronal degenerations like AD. A number of fucose and sialic acid containing natural ligands are involved in trafficking of

leukocytes and the inflammatory cell process and could contribute to similar phenomena described in neuronal degeneration.[1-8]

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Alzheimer's Disease

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. 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 Mg^{++} deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipase A_2 and D. The cholesterol: phospholipid ratio of the RBC membrane was decreased in AD. The concentration of total GAG, cholesterol, hexose and fucose of glycoprotein decreased in the RBC membrane and increased (unaltered in the case of cholesterol) serum suggesting their reduced incorporation into the membrane and defective membrane formation. The glycoproteins, GAG and glycolipids of the 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 Mg^{++} deficiency. The change in membrane structure produced by alteration in glycoconjugates and cholesterol: phospholipid ratio can produce changes in the conformation of Na^{+}-K^{+} ATPase resulting in further membrane Na^{+}-K^{+} ATPase inhibition. The same changes can affect the structure of the 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 AD.[1-8]

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Alzheimer's Disease - 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. The concentration of ubiquinone decreased significantly in AD which may be the result of low tyrosine levels, consequent to digoxins effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from the 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 Ca^{++} can open the mitochondrial PT pore causing a collapse of the H^{+} gradient across the inner membrane and uncoupling of the respiratory chain. Intracellular Mg^{++} deficiency can lead to a defect in the function of ATP synthase. All this leads to a defect in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of the 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 the superoxide radical to form peroxynitrite. Increased calcium also can 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 again.

There was 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 decrease in ubiquinone, reduced glutathione and alpha tocopherol in AD. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in AD suggesting reduced free radical scavenging. In our study the iron binding capacity and serum ceruloplasmin are reduced suggesting increased amounts of free iron and copper, promoting free radical generation. Ceruloplasmin is a 132 KD monomeric copper oxidase which has been implicated in iron metabolism because of its catalytic oxidation of Fe^{2+} to Fe^{3+} (ferroxidase activity). In the presence of iron in Fe^{2+} form the conversion of H_2O_2 to hydroxyl radical is greatly increased. Low ceruloplasmin results in more of the iron to be in Fe^{2+} form. It has been shown that ceruloplasmin increases iron uptake by cells increasing the apparent affinity for the substrate by three times. Low ceruloplasmin levels can result in decreased iron uptake and this results in increased amount of free iron. The intracellular magnesium deficiency can produce ribosomal dysfunction and inhibition of protein synthesis as noted by decrease in serum albumin in these cases. The low iron binding capacity and low serum ceruloplasmin levels may be a consequence of reduced ferritin and ceruloplasmin synthesis. The peroxisomal membrane is defective owing to the membrane Na^{+}-K^{+} ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Glutathione is synthesized by the enzyme glutathione synthetase which needs magnesium and ATP. The low intracellular Mg^{++} consequent to Na^{+}-K^{+} ATPase inhibition and the resulting low ATP can result in decreased synthesis of glutathione. Glutathione peroxidase, a selenium containing enzyme oxidises reduced glutathione (GSH) to oxidised glutathione (GSSG) which is then rapidly reduced to GSH by glutathione reductase. There is also a concomitant conversion of H_2O_2 to H_2O.

The activity of glutathione reductase needs NADPH for the regeneration of GSH. This NADPH comes mostly from the pentose phosphate pathway. Intracellular magnesium deficiency due to membrane Na^{+}-K^{+} ATPase inhibition leads to decreased formation of glucose 6-phosphate and down regulation of the pentose phosphate pathway with consequent decreased generation of NADPH. Thus the glutathione system of free radical scavenging is defective in the presence of membrane sodium potassium ATPase inhibition. Superoxide dismutase exists in a mitochondrial and cytoplasmic form. The opening of the mitochondrial PT pore produces hyperosmolality and matrix expansion rupturing the outer membrane producing loss of the mitochondrial dismutase and decrease in its activity. The reduction in catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase suggests reduced free radical protection. Mitochondrial dysfunction related free radical generation has been implicated in the pathogenesis of the neuronal degeneration like AD. Mitochondrial dysfunction can remove the Mg^{++} block of the NMDA receptor leading on to excitotoxicity and neuronal degeneration.[1-8]

Archaeal Digoxin and Immunoregulation in Relation to Alzheimer's Disease - 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. Increased intracellular Ca^{++} activates the Ca^{++} dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin - 3, 4, 5, 6 and TNF alpha (Tumour necrosis factor alpha). This can also explain the immune activation in some neuronal degenerations like Alzheimer's disease. TNF alpha can also bring about apoptosis of the cell. It binds to its receptor and activates caspase-9 an ICE protease which converts

IL-1 beta precursor to IL-1 beta. IL-1 beta produces apoptosis of the neurons in neuronal degenerations. 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.

Cell death is also mediated by increased intracellular Ca^{++} and ceramide related opening of the mitochondrial PT pore causing a collapse of the H^{+} gradient across the inner membrane and uncoupling of the respiratory chain. 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. This results in procaspase-9 activation to caspase-9 which produces cell death. Caspase-9 activates CAD (caspase activated deoxyribonuclease) which cleaves the nuclear membrane lamins and several proteins involved in cytoskeletal regulation like elsolin which cleaves actin. Apoptosis has also been implicated in neuronal aging and neuronal death in neuronal degeneration. We have been able to demonstrate neuronal degeneration and apoptosis in the digoxin injected rat brain.

Intracellular Mg^{++} depletion can produce defective phosphorylation of MAP (microtubule sociated proteins). This results in defective microtubule polymerization / depolymerisation and axonal transport contributing to neuronal degeneration.[1-8]

Archaeal Digoxin and Lipid Metabolism - in Relation to Alzheimer's Disease

The archaeaon steroidelle contributes to lipid synthesis and metabolism. Low HDL cholesterol is another feature observed in AD. Low HDL cholesterol and increased triglycerides is suggestive of insulin resistance. Insulin resistance has been described in Alzheimer's disease. A low HDL cholesterol level indicates

reduced reverse cholesterol transport to the liver for degradation. HDL cholesterol has been related to neuronal regeneration and has a neurotrophic effect. Altered reverse transport of cholesterol and abnormal redistribution of HDL particles have been reported in neuronal degeneration. This may relate low HDL cholesterol to AD. Serum free fatty acids are significantly increased in AD. This may indicate increased lipolysis or decreased mitochondrial fatty acid oxidation or both. Magnesium deficiency can lead to activation of lipoprotein lipase and lipolysis. Increased intracellular calcium by opening up the mitochondrial PT pore can produce mitochondrial dysfunction. The mitochondrial dysfunction can cause reduced beta oxidation of fatty acid leading to an increase in the free fatty acid level. Free fatty acids themselves can produce Na^{+}-K^{+} ATPase inhibition. There was no alteration in serum cholesterol despite elevated HMG CoA reductase activity probably because most of HMG CoA was channelized for the synthesis of other isoprenoid metabolites like dolichol or digoxin. The RBC membrane cholesterol was reduced despite no alteration in the serum suggesting reduced uptake into the RBC membrane.[1-8]

Archaeal Digoxin and Hemispheric Dominance in Relation to Alzheimer's Disease

The archaeaon related organelle-steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus Alzheimer's disease and neuronal degeneration can happen due to a defect in the isoprenoid pathway. The following mechanisms are involved, (i) NMDA excitotoxicity consequent to hypomagnesemia, digoxin related membrane Na^{+}-K^{+} ATPase inhibition, ubiquinone deficiency and mitochondrial dysfunction removing the Mg^{++} block and elevated levels of positive modulators of the NMDA receptor (quinolinic acid, serotonin and strychnine), (ii) Dolichol related and Mg^{++} depletion related

altered glycoproteins and proteoglycan and possibly complexes formed between them which resist lysosomal digestion, (iii) defective membrane formation and structure contributing to Na^{+}-K^{+} ATPase inhibition, lysosomal instability and peroxisomal dysfunction, (iv) mitochondrial dysfunction due to low ubiquinone levels, altered Ca^{++}/Mg^{++} ratios intracellularly and ceramide leading to free radical generation, and (v) membrane Na^{+}-K^{+} ATPase inhibition related immune activation, apoptosis and defective presentation of neuronal antigens.

Alzheimer's disease can thus be considered as being due to hypothalamic archaeal digoxin hypersecretion consequent to an upregulated isoprenoid pathway. The biochemical patterns obtained in Alzheimer's disease correlated with those obtained in right hemispheric chemical dominance. In left handed / right hemispheric dominant individuals there was a derangement of the isoprenoid pathway. They had an upregulated HMG CoA reductase activity with increased digoxin and dolichol levels and reduced ubiquinone levels. The RBC membrane Na^{+}-K^{+} ATPase activity was reduced and serum magnesium depleted. The left handed/right hemispheric dominant individuals had increased levels of tryptophan, serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine, dopamine, noradrenailne and morphine were lower. Thus an upregulated isoprenoid pathway, increased level of tryptophan and its catabolites, decreased levels of tyrosine and its catabolites and hyperdigoxinemia is suggestive of right hemispheric dominance. In right handed/left hemispheric dominant individuals the biochemical patterns were reversed. Alzheimer's disease occurs in right hemispheric chemically dominant individuals and could be a reflection of altered brain function.[1-8]

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