Chapter 5

Archaeal Strychnine - Description of the Hypo - and Hyper - Strychninergic State in Relation to Neuropsychiatric Diseases

Introduction

Endogenous morphine has been described in the mammalian brain. Morphine can be synthesized from intravenously injected salutaridine, thebaine and codeine and thebaine can be converted to morphine when incubated with microsomal preparation from the liver, kidney and brain of rats. Strychnine is biosynthesized from tryptophan and a terpenoid C_{10} unit. There are reports of strychnine binding sites in the brain. But so far no endogenous strychnine has been identified in mammalian brain and other tissues. Strychnine causes a blockade of central nervous system inhibition by selectively antagonizing the effect of glycine. Glycine is a inhibitory transmitter of 4-aminobutryrate receptor in the central nervous system of vertebrates, particularly in the spinal cord. Ishimaru et al. studied the strychnine insensitive glycine binding sites in the cerebral cortex for chronic schizophrenia and suggested that an NMDA associated glycine binding site may be implicated in the pathophysiology of schizophrenia.

In this context it was considered pertinent to look for endogenous strychnine in neuropsychiatric disorders. The disorders studied include - manic depressive psychosis, schizophrenia, primary generalised epilepsy, Parkinson's disease, multiple sclerosis and CNS glioma. The serum levels of tyrosine and tryptophan were also estimated.

Results

No strychnine could be estimated in the serum of control subjects. Serum of patients with epilepsy, PD and MDP contained 11.44, 9.54 and 11.51 µg/dL of strychnine respectively. Serum of patients with schizophrenias multiple sclerosis and syndrome X contained traces of strychnine (0.60, 1.02 and 2.92 µg/dL respectively). No strychnine could be detected in the serum of patients with CNS glioma.

Serum tryptophan was found to be elevated in primary generalised epilepsy, Parkinson's disease, multiple sclerosis, CNS glioma, schizophrenia, MDP, syndrome X with multiple lacunar state, Serum tyrosine levels were found to be decreased in primary generalised epilepsy, Parkinson's disease, schizophrenia, CNS glioma, syndrome X multiple lacunar state. Dopamine levels also found to be low in multiple sclerosis, CNS glioma, syndrome X with multiple lacunar state, Primary generalised epilepsy, Parkinson's disease and schizophrenia.

RBC Na^+-K^+ ATPase was found to be reduced in primary generalised epilepsy, Parkinson's disease, multiple sclerosis, CNS glioma, schizophrenia and syndrome X with multiple lacunar state but was normal in MDP.

Discussion

The increase in serum tryptophan and decrease in tyrosine in the serum of patients of many of these disorders is a significant observation in the light of altered levels of the alkaloids. It is known that tryptophan is the precursor for strychnine. The presence of strychnine in the serum of patients of most disorders studied may be a reflection of their synthesis from tryptophan. The absence of strychnine in CNS glioma in spite of the increased tryptophan level may be a reflection of some block in their synthesis in spite of the increased availability of tryptophan.

The inhibition of membrane Na^+-K^+ ATPase activity in most of the disorders studied is another significant observation. This inhibition can result from increased depolarising strychninergic transmission. It is known that inhibition of this enzyme leads to increase in intracellular calcium due to increase in sodium calcium exchange, increased entry of calcium via voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. The increase in intracellular calcium by the displacement of magnesium from its binding sites leads to a decrease in

functional availability of magnesium. Decrease in magnesium inhibits Na^+-K^+ ATPase further as the ATP magnesium complex is the actual substrate for the reaction. Thus there is progressive inhibition of Na^+-K^+ ATPase.

Strychnine displaces glycine from its binding site and inhibits glycinergic inhibitory transmission in the brain. The glycine is free to bind to the strychnine insensitive site of the NMDA receptor and promotes excitatory NMDA transmission with a consequent increase in calcium load. This results in Na^+-K^+ ATPase stimulation and reduced opening of the voltage gated calcium channel and decrease in intracellular calcium. Thus both increased nicotine and strychnine and reduced morphine can lead on to an intraneuronal calcium overloaded state and functional magnesium deficiency owing to Na^+-K^+ ATPase inhibition.

The changes discussed above are with respect to the RBC membrane. It has been suggested that the changes in the RBC membrane may be reflective of neuronal membrane changes. If similar changes take place in the neuronal membrane also (this can be studied only with isolated neuronal membrane) then the consequence of an inhibition of neuronal membrane Na^+-K^+ ATPase and the resultant increase in neuronal calcium load and magnesium depletion can be manifold. Na^+-K^+ ATPase inhibition can produce neurotransmitter transport dysfunction, apoptosis and mitochondrial dysfunction, protein processing defects, immune activation and activation of oncogenes as discussed below.

The increased presynaptic neuronal Ca^{++} can produce cyclic AMP dependent phosphorylation of synapsins in the presynaptic neuron resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling. Increased intracellular Ca^{++} in the post synaptic neuron can also activate the G-protein coupled neurotransmitter signal transduction system of monoamine neurotransmitters and also Ca^{++} dependent NMDA signal (glutamate receptor) transduction. The plasma membrane neurotransmitter transporter (on the surface of the glial cell and presynaptic neuron) is coupled to a Na^+ gradient which is

disrupted by the inhibition of Na^{+}-K^{+} ATPase, resulting in decreased clearance of neurotransmitter (monoamines and glutamate) by presynaptic and glial uptake at the end of synaptic transmission. By these mechanisms, inhibition of Na^{+}-K^{+} ATPase can promote monoaminergic and glutamatergic transmission. Increased glutamatergic transmission resulting in excitotoxicity has been implicated in the neuronal degeneration observed in Parkinson's disease, primary generalized epilepsy and schizophrenia. Increased monoaminergic transmission, particularly of dopamine in the mesolimbic systems has been implicated in schizophrenia. A biphasic response with an increase in monoaminergic transmission in the manic phase and decrease in the depressive phase has been reported in the MDP. Inhibition of Na^{+}-K^{+} ATPase can also result in defective neuronal membrane repolarisation and a paroxysmal depolarization shift resulting in epileptogenesis.

Increased intracellular Ca^{++} activates the Ca^{++} dependent calcineurin signal transduction pathway which can produce T-cell activation and secretion of interleukin-3,4,5,6 and TNF alpha. This can explain the immune activation in MS. TNF alpha binds to its receptor and in turn can activate the caspase cascade, especially the downstream caspase-9 and produce apoptosis. Caspase-9 is an ICE protease which converts IL-1 beta precursor to IL-1 beta. IL-1 beta produces apoptosis of the neuron in Parkinson's and Alzheimer's disease and the oligodendrocyte, the myelin forming cell in MS.

Increased intracellular Ca^{++} can open the mitochondrial PT pore causing a collapse of the H^{+} gradient across the inner membrane and uncoupling of the respiratory chain. This also leads to volume dysregulation and rupture of the Outer membrane of mitochondria resulting in the release of AIF (apoptosis inducing factor) and cyto C (cytochrome C) to the cytoplasm. This results in activation of caspase-9 which produces cell death. Apoptosis has been implicated in neuronal degeneration. Increased neuronal apoptosis can produce

defective synaptogenesis and synaptic connectivity contributing to functional disorders like schizophrenia and epilepsy.

The magnesium deficiency related ATP synthase defect and increased calcium related opening of the mitochondrial PT pore produces a mitochondrial dysfunction. This results in incomplete reduction of O_2 and increased production of free radical, the superoxide ion. Mitochondrial dysfunction has been implicated in the pathogenesis of neuronal degeneration like Parkinson's disease. Increased intracellular Ca^{++} can also activate NOS (nitric oxide synthase) causing increased production of NO which combines with the superoxide radical to form the peroxynitrite ion promoting lipid peroxidation. Free radical damage has been implicated in oncogenesis and neuronal degeneration.

Intracellular magnesium deficiency also results in defective ubiquitin dependent proteolytic processing of glycoproteins and antigens as it requires magnesium for its function. The protein processing defect can result in defective glycosylation of endogenous myelin glycoprotein antigens with consequent defective formation of the MHC-antigen complex. The MHC linked peptide transporter is a P-glycoprotein which transports the MHC-antigen complex to the antigen presenting cell surface and requires magnesium for its function. Intracellular Mg^{++} deficiency results in dysfunction of the MHC linked peptide transport. Defective presentation of the endogenous myclin glycoprotein antigen can explain the immune dysregulation in MS. A CD_8 MHC class-1 restricted immune dysregulatory defect has been described in MS. Defective tumour antigen presentation to the NK cell will lead to oncogenesis as cancer cell immunosurveillance becomes dysfunctional. Defectively processed glycoproteins like membrane beta amyloid resist lysosomal digestion and accumulate, producing neuronal degeneration. Ubiquitin dependent proteolytic dysfunction has been reported in neuronal degeneration especially Parkinson's disease. Defective glycoproteins and glycosaminoglycans of the neuronal

membrane can produce defective synaptic connectivity producing functional disorders like epilepsy, MDP and schizophrenia. Defective glycosylation of proteins consequent to Na^{+}-K^{+} ATPase inhibition can result in loss of contact inhibition and oncogenesis.

Increased intra cellular calcium activates phospholipase C beta which results in production of diacyl glycerol (DAG) which activates protein kinase C. The protein kinase C (PKC) activates the MAP kinase cascade resulting in cellular proliferation. The decreased intracellular Mg^{++} can produce dysfunction of GTPase activity of the alpha-subunit of G-protein. The results in ras oncogene activation, as more of the ras is bound to GTP rather than GDP. Phosphorylation mechanism are required for the activation of the tumours, suppressor, gene P_{53}. The activation of P_{53} is impaired due to intracellular magnesium deficiency producing a phosphorylation defect.

In syndrome X there is an increase in depolarising strychninergic transmission. This can lead to Na^{+}-K^{+} ATPase inhibition. The consequent increase in calcium within the cell especially the beta cell can displace magnesium from the binding site. Magnesium depletion within the beta cell can lead to increased release of insulin from the beta cell. A cellular magnesium deficiency and increase in a calcium overloaded state can have the following consequences. Increase in intracellular calcium can lead to immune activation and increased production of TNF alpha leading to insulin resistance. Intracellular cellular magnesium deficiency can lead to protein tyrosine kinase dysfunction, an insulin receptor defect. Increased intracellular calcium can lead to an increase in G-protein coupled signal transduction of the contrainsulin hormones-glucagon, growth hormone and adrenaline. This leads to hyperglycemia. Increased intra cellular calcium can open up the mitochondrial PT pore producing a mitochondrial dysfunction and uncoupling oxidative phosphorylation. Decreased intra cellular magnesium can inhibit ATP synthase

producing a decrease in the synthesis of ATP and a mitochondrial dysfunction. Decreased intracellular magnesium can lead to inhibition of glycolysis and the citric acid cycle. Thus glucose utilization as a whole is decreased. Intra cellular magnesium deficiency can produce decreased dolichol phosphate synthesis and N-linked glycosylation. Generation of ATP for synthesis of nucleoside diphosphate sugars for O-linked glycosylation is also defective, leading to altered glycoproteiis. Intracellular magnesium deficiency can also upregulate GAG synthesis. Both these contribute to the microangiopathy and macroangiopathy of syndrome X. Increased intracellular calcium can increase the signal transduction of the G protein coupled platelet activating factor receptor and thrombin receptor producing thrombosis. Intracellular magnesium deficiency can also produce vasospasm, as described in syndrome X.

Strychnine, by displacing glycine from its binding sites and decreasing inhibitory transmission in the brain, promotes epileptogenesis. The increase in depolarising strychninergic transmission producing net Na^{+}-K^{+} ATPase inhibition can contribute to epileptogenesis. Inhibition of Na^{+}-K^{+} ATPase can lead to a paroxysmal depolarisation shift and epileptogenesis.

In schizophrenia the glutamatergic excitotoxic mechanism has been described. Strychnine, by blocking glycinergic transmission, can contribute to the decreased inhibitory transmission in schizophrenia. The glycine is free to bind to the NMDA receptor and promote NMDA transmission resulting in glutamatergic excitotoxicity.

Increased levels of strychnine have been detected in the serum of manic depressive psychosis patients. Strychnine may also act in a similar biphasic manner contributing to the bipolar mood disorder. Strychnine was detected in Parkinson’s disease. As mentioned earlier it can promote NMDA receptor activity, resulting in glutamatergic excitotoxicity important in neuronal degeneration in PD. Strychnine was detected in syndrome X contributing to decreased Na^{+}-K^{+} ATPase

and altered calcium / magnesium ratios. Serum of patients with MS showed strychnine, which can produce an increase in the intraneuronal calcium load producing oligodendrocyte apoptosis and immune activation.

In this context it is pertinent to note the interrelationship between these diseases, as documented in literature. The presence of autoantibodies has been demonstrated in MS, SLE, motor neuron disease (MND), Alzheimer's disease, Down's syndrome, paraneoplastic disease and AIDS dementia. Psychosis have been described in neurolupus, MS, Alzheimer's disease, Parkinson's disease, cancer related psychosis and AIDS dementia. The relationship between Hodgkin's lymphoma and MS, lymphoma and MND, CNS lymphoma and HIV infection and lymphomatous transformation in SLE and rheumatoid arthritis have been documented. Viral persistence as an etiological factor has been documented in MS, Parkinson's disease, non-Hodgkin's lymphoma and schizophrenia. Hyperinsulinemia has been documented in Alzheimer's disease and immune mediated neuropathies as described in syndrome X. This interrelationship is possibly dependent on increased strychnine contributing to Na^+-K^+ ATPase inhibition.

References

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