Chapter 5

Archaeal Digoxin and Mitochondrial Function - Relation to Free Radical Metabolism

Introduction

Global warming induces a genomic change in humans. Global warming induces endosymbiotic archaeal and RNA viroidal growth. The porphyrins form a template for the formation of RNA viroids, DNA viroids, prions, isoprenoids and polysaccharides. They can symbiose together to form primitive archaea. The archaea can further induce HIF alpha, aldose reductose and fructolysis resulting in further porphyrinogenesis and archaeal self replication. 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. The archaeaon can express the fructolytic pathway constituting an organelle called the fructosome, cholesterol catabolic pathway and digoxin synthetic forming an organelle called the steroidelle, the shikimic acid pathway forming an organelle called the neurotransminoid, antioxidant vitamin E and vitamin C synthetic organelle called the vitaminocyte as well as the glycosaminoglycan synthetic organelle called glycosaminoglycoid. The archaeaon secreting RNA viroids is called the viroidelle.

The increase in endogenous EDLF, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The results showed increased endogenous EDLF synthesis as evidenced by increased HMG CoA reductase activity, which functions as the rate limiting step of the isoprenoid pathway.

Studies in our laboratory have demonstrated that EDLF is synthesized by the isoprenoid pathway. The endosymbiotic archaeal sequences in the human genome get expressed by redox stress and osmotic stress of global warming. This results in induction of HIF alpha which will upregulate fructolysis and glycolysis. In the setting of redox stress all glucose gets converted to fructose by the induction of enzymes aldose reductase and sorbitol dehydrogenase. Aldose reductase converts glucose to sorbitol and sorbitol dehydrogenase converts sorbitol to fructose. Since fructose is preferentially phosphorylated by ketohexokinases the cell is depleted of ATP and glucose phosphorylation comes to a halt. Fructose becomes the dominant sugar that is metabolized by fructolysis in expressed archaeal particles in the cell functioning as organelle called fructosoids. The fructose is phosphorylated to fructose 1-phosphate which is acted upon by aldolase B which converts it into glyceraldehyde 3-phosphate and dihydroxy acetone phosphate. Glyceraldehyde 3-phosphate is converted to D1,3-biphosphoglycerate which is then converted to 3-phosphoglycerate. The 3-phosphglycerate is converted to 2-phosphoglycerate. 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme enolase. Phosphoenol pyruvate is converted to pyruvate by the enzyme pyruvic kinase. The archaeaon induces HIF alpha which upregulates fructolysis and glycolysis but inhibits pyruvate dehydrogenase. The forward metabolism of pyruvate is stopped. The dephosphorylation of phosphoenol pyruvate is inhibited in the setting of pyruvic kinase inhibition. Phosphoenol pyruvate enters the shikimic acid pathway where it is converted to chorismate. The shikimic acid is synthesized by a pathway starting from glyceraldhyde 3-phosphate. Glyceraldehyde 3-phosphate combines with the pentose phosphate pathway metabolite sedoheptulose 7-phosphate which is converted to erythrose 4-phosphate. The pentose phosphate pathway is upregulated in the presence of the suppression of glycolytic pathway. Erythrose 4-phosphate combines with

phosphoenol pyruvate to generate shikimic acid. Shikimic acid combines with another molecule of phosphoenol pyruvate to generate chorismate. The chorismate is converted to prephenic acid and then to parahydroxy phenyl pyruvic acid. Parahydroxy phenyl pyruvic acid is converted to tyrosine and tryptophan as well as neuroactive alkaloids. The shikimic acid pathway is structured in expressed archaeaon organelle called the neurotransminoid. The fructolytic intermediates glyceraldehydes 3-phosphate and pyruvate are the starting points of the DXP pathway of cholesterol synthesis. Glyceraldehyde 3-phosphate combines with pyruvate to form 1-deoxy D-xylulose phosphate (DOXP) which is then converted to 2-C methyl erythritol phosphate. 2-C methyl erythritol phosphate can be synthesized from erythrose 4-phosphate a metabolite of the shikimic acid pathway. DXP combines with MEP to form isopentenyl pyrophosphate which is converted to cholesterol. Cholesterol is catabolized by archaeal cholesterol oxidases to generate digoxin. The digoxin sugars digitoxose and rhamnose are synthesized by the upregulated pentose phosphate pathway. Glycolytic suppression leads to upregulation of the pentose phosphate pathway. The expressed archaeaon organelle concerned with cholesterol catabolism and digoxin synthesis is called the steroidelle. The suppression of glycolysis and stimulation of fructolysis results in upregulation of the hexosamine pathway. Fructose is converted to fructose 6-phosphate by ketohexokinases. The fructose 6-phosphate is converted to glucosamine 6-phosphate by the action of glutamine fructose 6-phosphate amidotransferase (GFAT). Glucosamine 6-phosphate is converted to UDP N-acetyl glucosamine which is then converted to N-acetyl glucosamine and various amino sugars. UDP glucose is converted to UDP D-glucuronic acid. UDP D-glucuronic acid is converted to glucuronic acid. This forms the uronic acid synthetic pathway. Uronic acids and hexosamines form repeating units of glycosaminoglycans. In the setting of glycolytic suppression and fructolytic metabolism fructolysis

leads to increase synthesis of hexosamines and GAG synthesis. The GAG synthesizing archaeaon particles are called the glycosaminoglycoids. The expressed archaeaon particles are capable of synthesizing antioxidant vitamin C and E. The UDP D-glucose is converted to UDP D-glucuronic acid. UDP D-glucuronic acid is converted to D-glucuronic acid. D-glucuronic acid is converted to L-gulonate by enzyme aldoketo reductases. L-gulonate is converted to L-gulonolactone by lactonase. L-gulonolactone is converted to ascorbic acid by the action of archaeal L-Gulo oxidase. The vitamin E is synthesized from shikimate which is converted to tyrosine and then to parahydroxy phenyl pyruvic acid. Parahydroxy phenyl pyruvic acid is converted to homogentisate. Homogentisate is converted to 2-methyl 6-phytyl benzoquinone which is converted to alpha tocopherol. 2-methyl 6-phytyl benzoquinone is converted to 2,3-methyl 6-phytyl benzoquinone and gamma tocopherol. Vitamin E can also be synthesized by the DXP pathway. Glyceraldehyde 3-phosphate and pyruvate combined to form 1-deoxy D-xylulose 5-phosphate which is converted to 3-isopentenyl pyrophosphate. 3-isopentenyl pyrophosphate and dimethyl allyl pyrophosphate combined to form 2-methyl 6-phytyl benzoquinone which is converted to tocopherols. The ubiquinone another important membrane antioxidant and part of the mitochondrial electron transport chain is synthesized by the shikimic acid pathway and DXP pathway. The isoprenoid moiety of ubiquinone is contributed from the DXP pathway and the rest of it by tyrosine catabolism. The tyrosine is generated by the shikimic acid pathway. The archaeaon particles concerned with the synthesis of vitamin C, vitamin E and ubiquinone which are all antioxidants are called the vitaminocyte.

Fructolysis in the archaeaon vitaminocyte can contribute to vitamin C and vitamin E synthesis. The Neanderthals have a higher density of archaeal symbiosis resulting in increasing number of vitaminocyte organelle. This results

in increased synthesis endogenous ascorbic acid and tocopherol in Neanderthals which function as free radical scavengers. Free radicals are important in neuronal function and NMDA activity. Free radicals increase NMDA activity. Free radicals are also important as messengers of human endogenous retroviruses. Free radicals mediate the expression and reintegration into the genome where it functions as jumping genes contributing to genomic plasticity and dynamicity. Genomic dynamicity is consequently absent in Neanderthals due to higher synthesis of ascorbic acid and tocopherol by the vitaminocyte and free radical deficiency. Genomic dynamicity and HERV sequences contribute to development of synaptic connectivity, formation of cerebral cortex and brain size. This leads onto defective NMDA transmission, cerebral cortical dysfunction and cerebellar dominance in Neanderthals. The brain size in Neanderthals is bigger than the newer species of homo sapiens. The homo sapiens on the other hand has less of archaeal symbiotic density and fewer archaeal vitaminocyte organelle. The gene for vitamin C synthesis is already mutated in all human species and in the presence of decreased density of archaeal vitaminocyte organelle in homo sapiens there is deficiency of ascorbic acid and tocopherols in homo sapiens. This results in reduced free radical scavenging, increased free radicals in the system, increased expression and reintegration of HERV sequences in to the genome. There is increased genomic dynamicity and plasticity and a dominant cerebral cortical function in homo sapien population and a smaller brain size. Thus the archaeal symbiosis and the resultant vitaminocyte organelle decides the human species type, brain size, cerebral cortical versus cerebellar dominance and the human consciousness.

Two substances, which are products of the isoprenoid pathway, can participate in lipid peroxidation. One is digoxin, which by inhibiting membrane Na^{+}-K^{+} ATPase causes increase in intracellular Ca^{++}, and depletion of intracellular Mg^{++} both contributing to increase in lipid peroxidation.

Ubiquinone, another product of the pathway, is a powerful membrane antioxidant and its deficiency can also result in defective electron transport and generation of reactive oxygen species.

In view of this and also in the light of some preliminary reports on alteration in lipid peroxidation in neuropsychiatric disorders, a study was undertaken on the following aspects in some of these disorders (primary generalised epilepsy, schizophrenia, multiple sclerosis, Parkinson's disease and CNS glioma): (1) concentration of digoxin, ubiquinone, activity of HMG CoA reductase and RBC membrane Na^+-K^+ ATPase, (2) activity of enzymes involved in free radical scavenging, (3) parameters of lipid peroxidation and (4) antioxidant status.

The result obtained indicates an increase in the concentration of archaeal digoxin and activity of HMG CoA reductase, decrease in ubiquinone levels and in the activity of membrane Na^+-K^+ ATPase [There oxides and NO with decreased antioxidant protection as indicated by decrease in ubiquinone, vitamin E and reduced glutathione in schizophrenia, Parkinson's disease and glutathione reductase is decreased in the above diseases]. However, there is no evidence of any increase in lipid peroxidation in epilepsy in MS. The role of increased operation of the isoprenoid pathway as evidenced by alteration in the concentration of digoxin and ubiquinone in the generation of free radicals and protection against them in these disorders is discussed.

Apart from cholesterol, the isoprenoid or mevalonate pathway produces digoxin (a potent inhibitor of Na^+-K^+ ATPase) and ubiquinone (a cell membrane antioxidant and a component of the mitochondrial electron transport chain). Endogenous digoxin, reported to be synthesized by the hypothalamus is known to be altered in many neuropsychiatric disorders. Increased serum digoxin levels have been observed by us in primary generalised epilepsy, schizophrenia,

Parkinson's disease and multiple sclerosis. Involvement of endogenous digoxin like activity (EDLA) has also been reported by others in the brain in manic depressive psychosis and epilepsy. Digoxin by its inhibitory action on membrane Na^+-K^+ ATPase is known to cause an increase in intracellular calcium contributing to calcium mediated free radical generation of which NO is an example. NO combines with superoxide radical to form peroxynitrite which promotes lipid peroxidation. Increased intracellular calcium leads to opening of the mitochondrial PT pore resulting in uncoupling of the oxidative phosphorylation chain and consequent increased free radical generation. Intracellular calcium also activates phospholipase A_2 leading to increased generation of arachidonic acid and consequent increased lipid peroxidation. Further, inhibition of membrane sodium potassium ATPase by digoxin apart from increasing intracellular calcium also leads to intracellular magnesium depletion. Magnesium deficiency can affect mitochondrial electron transport and oxidative phosphorylation resulting in incomplete reduction of molecular oxygen and generation of reactive oxygen species.

Ubiquinone, another product of the isoprenoid pathway, which apart from its role in electron transport pathway which apart from - its role in electron transport is a powerful membrane and its deficiency can contribute to decreased antioxidant protection. Its deficiency can also contribute to defective electron transport leading to generation of reactive oxygen species like superoxide and hydrogen peroxide. Decreased ubiquinone levels have been reported in Parkinson's disease, schizophrenia, multiple sclerosis and epilepsy. It is therefore evident that alteration of the isoprenoid pathway can affect free radical generation and be damaging.

In connection to this, free radical damage has been implicated in the pathogenesis of Parkinson's disease, neoplasm and immune mediated disorders like multiple sclerosis. Increased lipid peroxidation and decreased activity of

superoxide dismutase (SOD) have been reported in the blood of epileptic patients. There is also a report of abnormal lipid peroxidation and activity of critical antioxidant enzymes and free radical damage in schizophrenia.

In Parkinson's disease the free radical generation has been reported to be due to formation of H_2O_2 from dopamine by the action of monoamine oxidase and the subsequent reaction of H_2O_2 with iron to generate the hydroxyl radical by the Fenton reaction. A critical role for iron in free radical generation has been suggested in neurodegenerations like Parkinson's disease and Alzheimer's disease. Another report associates manganese toxicity promoting autooxidation of catecholamines and free radical generation in Parkinson'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 neurodegenerative disorders. Increased formation of NO which forms peroxynitrite with superoxide promoting lipid peroxidation has also been reported in neurodegeneration.

The interrelationship between neuronal degeneration, psychiatric manifestation, immune activation and malignant transformation disorders has been well documented in literature. Autoantibodies have been demonstrated in multiple sclerosis, motor neuron disease, paraneoplastic disease and schizophrenia. Psychosis has been described in MS, Alzheimer's disease, Parkinson's disease and in neoplastic disorders. The relationship between Hodgkin's lymphoma and MS and lymphoma coexisting with MND has been documented in literature. A family with coexistence of many of these disorders has been reported by us. This interrelationship is probably dependent upon a central dysfunction which could play a role in the pathophysiology of these diseases. A dysfunction of the isoprenoid pathway with consequent aberration in free radical generation with damage may be a possibility in this respect. Support for this view comes from the reports on the alteration in some of the

products of this pathway in a few neuropsychiatric disorders. In view of this the following aspects have been studied in some neuropsychiatric disorders: the activity of the major regulatory steps in the isoprenoid of this pathway, plasma digoxin and ubiquinone levels, RBC membrane sodium potassium ATPase activity, parameters of lipid peroxidation-activity of enzymes involved in free radical scavenging, concentration of various antioxidants, concentration of products of lipid peroxidation, viz. malondialdehyde, conjugated dienes and hydroperoxides, concentration of NO, ceruloplasmin and iron binding capacity and concentration of serum magnesium and tyrosirle (since tyrosine is the precursor for melanin polymers which entrap free radicals and is also required for the synthesis of the ring system of ubiquinone).

The disorders studied include Parkinson's disease, primary generalised epilepsy, schizophrenia, multiple sclerosis and CNS glioma.

Results

HMG CoA Reductase RBC Membrane Na^+-K^+ ATPase Activity, Concentration of Digoxin and Ubiquinone

Activity of HMG CoA reductase in the plasma showed significant increase in epilepsy, schizophrenia, PD and glioma but not in MS when compared to the controls. Concentration of digoxin increased significantly in the plasma in all of these disorders when compared to controls except in glioma. Level of ubiquinone decreased in the serum in all these disorders when compared to controls except in the case of glioma. Activity of RBC membrane Na^+-K^+ ATPase activity also decreased significantly in all the disorders studied.

SOD, Catalase, Glutathione Reductase and Glutathione Peroxidase in the Erythrocytes

The activity of these enzymes decreased significantly in schizophrenia, Parkinson's disease and CNS glioma when compared to controls. None of these enzyme activities showed any significant alteration in primary generalized epilepsy and MS.

Malondialdehyde (MDA), Hydroperoxides, Conjugated Dienes, Glutathione, Alpha Tocopherol, NO, Iron Binding Capacity and Ceruloplasmin

None of these parameters was affected in primary generalized epilepsy and MS when compared to controls. In schizophrenia, Parkinson's disease and CNS glioma, concentration of MDA, hydroperoxides, conjugated dienes and NO increased significantly. Glutathione levels decreased significantly in schizophrenia, Parkinson's disease and CNS glioma. Concentration of alpha tocopherol decreased only in Parkinson's disease. Iron binding capacity decreased significantly only in Parkinson's disease and CNS glioma while concentration of ceruloplasmin decreased in schizophrenia, Parkinson's disease and CNS glioma.

Serum Albumin, Magnesium and Tyrosine

Concentration of albumin, magnesium and tyrosine showed significant decrease in the serum in all the disorders studied when compared to controls (except in the case of CNS glioma).

Discussion

Archaeal Digoxin and Membrane Na^{+}-K^{+} ATPase Inhibition in Relation to Mitochondrial Function

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The results obtained indicate an upgradation of the isoprenoid (mevalonate) pathway in most of these disorders as is evident from the increased activity of HMG CoA reductase. This enzyme catalyses a major rate limiting step in this pathway ie, conversion of HMG CoA to mevalonate and the level of activity of this enzyme can be taken as a measure of the operation of this pathway. Two products of this pathway are important from the point of free radical generation and damage. One is digoxin which is now known to be synthesized in the mammalian hypothalamus and is a very potent inhibitor of membrane Na^{+}-K^{+} ATPase. Inhibition of this enzyme is known to be associated with increased intracellular calcium and decreased intracellular magnesium concentration. The increase in intracellular calcium results from increased Na^{+}-Ca^{++} exchange, increased entry of Ca^{++} via voltage gated Ca^{++} channels and increased release of Ca^{++} from intracellular endoplasmic reticulum Ca^{++}. This increase in intracellular Ca^{++} by displacing Mg^{++} from its binding sites causes a decrease in functional availability of Mg^{++}. Increased intracellular Ca^{++} also brings about increased leakage of Mg^{++} from the cells (Mg^{++} being easily permeable). Renal tubular Mg^{++} re-absorption is also decreased in the presence of increased Ca^{++} with consequent increased renal excretion of Mg^{++}. Intestinal absorption of Mg^{++} is also decreased in the presence of high intracellular Ca^{++}. The consequence of both increased intracellular calcium and decreased intracellular magnesium is to increase free radical generation and damage, as will be discussed later.

The increase in the concentration of digoxin obtained in these studied disorders (except in glioma) may be the result of increased activity of HMG CoA reductase and increased channelling of intermediates for its synthesis in the hypothalamus. In this connection it has been shown in this lab that administration of ^{14}C acetate to rats resulted in incorporation of label into digoxin indicating that acetyl CoA is the precursor for digoxin also.

Archaeal Digoxin and Regulation of Tyrosine/Tryptophen Metabolism in Relation to Mitochondrial Function

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters and neuroactive alkaloids. The decrease in membrane Na^+-K^+ ATPase activity obtained in all these cases may be the result of increased levels of digoxin except in glioma. In glioma inhibition of this enzyme activity takes place even though the digoxin level is not increased. The lack of increase in digoxin in spite of increased activity of HMG CoA reductase in this case is probably due to more substrates being channelled for the synthesis of other products. For example, in glioma there is increase in the concentration of dolichol (another product of the isoprenoid pathway). The inhibition of membrane Na^+-K^+ ATPase activity in glioma in spite of digoxin being unaltered may be due to other substances. In this connection serotonin and quinolinic acid, both products of tryptophan catabolism, have been reported to inhibit this enzyme activity. Increase in the concentration of both these substances has been observed in glioma in this lab. In MS even though there is no significant alteration in the activity of HMG CoA reductase, digoxin in increased. This may again be due to more of the intermediates of the isoprenoid pathway being utilised for digoxin synthesis rather than for other substances of this pathway.

However the concentration of ubiquinone, another product of the isoprenoid pathway, shows decrease in these disorders except in glioma. This is probably due to the fact that the isoprenoid pathway provides only the side chain of ubiquinone, while its ring structure is derived from the aromatic amino acids particularly tyrosine. The decrease in the concentration of tyrosine in the serum in all these studied disorders may result in its decreased utilisation for the synthesis of the ring structure of ubiquinone thus explaining its decreased level in spite of increased operation of the isoprenoid pathway. In glioma, tyrosine levels are not significantly altered and this may explain the lack of alteration in ubiquinone concentration in this case. Digoxin has also been reported to have an inhibitory effect on neutral amino acid transport especially that of tyrosine and this may be the reason for the low tyrosine concentration observed in these disorders.

Archaeal Digoxin and Free Radical Metabolism

The results of studies on lipid peroxidation indicate an increase in free radical generation in schizophrenia, Parkinson's disease and CNS glioma as is evident from the increased concentration of MDA, hydroperoxides and conjugated dienes. NO, another important participant in cellular lipid peroxidation, is also increased in these disorders. However there is no evidence of any increase in lipid peroxidation in primary generalised epilepsy and MS. NO is formed from arginine by the action of nitric oxide synthase which requires calcium for its activation. The increase in intracellular calcium may lead to increased generation of NO which combines with the superoxide radical to form peroxynitrite. Peroxynitrite is known to promote lipid peroxidation. The decrease in SOD which breaks down the superoxide radical in many of these disorders may result in more of the superoxide radical being available to combine with NO.

In addition to increased generation of free radical, there appears to be a decrease in antioxidant protection in many of the disorders studied. This is particularly evident in the case of schizophrenia, Parkinson's disease and glioma where there is both increased free radical generation and decreased antioxidant protection. The activity of enzymes which participates in free radical scavenging like SOD, catalase, glutathione reductase and glutathione peroxidase shows a similar decrease in schizophrenia, Parkinson's disease and glioma. There is also decrease in the level of antioxidants-reduced glutathione, vitamin E and ubiquinone in Parkinson's disease and decrease in reduced glutathione in schizophrenia. The level of iron binding capacity is decreased in Parkinson's disease and glioma while ceruloplasmin is decreased in schizophrenia, Parkinson's disease and glioma. The decrease in iron binding capacity may indicate more free iron being available to catalyse the Fenton's reaction. The decrease in ceruloplasmin in schizophrenia, Parkinson's disease and glioma may indicate availability of more free Cu to participate in free radical generation.

The isoprenoid pathway can influence free radical generation and damage via digoxin and ubiquinone. As discussed earlier the decrease in ubiquinone can result in less antioxidant protection since ubiquinone is a very important cell membrane antioxidant. Ubiquinone also affects the generation of reactive oxygen species like superoxide and hydrogen peroxide. Being a component of the mitochondrial electron transport chain, its deficiency can result in defective electron transport and consequent incomplete reduction of molecular oxygen to form reactive oxygen species.

Archaeal Digoxin and Mitochondrial Function

Digoxin as mentioned earlier by its inhibitory effect on membrane Na^+-K^+ ATPase, contributes to increase in intracellular calcium and decrease in

intracellular magnesium. Increased calcium can result in opening of the mitochondrial PT pore resulting in collapse of the proteon gradient across the inner membrane and consequent uncoupling of oxidative phosphorylation. This also results in increase in generation of reactive oxygen species. The role of calcium on NO generation has already been discussed. Increased calcium also can activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Apart from calcium, decreased intracellular magnesium resulting from Na^+-K^+ ATPase inhibition by digoxin can also contribute to increased generation of free radicals. Magnesium is a cofactor for ATP synthesis taking place during coupling of electron flow to oxidative phosphorylation. The deficiency of magnesium can affect electron transport with consequent incomplete reduction of molecular oxygen to free reactive oxygen species.

Increased generation of free radicals like the superoxide ion, and hydroxyl radical can produce cell membrane damage. This damage to the cell membrane can further inactivate membrane Na^+-K^+ ATPase with entry of more calcium into the cell and further triggering of the mechanisms described above. The free radical related Na^+-K^+ ATPase inhibition can produce membrane depolarisation and opening of the voltage gated calcium channels resulting in increased intracellular calcium load. This Na^+-K^+ ATPase inhibition leads to free radical generation, which in turn produces further enzyme inhibition.

Free radical mediated mitochondrial dysfunction can result in decreased production of ATP. Cytosolic free calcium is normally buffered by two mechanisms: ATP dependent calcium extrusion from cell ATP dependent subquestration of calcium within the endoplasmic reticulum. The free radical related mitochondrial dysfunction results in defective extrusion of calcium from the cell. This calcium overload in the cell can trigger further free radical production.

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+} (feroxidase activity). In the presence of iron in Fe^{2+} from 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. Another recent study has 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 intracellular magnesium deficiency can produce ribosomal dysfunction and inhibition of protein synthesis as noted by the 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. Nigral iron accumulation in PD is primarily within neuromelanin granules. Neuromelanin binds to iron and is relatively protective. Neuromelanin is synthesized from tyrosine and digoxin related tyrosine transport defect may lead to decreased neuromelanin synthesis. 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 Na^+-K^+ 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 SOD, glutathione peroxidase and glutathione reductase suggests reduced free radical protection.

Archaeal Isoprenoids and Mitochondrial Protein Glycosylation

Apart from involvement in the free radical generation and antioxidant protection, the isoprenoid pathway may also contribute to the pathogenesis of neurological disorders in other ways. Dolichol, a product of the isoprenoid pathway is involved in protection glycosylation. Increase in serum dolichol has been observed by us in many neuropsychiatric disorders which may be the result of it being less utilised for the formation of dolichol 1-phosphate due to magnesium deficiency. This may result in altered protein glycosylation patterns. In this connection altered glycoprotein accumulation in the serum and brain has been reported in Alzheimer's disease, epilepsy, and schizophrenia. Defective N-glycosylated proteins can manifest as alteration in immune response. In the case of endogenous myelin glycoprotein antigen, defective glycosylation can cause its defective loading to HLA class-I or II molecules resulting in defective transport of the complex to the antigen presenting cell surface for recognition by CD_8 cell. A defective MHC class-1 restricted CD_8 response to myelin has been reported in MS. Defective glycoproteins can also lead to defective contact inhibition and cell proliferation leading to CNS glioma. Defective glycoproteins can also result in disordered synaptic connectivity and functional disorders like epilepsy and schizophrenia.

Archaeal Digoxin and Disease Pathology

The increase in digoxin can have the following consequence: - increased calcium can activate proteases, lipases and endonucleuses which can damage cell membrane, DNA and cytoskeleton of cell. Digoxin has been reported to inhibit glutamate transport via the dicarboxylate carrier system into the glial cells. This can result in synaptic accumulation of glutamate and increased NMDA transmission. The Na^{+}-K^{+} ATPase inhibitory action of digoxin can deplete the cells of Mg^{++} thus removing the magnesium-block on the NMDA receptor. All these lead to increased glutamatergic transmission and glutamate excitotoxicity. Glutamate excitotoxic mechanism is crucial in epileptogenesis, schizophrenia and neuronal degeneration. Digoxin has been shown to promote dopamine release by both exocytic and carrier mediated process in the rat brain and to inhibit serotonin uptake by mouse brain synaptosomes. The monoamine neurotransmitters dopamine, noradrenaline, adrenaline and serotonin act via the cyclic AMP/cyclic GMP/inositol second messenger system. Digoxin, by increasing cytosolic calcium, can activate the signal transduction system of these second messengers. Hyperdopaminergic transmission in the mesolimbic system has been shown to produce the psychotic symptoms of schizophrenia. Schizophrenia thus could be related to hypothalamic archaeal digoxin hypersecretion. Digoxin by its Na^{+}-K^{+} ATPase inhibitory action can prevent neuronal membrane repolarisation resulting in paroxysmal depolarization shift and epileptogenesis. The inhibition of Na^{+}-K^{+} ATPase caused by digoxin and the consequent Ca^{++} activation of inositol mediated signal transduction is also involved in ras oncogene activation. The increased cytosolic Ca^{++} caused by digoxin can upregulate the Ca^{++} mediated signal transduction system involved in T-cell activation. Immune activation may play a role in disorders like MS and neuronal degeneration. The question of how the upregulation of the isoprenoid pathway takes place in these disorders is important. Our studies have shown that

there is increased tryptophan catabolism in these disorders, resulting in increased levels of serotonin and quinolinic acid. Both these are known inhibitors of membrane Na^{+}-K^{+} ATPase. The Mg^{++} depletion resulting from this inhibition can cause stimulation of HMG CoA reductase and upregulation of the isoprenoid pathway with increase in digoxin synthesis. The endogenous digoxin can further inhibit membrane Na^{+}-K^{+} ATPase. Thus the inhibition of this enzyme proceeds in a cascade like fashion.

Thus apart from its role in generation of free radicals, the isoprenoid pathway can also influence the pathogenesis of various neuropsychiatric disorders.

References

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