Chapter 4

Archaeal Digoxin, Cerebral Dominance and Mitochondrial Function / Free Radical Metabolism

Introduction

There is a specialisation of function in the right and left hemispheres of the brain as manifested in cognitive dysfunctions noticed in lesions of the same. Typical cerebral lateralization is associated with left cerebral dominance for language, praxis and serial processing, whereas the right cerebral hemisphere is dominant for externally directed attention, visuospatial tasks and gestalt processing. The isoprenoid pathway is a key regulatory pathway in the cell. It produces digoxin (an endogenous membrane Na^+-K^+ ATPase inhibitor), dolichol (important in N-glycosylation of proteins), ubiquinone (a component of the mitochondrial electron transport chain) and cholesterol (a component of cellular membranes). Since digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of cerebral dominance. Cerebral dominance could also possibly influence cellular structure and function through changes in the isoprenoid pathway. The present study assessed the changes in the synthesis of an endogenous membrane Na^+-K^+ ATPase inhibitor, archaeal digoxin and changes in mitochondrial function in right hemispheric dominant and left hemispheric dominant individuals. The results are presented in this paper.

Results

The results showed that HMG CoA reductase activity and serum digoxin were increased and RBC membrane Na^+-K^+ ATPase activity, serum magnesium and ubiquinone were reduced in left handed / right hemispheric dominant individuals. The results also showed that HMG CoA reductase activity and serum digoxin were decreased and RBC membrane Na^+-K^+ ATPase activity, serum magnesium and ubiquinone were increased in right handed / left hemispheric dominant individuals.

The results showed that the concentration of tryptophan was found to be higher in the plasma of left handed / right hemispheric dominant individuals while that of tyrosine, was lower. The results also showed that the concentration of tryptophan was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine was higher.

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 and reduced glutathione in left handed / right hemispheric dominant individuals. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in left handed / right hemispheric dominant individuals. There was a decrease in lipid peroxidation as evidenced from the decrease in the concentration of MDA, conjugated dienes, hydroperoxides and NO with increased antioxidant protection as indicated by increase in ubiquinone and reduced glutathione in right handed left hemispheric dominant individuals. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is increased in right handed / left hemispheric dominant individuals.

Discussion

Archaeal Digoxin Synthesis / Hemispheric Dominance

The recent reports on endogenous digoxin, a potent inhibitor of Na^+-K^+ ATPase synthesized by the hypothalamus. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity in left handed / right hemispheric dominant individuals. In left handed / right hemispheric dominant there was significant inhibition of the RBC membrane Na^+-K^+ ATPase. The inhibition of Na^+-K^+ ATPase by digoxin is

known to cause an increase in intracellular calcium resulting from increased Na^{+}-Ca^{++} exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. This increase in intracellular calcium by displacing magnesium from its binding sites causes a decrease in the functional availability of magnesium. This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation which along with low magnesium can cause further inhibition of Na^{+}-K^{+} ATPase, since ATP-magnesium complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms, ATP dependent calcium extrusion from the cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus a progressive inhibition of Na^{+}-K^{+} ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to Na^{+}-K^{+} ATPase inhibition is seen in right hemispheric dominant / left handed individuals. The intracellular positive calcium signal and negative magnesium signal can regulate a diverse cellular process. Calcium on entry into the cell is used to charge up the internal endoplasmic reticulum stores which then release a burst of signal calcium responsible for activating a large variety of calcium dependent cellular processes. The information processing capability of the calcium signalling system is enhanced by amplitude and frequency modulation. The calcium is released from channels on internal ER individually or in small groups (bip/quark and puffs/sparks). Further diversity of calcium signalling is produced by compartmentalization as cytosolic calcium signal and nuclear calcium signal. There is evidence for increased digoxin synthesis in left handed / right hemispheric dominant individuals from the increase in HMG CoA reductase in activity that is noticed. HMG CoA reductase is the rate limiting enzyme of the isoprenoid pathway. In this connection,

incorporation of ^{14}C-acetate into digoxin in a rat brain has been shown by us indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. Serum magnesium was assessed in left-handed/right hemispheric dominant individuals and was found to be reduced.

The decrease in the activity of HMG CoA reductase in right handed individuals / left hemispheric dominant suggests a downregulation of the isoprenoid pathway. There is a marked decrease in plasma digoxin levels consequent to its reduced synthesis in the left hemispheric dominant state. The decrease in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can increase this enzyme activity. In right handed / left hemispheric dominant individuals there was significant stimulation of the RBC membrane Na^+-K^+ ATPase. The stimulation of Na^+-K^+ ATPase by decrease in digoxin synthesis is known to cause a decrease in intracellular calcium resulting from decreased Na^+-Ca^{++} exchange, decreased entry of calcium via the voltage gated calcium channel and decreased release of calcium from intracellular endoplasmic reticulum calcium stores. 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 increased intracellular magnesium related mitochondrial ATP synthesis results in increased calcium extrusion from the cell. There is thus a progressive stimulation of Na^+-K^+ ATPase activity. High intracellular magnesium and low intracellular calcium consequent to Na^+-K^+ ATPase stimulation is seen in right handed left hemispheric dominant individuals. The intracellular negative calcium signal and positive magnesium signal can regulate a diverse cellular process. Serum magnesium was assessed in right handed / left hemispheric dominant individuals and was found to be increased.

There is an increase in tryptophan and a reduction in tyrosine in the serum of left handed / right hemispheric dominant individuals. 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.

The results showed that the concentration of tryptophan was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine was higher. 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 and because digoxin levels are low in right handed / left hemispheric dominant individuals.

Archaeal Digoxin, Cerebral Dominance and Mitochondrial Function

The concentration of ubiquinone decreased significantly in left handed / right hemispheric dominant individuals which may be the result of low tyrosine levels as reported in most of the disorders, consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from tyrosine. Ubiquinone, which is an important component of the mitochondrial electron transport chain, is a membrane antioxidant and contributes to free radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore causing a collapse of the hydrogen gradient across the Inner membrane and uncoupling of the respiratory chain. Intracellular magnesium deficiency can lead to a defect in the function of ATP synthase. AU this leads to defects 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 demage, which can further inactivate Na^+-K^+ ATPase, triggering the cycle of free radical generation once again. Magnesium deficiency can affect glutathione synthetase and glutathione reductase function. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with calcium related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective owing to the membrane Na^+-K^+ ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Mitochondrial dysfunction related free radical generation is noticed in right hemispheric dominant individuals. The increased intracellular calcium and ceramide related opening of the mitochondrial PT pore also leads to volume dysregulation of the mitochondria, causing hyperosmolality of the matrix and expansion of the matrix space. The outer membrane of the mitochondria ruptures and releases an apoptosis inducing factor and cytochrome C into the cytoplasm. This results in activation of caspase-9 and caspase-3. Caspase-9 can produce apoptosis of the cell. Caspase-3 activation can cleave P_{21} involved in linking DNA duplication to cell division resulting in a polyploid cell and oncogenesis. We have been able to demonstrate neuronal degeneration and apoptosis in a digoxin injected rat brain. Thus in the hyperdigoxinemic right hemisphere dominant state there is a defect in mitochondrial function and increased free radical generation and reduced scavenging. There is also increased apoptosis.

The concentration of ubiquinone increased significantly in right handed / left hemispheric dominant individuals which may be the result of increased tyrosine levels, consequent to digoxin deficiency promoting tyrosine transport over tryptophan. The decrease in intracellular calcium can stabilise the mitochondrial PT pore and improve mitochondrial function. Intracellular magnesium excess

can lead to an increase in the activity of ATP synthase. All this leads to improved efficiency in mitochondrial oxidative phosphorylation and reduced free radical generation. Ubiquinone excess also leads to increased free radical scavenging. The decrease in intracellular calcium may lead to decreased generation of NO by inhibiting the enzyme nitric oxide synthase and reduced peroxynitrite formation. Decreased calcium also can inhibit phospholipase [A_2 resulting in decreased generation of arachidonic acid and free radical formation]. Decreased generation of free radicals like the superoxide ion and hydroxyl radical can stabilise the cell membrane and stimulate membrane Na^+-K^+ ATPase. There was a decrease in lipid peroxidation as evidenced from the decrease in the concentration of MDA, conjugated dienes, hydroperoxides and NO with increased antioxidant protection as indicated by the increase in ubiquinone and increased reduced glutathione in hypodigoxinemic left hemisphere dominant individuals. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase is increased suggesting increased free radical scavenging. The peroxisomal membrane is stabilised owing to membrane Na^+-K^+ ATPase stimulation related alteration in membrane formation. This leads to increased catalase activity. Glutathione is synthesized by the enzyme glutathione synthetase which needs magnesium and ATP. The high intracellular magnesium consequent to Na^+-K^+ ATPase stimulation and the resulting increased ATP can result in increased synthesis of glutathione. Glutathione peroxidase, a selenium containing enzyme oxidases reduced glutathione (GSH) to oxidised glutathione (GSSG) which is 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 excess due to membrane Na^+-K^+ ATPase stimulation leads to increased formation of

glucose 6-phosphate and upregulation of the pentose phosphate pathway with consequent increased generation of NADPH. Thus glutathione system of free radical scavenging is activated in the presence of membrane Na^+-K^+ ATPase stimulation. Superoxide dismutase exists in a mitochondrial and cytoplasmic. The stabilisation of the mitochondrial PT pore consequent to reduced intracellular calcium produces increased efficiency of superoxide dismutase activity. The increase in catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase suggests increased free radical protection. This leads to decreased generation of free radicals in the hypodigoxinemic individuals. The decreased intracellular calcium and ceramide related stabilisation of the mitochondrial PT pore also leads to down regulation of the apoptotic program and reduced apoptosis. The stabilisation of the mitochondria leads to reduced release of the apoptosis inducing factor and cytochrome C into the cytoplasm. This results in inactivation of caspase-9 and caspase-3. Inhibition of apoptosis occurs in right hemispheric dominant individuals. Caspase-3 inactivation inhibits P_{21} cleavage and protects against oncogenesis. Thus the hypodigoxinemic left hemisphere dominant state has improved efficiency of mitochondrial oxidative phosphorylation, reduced generation of free radicals, increased free radical scavenging and reduced apoptosis.

References

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