3

Archaeal Digoxin Mediated Model for OCD / TIC Syndrome

Introduction

The study focuses on alterations in the isoprenoid pathway in obsessive compulsive disorder (OCD) and la tourette's syndrome (TS). Alteration in the cation pump has been described in several neuropsychiatric disorders and an endogenous inhibitor of membrane Na^+-K^+ ATPase has been described. Digoxin is a steroidal glycoside synthesized by the isoprenoid pathway and is reported to be secreted by the human hypothalamus. Digoxin is also reported to modulate neutral amino acid and neurotransmitter transport. In OCD PET studies have shown abnormalities in the orbitofrontal cortex, striatum and cingulate cortex. A series of discrete, parallel, neuroanatomic circuits connecting the prefrontal cortex, striatum, globus pallidus and thalamus, have been described forming a fronto-striato-pallido-thalamo-cortical loop. It involves both direct and indirect pathways the former being facilitatory and the latter inhibitory. The proposed model of OCD pathophysiology is an imbalance of direct > indirect pathway tone in the fronto-subcortical circuits. Serotoninergic drugs change the relative balance of tone through the indirect versus direct orbitofrontal-subcortical pathways thereby normalising the pathological state seen in OCD. Drugs with selective D_1 blockade which specifically decreases activity in the direct pathway also reduce OCD symptoms. The relative preponderence of serotoninergic versus dopaminergic transmission would play an important role in the genesis of OCD symptoms. There is no agreement regarding the pathophysiology of tourette syndrome (TS). The prevailing view is the so-called dopamine hypothesis, which in essence suggests that the syndrome is caused either by disorders in presynaptic release of dopamine or by a dysfunction of its postsynaptic receptors. Other pathogenetic mechanisms have been proposed such as abnormal serotonin uptake, and a hyperactive endogenous opioid system known to influence the sensitivity of dopamine receptors. Other authors have implicated the noradrenergic system and

Kurlan has suggested that TS may occur following an inhibition of the excitatory amino acid, glutamate that regulates the dopamine uptake in neurons within the basal ganglia. Because of the reports implicating digoxin in regulating neurotransmitter transport, the isoprenoidal pathway and digoxin synthesis were studied in TS and OCD. The other metabolites of the isoprenoid pathway of significance are ubiquinone, cholesterol and dolichol. Membrane Na^+-K^+ ATPase inhibition is reported to be lead to magnesium depletion and intracellular calcium excess. Hypomagnesemia and dolichol can affect glycoconjugate metabolism. Alteration in ubiquinone levels and intracellular calcium\magnesium ratios can affect mitochondrial function and free radical metabolism. Therefore the following parameters were studied in OCD and TS: plasma HMG CoA reductase activity, serum cholesterol, digoxin, dolichol, ubiquinone, magnesium and RBC Na^+-K^+ ATPase activity. The levels of serum tyrosine, tryptophan and their catabolites, glycoconjugate metabolism, free radical metabolism and membrane composition were also assessed. The results are presented in this paper and a hypothesis regarding the role of membrane Na^+-K^+ ATPase activity in the pathogenesis of these disorders is discussed. The parameters were also assessed in right hemispheric, left hemispheric and bihemispheric dominant individuals to find out whether hemispheric dominance has any correlation with these neuropsychiatric syndromes.

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 psychiatric disease.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were decreased in the serum of OCD and TS patients. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na^+-K^+ ATPase and serum magnesium were increased.

(2) The concentration of serum tryptophan, quinolinic acid and serotonin was decreased in the plasma of OCD and TS patients while that of tyrosine, dopamine and noradrenaline was increased.

(3) Nicotine and strychnine were not detected in the plasma of OCD and la Tourette's syndrome patients. Morphine was detected in the plasma of OCD and TS patients.

(4) The concentration of total glycosaminoglycans (GAG) decreased in the serum of OCD and TS patients. The concentration of heparan sulphate (HS) heparin (H), dermatan sulphate (DS), chondroitin sulphates (ChS)

and hyaluronic acid (HA) was decreased. The concentration total hexose, fucose and sialic acid were decreased in the glycoproteins of the serum of TS and OCD patients. The concentration of gangliosides, glycosyl-diglycerides, cerebrosides and sulphatides showed significant decrease in the serum of OCD and TS patients.

(5) The activity of glycosaminoglycan (GAG) degrading enzymes - beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D was decreased in the serum of OCD and TS patients when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase decreased in the serum of OCD and TS cases.

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

(7) The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes increased significantly in the serum of OCD and TS cases. The concentration of malon dialdehyde (MDA), hydroperoxides, conjugated dienes and nitric oxide (NO) decreased significantly in the serum of OCD and TS cases. The concentration of reduced glutathione increased in OCD and TS cases. Iron binding capacity and ceruloplasm increased significantly in OCD and TS cases.

(8) The results showed that HMG CoA reductase activity serum digoxin and dolichol were increased and serum ubiquinone, RBC membrane sodium-potassium ATPase activity and serum magnesium were reduced in left handed / right hemispheric dominant individuals. The results showed

that HMG CoA reductase activity serum digoxin and dolichol were decreased and serum ubiquinone, RBC membrane sodium-potassium ATPase activity and serum magnesium 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 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 OCD / la Tourette Syndrome

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The decrease in the activity of HMG CoA reductase in OCD / TS patients suggests a downregulation of the isoprenoid pathway. There is a marked decrease in plasma digoxin and dolichol and this decrease may be a consequence of decreased channelling of intermediates of the isoprenoid pathway for their biosynthesis. In this connection, incorporation of ^{14}C-acetate into digoxin in the rat brain has been shown by us indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The decrease in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can increase this enzyme activity in OCD/TS cases where there was significant stimulation of the RBC membrane Na^+-K^+ ATPase. The stimulation of Na^+-K^+ ATPase by digoxin deficiency 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. This decrease in intracellular calcium causes an increase in the functional availability of magnesium. This increase in the availability of magnesium can cause increased mitochondrial ATP formation which along with increased magnesium can cause further stimulation of Na^{+}-K^{+} ATPase, since the 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 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 appear to be crucial to the pathophysiology of OCD/TS cases. The intracellular negative calcium signal and positive magnesium signal can regulate diverse cellular process. Serum magnesium was and assessed in OCD/TS cases and was found to be increased.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to OCD / la Tourette 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

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 result showed that the concentration of tryptophan, quinolinic acid and serotonin was found to be lower in the plasma of patients with OCD/TS cases while that of tyrosine, dopamine and norepinephrine was higher. Serum of OCD/TS cases showed the absence of strychnine and nicotine. Morphine could be detected in the serum of OCD/TS patients. Thus there is a decrease in tryptophan and its catabolites and increase in tyrosine and its catabolites in the serum of OCD/TS cases. This could be due to the fact that digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine and that digoxin levels are low in OCD/TS cases. The increase in membrane Na^{+}-K^{+} ATPase activity in OCD/TS cases could be due to the fact that hyperpolarising neurotransmitters (dopamine, morphine and noradrenaline) are increased and the depolarising neuroactive compounds (serotonin, strychnine, nicotine and quinolinic acid) are decreased.

TS has been postulated to be due to an hyperactive dopaminergic system, the reasons for which are manifold. There is an increased level of dopamine and morphine in TS, due to increased synthesis from tyrosine in our study. Morphine can increase the sensitivity of dopamine receptors. A hyperactive noradrenergic system has also been postulated. Our study shows increased noradrenaline synthesis from tyrosine in TS. Thus most of the neurotransmitter changes producing TS can be attributed to digoxin induced upregulation of tyrosine transport and increased tyrosine catabolism.

In OCD there is an abnormality in the striato-pallido-thalamo-cortical loop especially involving the balance between the facilitatory direct and indirect inhibitory pathway. There is a hyperactive facilitatory direct pathway due to decreased serotoninergic transmission and increased dopaminergic transmission via the D_1 receptor. Our studies show reduced tryptophan levels and consequently

reduced serotonin synthesis in OCD. On the other hand the tyrosine levels and the synthesis of dopamine and morphine from tyrosine is upregulated. Thus there is reduced serotoninergic and increased dopaminergic transmission in OCD owing to low digoxin levels leading on to a hyperactive facilitatory direct pathway.

The low level of quinolinic acid, serotonin and strychnine can contribute to reduced excitatory glutamatergic transmission as they are all positive modulators of the NMDA receptor. In the presence of hypermagnesemia, the magnesium block on the NMDA receptor is strengthened leading on to reduced NMDA transmission. The decreased presynpatic neuronal calcium can produce reduced cyclic AMP dependent phosphorylation of synapsins resulting in decreased glutamate release into the synaptic junction and vesicular recycling. Decreased intracellular calcium in the post synaptic neuron can also inhibit the calcium dependent NMDA signal transduction. The plasma membrane glutamate transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a sodium gradient which is activated by the stimulation of Na^+-K^+ ATPase, resulting in increased clearance of glutamate by presynaptic and glial uptake at the end of synaptic transmission. By these mechanisms, stimulation of Na^+-K^+ ATPase can inhibit glutamatergic transmission. Reduced glutamatergic transmission can lead on to mental subnormality which has been associated with OCD and TS cases, The reduced glutamatergic transmission could also be related to the attention deficit disorder associated with both OCD and TS syndrome.

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to OCD / la Tourette Syndrome

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The membrane Na^+-K^+ ATPase stimulation related increased intracellular magnesium levels can affect the metabolism of glycosaminoglycans,

glycoproteins and glycolipids. The decrease in the levels of dolichol may suggest its decreased availability for N-glycosylation of proteins. Magnesium excess can lead on to increased catabolism of sphinganine leading on to decreased cerebroside and ganglioside synthesis. In magnesium excess the glycolysis, citric acid cycle and oxidative phosphorylation are activated and less of glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results show a decrease in the Concentration of serum total GAG, glycolipids (ganglioside, glycosyl-diglyceride, cerebrosides and sulphatides) and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in OCD/TS cases. The individual GAG fractions in the serum - heparan sulphate (HS), chondroitin sulphates (ChS), heparin (H), hyaluronic acid (HA) and dermatan sulphate (DS) are decreased in OCD/TS cases. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) and that of glycohydrolases (beta galactosidase, beta fucosidase and beta glucosidase) showed significant decrease in the serum in OCD/TS cases. Intracellular magnesium excess also results in increased ubiquitin dependent proteolytic processing of glycoconjugates as it requires magnesium for its function. The decrease in the activity of glycohydrolases and GAG degrading enzymes could be due to increased lysosomal stability. Increased lysosomal stability would lead to decreased phagocytosis and increased incidence of respiratory infections. A number of fucose and sialic acid containing natural ligands have been implicated in inflammatory responses. The decrease in fucose and sialic acid noted in these cases could inhibit a protective inflammatory response to the virus or bacteria leading on to recurrent respiratory infection. This could account for the linkage between OCD and streptococcal infection. Altered glycoconjugates in OCD play an important role in the pathogenesis of the syndrome. This could lead to altered synaptic

connectivity in the frontostriato-pallido-thalamo cortical loops important in the pathogenesis of OCD.

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to OCD / la Tourette Syndrome

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contributes 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 downregulation of the isoprenoid pathway can lead to decreased cholesterol synthesis and magnesium excess can stimulate phospholipid synthesis. Phospholipid degradation is decreased owing to decrease in intracellular calcium inhibiting phospholipase A_2 and D. The cholesterol: phospholipid ratio of the RBC membrane was decreased in OCD/TS cases. The concentration of total GAG, hexose and fucose of glycoprotein increased in the RBC membrane and decreased in the serum suggesting their increased 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 activated in magnesium excess. The change in membrane structure produced by alteration in glycoconjugates and the cholesterol: phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase resulting in further membrane Na^+-K^+ ATPase stimulation. This leads to increased intracellular hypermagnesemia. Elevated magnesium levels inhibit HMG CoA reductase activity and reduced digoxin synthesis. This leads

to further membrane Na^+-K^+ ATPase stimulation and the amino acid transport defect gets accentuated. The tyrosine/tryptophan defect in OCD / TS could be due to the neuronal membrane abnormality. Similar membrane abnormalities have been described in choreoacanthocytosis-tic syndrome. The same changes can affect the structure of the organallae membrane. This results in increased lysosomal stability. Altered peroxisomal membranes could lead to catalase hyperactivity which has been documented in OCD/TS cases.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to OCD / la Tourette 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 increased significantly in OCD/TS cases which may be the result of increased tyrosine levels, consequent to digoxin deficiency promoting tyrosine transport over tryptophan. The aromatic ring promotion of ubiquinone is derived from lyrosine. Ubiquinone, which is an important component of the mitochondrial electron transport chain, is a membrane antioxidant arid contributes to free radical scavenging. 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 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 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 OCD/TS cases. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase is increased in OCD/TS cases suggesting increased free radical scavenging. The peroxisomal membrane is stabilised owing to membrane Na^+-K^+ ATPase stimulation related alteration in membrane formation and 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 synthesis can result in increased 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 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 form. 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. Free radicals are required for lymphocyte activation and this leads to a hypoimmune response and increased respiratory infection owing to immunodeficiency.

Archaeal Digoxin and Immune Function in OCD / la Tourette Syndrome

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. Decreased intracellular calcium inactivates the calcium dependent calcineurin signal transduction pathway involved in T-cell inactivation and decreased secretion of interleukin-3, 4, *5,* 6 and TNF alpha. TNF alpha can also bring about apoptosis of the cell and this is inhibited. TNF alpha binds to its receptor TNFR1 and activates the transcription factors NFkB and AP-l leading to induction of proinflammatory and immunomodulatory genes. Low levels of TNF alpha can lead to immunosuppression. This can explain the immunosuppression and increased rate of respiratory infection noted in OCD/TS cases. Morphine has also got an immunosuppressive action. This could also contribute to increased incidence of respiratory infections.

Thus elevated membrane Na^{+}-K^{+} ATPase can lead on to an obsessive compulsive personality and TS syndrome. It can produce a hypoimmune state and recurrent respiratory infections. This could be due to three factors - altered levels of sialo and fucoligands, decreased T-cell activation consequent to inhibition of calcineurin signal transduction system and reduced free radical generation leading on to defective phagocytosis.

Archaeal Digoxin and Hemispheric Dominance in Relation to OCD / la Tourette Syndrome

The archaeaon related organelle - steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. The biochemical patterns in left hemispheric dominant individuals correlated with OCD and la tourette's syndrome. In left hemispheric dominant individuals there is a downregulated isoprenoid pathway and reduced digoxin synthesis. In right hemispheric dominant individuals there is an upregulated isoprenoid pathway and increased digoxin synthesis. The neurotransmitter patterns are also different in right hemispheric and left hemispheric dominant individuals. In left hemispheric dominant individuals there is upregulated tryptophan catabolism and down regulated tyrosine catabolism. In right hemispheric dominant individuals there is downregulated tryptophan catabolism and upregulated tyrosine catabolism.

References

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