Chapter 10

Archaeal Digoxin, Cerebral Dominance and Nuclear Function

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, which functions as an endogenous membrane Na^+-K^+ ATPase inhibitor. Since digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of cerebral dominance. Membrane Na^+-K^+ ATPase inhibition leads to an increase in intracellular calcium and a depletion of intracellular magnesium. Intracellular magnesium functions as a co-factor for several enzymes important in nuclear function - DNA polymerase, DNA ligase, DNA dependent RNA polymerase and amino acyl tRNA synthetase. Magnesium is also required for protein synthesis and ribosomal integrity. Cerebral dominance could also possibly influence nuclear functions. The present study assessed the changes in the synthesis of an endogenous membrane Na^+-K^+ ATPase inhibitor, digoxin and neurotransmitter patterns in neurogenetic syndromes - Huntington's disease and trisomy 21 as well as in tumours like glioblastoma multiforme and non-Hodgkin's lymphoma. The digoxin status and neurotransmitter patterns were also assessed in individuals with differing hemispheric dominance. The results are presented in this paper and a hypothesis regarding the role of endogenous digoxin and cerebral dominance in the regulation of nuclear function and cell differentiation is put forward.

Results

The results showed that HMG CoA reductase activity and serum digoxin were increased in left handed / right hemispheric dominant individuals. The RBC membrane Na^+-K^+ ATPase activity and serum magnesium was reduced. The results also showed that HMG CoA reductase activity and serum digoxin were decreased in right handed / left hemispheric dominant individuals. The RBC membrane Na^+-K^+ ATPase activity and serum magnesium were increased. The results showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be higher in the plasma of left handed / right hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was lower. The results also showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was higher.

The activity of serum HMG CoA reductase and the concentration of serum digoxin and dollchol were increased in HD and trisomy 21. The concentration of serum ubiquinone, magnesium and the activity of erythrocyte membrane Na^+-K^+ ATPase were decreased. The concentration of serum tryptophan, quinolinic acid and serotonin was increased in these patients while that of tyrosine, dopamine and noradrenaline was decreased. Nicotine and strychnine were detected in the serum of patients with trisomy 21 and HD but were not detectable in control serum. Morphine was not detected in the serum of these patients.

The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in CNS astrocytomas and NHL when compared with controls. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na^+-K^+ ATPase and serum magnesium were decreased. The concentration of serum tryptophan, quinolinic acid and serotonin was increased

in the plasma while that of tyrosine, doparnine and noradrenaline was decreased in CNS astrocytomas and NHL. Nicotine and strychnine could be detected in the plasma of patients with CNS astrocytomas and NHL but was not detectable in the control serum. Morphine was not detected in the plasma of these patients.

Discussion

Archaeal Digoxin and Membrane Na^+-K^+ ATPase Inhibition - Relation to Genomic Function

Na^+-K^+ ATPase is an active transport system present in the cell membrane of nearly all animal cells. This enzyme is particularly important in the brain because about 2/3 of the energy demand of this organ is used to maintain the characteristic transmembrane potential across the neuronal membrane by the action of Na^+-K^+ ATPase. Na^+-K^+ ATPase contains two sub units, α and β. The α sub unit contains the catabolytic site for ATP hydrolysis and for digoxin binding. There are three types of α sub units, α_1 which is widely distributed and is digoxin resistant, α_2 and α_3 are digoxin sensitive and are restricted to the excitable tissues. α_3 is the predominant subunit is neuron while α_2 is predominant in the muscle, heart and glial cell. The β subunits, which are glycosylated polypeptides, also exist in two forms β_1 and β_2. One isoform of β_2 (AMOG) is the adhesion molecule of glial cell. This isoform promotes neuritic outgrowth and neuronal migration. Thus normal functioning of Na^+-K^+ ATPase is important for neuronal function.

There are recent reports on endogenous digoxin, a potent inhibitor of Na^+-K^+ ATPase synthesized by the hypothalamus. The increase in endogenous digoxin, a potent inhibitor or membrane Na^+-K^+ ATPase, can decrease this enzyme activity in right hemispheric dominant individuals. The increase in HMG CoA reductase activity, the rate-limiting enzyme of the isoprenoid pathway, suggests increased digoxin synthesis in right hemispheric dominant individuals. 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 Ca^{++} via the voltage gated calcium 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 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-magnesium complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two 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. The intracellular positive Ca^{++} signal and negative Mg^{++} signal can regulate diverse cellular processes. Ca^{++} 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 Ca^{++} is released from channels on internal ER individually or in small groups (blip/quark and puffs/sparks). Further diversity of calcium signalling is produced by compartmentalization as a cytosolic calcium signal and nuclear calcium signal.

The decrease in the activity of HMG CoA reductase in right handed individuals / left hemispheric dominant suggests a downregulation of the isoprenoid pathway and decreased digoxin synthesis. There is a marked decrease in plasma digoxin levels in left hemispheric dominant individuals. 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 a 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 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 diverse cellular processes. Serum magnesium was assessed in right handed / left hemispheric dominant individuals and was found to be increased.

Archaeal Digoxin and Tryptophan/Tyrosine Metabolism - Relation to Genomic Function

There is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the serum of left-handed/right hemispheric dominant individuals. This could be due to the fact that digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^{+}-K^{+} ATPase activity in left handed/right hemispheric dominant individuals 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. Studies from our laboratory have demonstrated the synthesis of endogenous strychnine and nicotine from

tryptophan and endogenous morphine from tyrosine. In the presence of hypomagnesemia, the magnesium block on the NMDA receptor is removed leading to NMDA excitotoxicity. The increased presynaptic neuronal calcium can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling. Increased intracellular calcium in the postsynaptic neuron can also activate the calcium dependent NMDA signal transduction. The plasma membrane neurotransmitter transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a sodium 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 Na^+-K^+ ATPase can promote glutamatergic transmission. The elevated levels of quinolinic acid, strychnine and serotonin can also contribute to NMDA excitotoxicity. Strychnine displaces glycine from its binding sites and inhibits glycinergic inhibitory transmission in the brain. The glycine is free to bind to the strychnine insensitive site of the NMDA receptor and promote excitatory NMDA transmission. Quinolinic acid and serotonin are also positive modulators of the NMDA receptor. Increased levels of nicotine can lead to increased cholinergic transmission by binding to the central nicotinic cholinergic receptor. Thus in the right hemisphere dominant hyperdigoxinemic state there is upregulated serotoninergic, cholinergic and glutamatergic transmission and downregulated dopaminergic, glycinergic and noradrenergic transmission resulting in net membrane Na^+-K^+ ATPase inhibition.

The results showed that the concentration of tryptophan, quinolinic acid, strychnine, nicotine and serotonin was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine, morphine, dopamine and norepinephrine was higher. Thus, there is a decrease in tryptophan and its catabolites and an increase in tyrosine and its catabolites in

the serum of right handed / left 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 and that digoxin levels are low in right handed / left hemispheric dominant individuals. The increase in membrane Na^+-K^+ ATPase activity in these cases could be due to the fact that the hyperpolarising neurotransmitters (dopamine, morphine and noradrenaline) are increased and the depolarising neuroactive compounds (serotonin, strychnine, nicotine and quinolinic acid) are decreased. 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 to reduced NMDA transmission. The decreased presynaptic neuronal calcium can produce reduced cyclic AMP dependent phosphorylation of synapsins resulting in decreased glutamate release into the synaptic junction and vesicular recycling. The plasma membrane glutamate transporter (on the surface of the glial cell and presynaptic neuron) is coupled to the 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. Decreased levels of nicotine can lead to down regulated central cholinergic transmission. Decreased production of nitric oxide occurs in right handed / left hemispheric dominant individuals consequent to inhibition of nitric oxide synthase by the reduced intracellular calcium levels. Thus, in the left hemisphere dominant hypodigoxinemic state there is downregulated serotoninergic, cholinergic and glutamatergic transmission and upregulated dopaminergic, glycinergic and noradrenergic transmission resulting in net membrane Na^+-K^+ ATPase stimulation.

Archaeal Digoxin and Genomic Function

Digoxin and membrane Na^+-K^+ ATPase activity changes can modulate all aspects of gene function by the alteration in the intracellular calcium/magnesium ratios that it produces. Magnesium is required as a co-factor for DNA polymerase, DNA ligase, DNA dependent RNA polymerase, amino acyl tRNA synthetase and also for ribosomal integrity. Thus it can regulate DNA replication, transcription and translation. It can also modulate DNA repair. The dielectric protein molecules - nucleosomes, which are combinations of the basic histones and nucleic acid, are excellent electric dipole oscillators which exist under a steep neuronal membrane voltage gradient consequent to membrane Na^+-K^+ ATPase inhibition produced by digoxin. Alteration in the charge of nucleosones can change its conformation resulting in alteration in the chromosomal structure. This can affect DNA transcription.

A DNA molecule with a chemical group - electron accepting metal complex of rhodium - tethered to one end of DNA appears to mediate a chemical change far down the helix, causing a patch of damaged DNA to be mended. The DNA damage repaired in the experiment - a small kink in the helix known as a thymine dimer - is the kind of damage caused by the sun's ultraviolet rays. Long range DNA repair of some kind might play a role in normal cells. DNA's unique structure allows it to behave like a conductive wire, unlike the insulating behavior of proteins. Electrons can flow freely through the channel that runs down the center of the joined bases of the helix - in this case, traveling from the thymine dimers to the added chemical groups and repairing the dimers in the process. As DNA readily transports electrons, the implications could go well beyond DNA repair. In living things, the transfer of electrons in DNA plays a crucial role in DNA regulation and other biological processes. The arrangement of bases on the complementary strands allows the electrons shared by multiple atoms to inhabit donut-shaped electron clouds above and below each ring of

bases. The interior of the helix can be thought of as a stack of these π orbitals. If electrons could be injected into this stack, they might easily tunnel from one end of the DNA to the other. While this could still be a quantum mechanical effect, the electron transfer would be as effortless as moving current through a wire. This is known as the π stack conductivity theory. The alteration in the neuronal and cellular membrane electric potential mediated by the endogenous membrane Na^+-K^+ ATPase inhibitor digoxin can affect the electron flow along the DNA changing its conformation and possibly regulating transcription.

Previous studies from this laboratory have demonstrated increased synthesis of digoxin in Huntington's disease and trisomy 21 signifying a possible role for endogenous digoxin in regulation of genomic function. DNA polymerase requires magnesium for its function. The 3' exonuclease activity of DNA polymerases I and III is the device for proofreading the newly made DNA strand and for correcting errors made by the polymerase activity. If a wrong nucelotide is inserted by the DNA polymerase, the enzyme can recognize its failure to form a correct base pair with the corresponding nucleotide in the template. If then backs up and hydrolyzes off the wrong nucleotide from the 3' end of the chain. The polymerase then proceeds to add the correct nucleotide as it resumes its normal progress in the 5'3" direction. Thus, addition of each nucleotide is checked as the replication fork moves along the template strand. The proofreading function of the DNA polymerase is very efficient and contributes a factor of at least 10^4 in guaranteeing fidelity of replication. The replication and proofreading machinery deals with DNA in lengths of a few hundred nucleotides at a time. Once the length of a nucleotide repeat becomes greater than this, the accuracy of the process breaks down. Unlike normal DNA, nucleotide repeats seem to have an inbuilt tendency to get bigger. It is the first few cell divisions that make them so vulnerable to replication errors. One explanation is that this is the time when cells are dividing most rapidly. If the

cells are trying to divide faster than normal, this could create extra problems. Part of the genome could still be replicating while the chromosomes are being pulled apart into the two new cells. This behaviour may cause a characteristic broken appearance.

Membrane Na^+-K^+ ATPase inhibition can produce intracellular magnesium depletion leading on to a defect in the proofreading function of DNA polymerase in the nucleus during DNA replication. Defective functions of DNA polymerase and the proofreading defect during DNA replication may possibly lead to the trinucleotide repeats described in HD. Trinucleotide repeat can lead to polyglutamine repeats in the proteins. This is exemplified by the protein described in HD. Intracellular magnesium depletion can also produce defective phosphorylation of MAP (microtubule associated proteins). This results in defective microtubule related spindle fibre dysfunction and chromosomal non-dysjunction probably contributing to trisomy 21. The same reason holds good for the broken appearance and fragile sites of the chromosome in fragile X syndrome. Thus the genetic defect described in these two syndromes may be partly be contributed to by hypothalamic archaeal digoxin-induced membrane Na^+-K^+ ATPase inhibition. Digoxin can regulate the function of heat-shock protein which coordinates the trafficking and regulation of diverse signalling proteins and which also functions as a molecular chaperone involved in protein folding and maturation. The heat - shock protein has an ATP/ADP switch domain that regulates HSP conformation. HSP is in the presence magnesium deficiency. Normally cellular mutations are masked by HSP90, one of the heat shock proteins. But when HSP90 is out of commission, it can no longer stabilise mutant proteins and keep them working properly. Increased mutations are revealed. Thus the brain can regulate genomic function by hypothalamic archaeal digoxin acting on the neuronal or cell membrane. Thus the hyperdigoxinemic state is associated with genomic instability owing to the

intracellular hypomagnesemia it produces. The reverse holds good for the hypodigoxinemic left hemisphere dominant state. Because of an increase in intracellular magnesium there is genomic stability.

Archaeal Digoxin, Genomic Function and Oncogenesis

We have reported elevated levels of serum digoxin and inhibition of RBC membrane Na^+-K^+ ATPase activity in neoplasms like non-Hodgkin's lymphoma and CNS glioma. In the hyperdigoxinemic right hemisphere dominant state, increased intracellular calcium activates phospholipase C beta which results in increased production of diacyglycerol (DAG) with resultant activation of protein kinase C. The protein kinase C (PKC) activates the MAP kinase cascade resulting in cellular proliferation. The decreased intracellular magnesium can produce dysfunction of GTPase activity of the alpha-subunit of the G-protein. This results in ras oncogene activation, as more of the ras are bound to GTP rather than GDP. Phosphorylation mechanisms are required for the activation of the tumour suppressor gene P_{53}. The activation of P_{53} is impaired owing to intracellular magnesium deficiency producing a phosphorylation defect. Upregulation of the isoprenoid pathway can result in increased production of farnesyl phosphate, which can farnesylate the ras oncogene producing its activation. The ubiquitin system of catabolic processing of proteins is important in the DNA repair mechanism. In the presence of intracellular magnesium deficiency, ubiquitin protein catabolic processing and DNA repair mechanisms are defective and this could contribute to oncogenesis. In the hyperdigoxinemic right hemisphere dominant state, there is oncogene activation and increased cell proliferation.

In the hypodigoxinemic left hemisphere dominant state, high intracellular magnesium and low intracellular calcium consequent to Na^+-K^+ ATPase stimulation appears to be crucial to protection against oncogenesis. Decreased intracellular calcium inactivates phospholipase C beta which results in

decreased production of diacyglycerol (DAG) with resultant inactivation of protein kinase C. The protein kinase C (PKC) activation of the MAP kinase cascade is inhibited resulting in a blockade of cellular proliferation. The increased intracellular magnesium can produce an increase in the GTPase activity of the alpha-subunit of the G-protein. This results in ras oncogene inactivation, as more of the ras is bound to GDP rather than GTP. Phosphorylation mechanisms required for the activation of the tumour suppressor gene P_{53} is increased owing to intracellular magnesium excess-producing increased phosphorylation. Downregulation of isoprenoid pathway can result in decreased production of farnesyl phosphate, which is required for ras oncogene activation. Therefore the ras oncogene is inactivated. In the hypodigoxinemic left hemisphere dominant state, there is a tendency for oncogene inactivation and inhibition of cellular proliferation.

Patients with neoplasms showed the right hemispheric neurotransmitter patterns with increased tryptophan, serotonin, quinolinic acid, strychnine and nicotine and reduced tyrosine, morphine, noradrenaline and dopamine. The neurotransmitter pattern of reduced dopamine and noradrenaline, and increased serotonin can contribute to cancer related psychosis. This neurotransmitter pattern is common to neoplasms and schizophrenia. A schizoid state of mind can predispose patients to the development of neoplasms. Alteration in natural killer cell activity has been reported in psychiatric disorders. Serotonin and acetyl choline promote cell proliferation and dedifferentiation by inhibiting adenyl cyclase or by activating phospholipase-C (PLC). Nicotine, by binding to the nicotinic receptor, promotes cholinergic transmission. Dopamine and noradrenaline elevate cyclic AMP levels and inhibit cell proliferation and differentiation. Increased glutamatergic transmission resulting in excitotoxicity has been implicated in cellular proliferation. Excitatory amino acids like glutamate can act as trophic factors and promote cellular proliferations.

Increased quinolinic acid can lead on to cancer related cachexia. Serotonin, dopamine and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation, serotonin is increased with the corresponding reduction in dopamine and noradrenaline in the brainstem monoaminergic nuclei. Thus elevated serotonin and reduced noradrenaline and dopamine can contribute to the immune activation and immunoproliferation in non-Hodgkin's lymphoma. Decreased morphine levels can lead to increased metastatic property of tumours as morphine has a suppressing effect on tumour metastasis and tumour growth. The hypodigoxinemic state with downregulated serotoninergic, glutamatergic and cholinergic transmission and upregulated morphinergic, dopaminergic and noradrenergic transmission can have an inhibitory effect on cellular proliferations.

References

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