4

Archaeal Digoxin Mediated Model for Reye's Syndrome

Reye's syndrome is characterised by vomiting and signs of progressive central nervous system damage, signs of hepatic injury, and hypoglycemia. There is a mitochondrial dysfunction and decrease in the activity of hepatic mitochondrial enzymes. The cause of Reye's syndrome is unknown although viral agents and drugs, especially salicylates, have been implicated. In fatal cases the liver is enlarged and yellow with striking diffuse fatty microvacuolization of cells. Peripheral zonal hepatic necrosis also has been present. Fatty changes of the renal tubular cells, cerebral edema and neuronal degeneration of the brain are the major extrahepatic changes. Electron-microscopic studies show structural alterations of mitochondria in liver, brain, and muscle.

The isoprenoid pathway is a key regulatory pathway in the cell. It produces 4 key metabolites important in regulation of cellular function - cholesterol, an important component of cellular membranes; ubiquinone, an important membrane antioxidant and component of the mitochondrial electron transport chain; dolichol, important in N-glycosylation of proteins and endosymbiotic archaeal digoxin, an endogenous membrane Na^+-K^+ ATPase inhibitor. Since mitochondrial dysfunction is important in the pathogenesis of Reye's syndrome it was considered pertinent to study the isoprenoid pathway in patients with Reye's syndrome. Hemispheric dominance can play a role in regulating cellular functions. Since digoxin can regulate multiple neurotransmitter systems, the pathway was also assessed in individuals with differing hemispheric dominance in order to find out the role of hemispheric dominance in the genesis of the disease.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in Reye's syndrome. 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 Reye's syndrome. Nicotine and strychnine were detected in the plasma of patients with Reye's syndrome but were not detectable in control serum. Morphine was not detected in the plasma of these patients.

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

(4) Concentration of total serum cholesterol was unaltered in Reye's syndrome. HOL cholesterol decreased significantly in Reye's syndrome. LDL cholesterol was also not significant. Plasma triglycerides were increased in Reye's syndrome. Concentration of free fatty acid increased in Reye's syndrome.

(5) 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.

(6) 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,

dopainine, 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 Reye's Syndrome

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 Reye's syndrome. 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 ATP-Mg^{++} complex is the actual substrate for this reaction. Cyrtosolic free calcium is normally buffered by 2 mechanisms, ATP dependent

calcium extrusion from 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 Reye's syndrome. Serum Mg^{++} was found to be reduced in Reye's syndrome. The increased digoxin synthesis in Reye's syndrome is significant. Digoxin administration in experimental animals has been reported to lead to brain oedema and vacuolar changes in the brain. The refractory brain oedema in Reye's syndrome could be due to increased digoxin levels.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Reye's Syndrome

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 Reye's syndrome patients while that of tyrosine, dopamixie and norepinephrlne was lower. Serum of patients with Reye's syndrome 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 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 decrease in membrane Na^{+}-K^{+} ATPase activity in Reye's syndrome 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 well 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 free fatty acids 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. Increased NMDA excitotoxicity could contribute to the seizures in Reye's syndrome. NMDA excitotoxicity could also contribute to the neuronal degeneration observed in Reye's syndrome.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Reye's Syndrome

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 Reye's syndrome 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 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 superoxide ions 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 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 an increase in lipid peroxidation as evidenced by 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 Reye's syndrome. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in Reye's syndrome 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 an increased amount of free iron. The intra cellular magnesium deficiency can produce ribosomal dysfunction and inhibition of protein synthesis as noted by a 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 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 concommitant 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 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 Reye's syndrome. Free radicals and mitochondrial dysfunction can also produce degenerative changes in the neurons.

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 gelsolin which cleaves actin. Apoptosis could contribute to hepatic dysfunction and hepatic necrosis in Reye's syndrome. Apoptosis can also contribute to neuronal cell death in Reye's syndrome.

The mitochondrial dysfunction can lead to reduced beta oxidation of fatty acids. This leads to fatty acid accumulation in cells. The digoxin induced hypomagnesemia can inhibit the function of lipoprotein lipase. Lipoprotein lipase is concerned with triglyceride catabolism. This leads to accumulation of triglycerides within the cells. This could be the basis for fatty micro vacuolization in renal tubular cells and liver cells in Reye's syndrome. The lipid abnormality in Reye's syndrome of increased triglyceride and low HDL cholesterol is similar to that obtained in syndrome X and insulin resistance states.

Archaeal Digoxin and Hemispheric Dominance in Relation to Reye's Syndrome

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. Thus the altered mitochondrial function in Reye's syndrome could be due to a defective isoprenoid pathway. The biochemical pattern in Reye's syndrome is correlated with those obtained in right hemispheric dominance. In right hemispheric dominant individuals there is an upregulated isoprenoid pathway and increased digoxin synthesis. In left hemispheric dominant individuals there is a downregulated isoprenoid pathway and reduced digoxin synthesis. Hemispheric dominance could decide the predisposition to the development of Reye's syndrome.

References

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