

HYPOTHALAMIC DIGOXIN, CEREBRAL CHEMICAL DOMINANCE AND MYALGIC ENCEPHALOMYELITIS

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The isoprenoid pathway was assessed in 15 patients with chronic fatigue syndrome. The pathway was also assessed in individuals with differing hemispheric dominance to assess whether hemispheric dominance had any correlation with these disease states. The isoprenoid metabolites—digoxin, dolichol, and ubiquinone—RBC membrane Na⁺-K⁺ ATPase activity, serum magnesium and tyrosine/tryptophan catabolic patterns were assessed. The free-radical metabolism, glycoconjugate metabolism, and RBC membrane composition was also assessed. Membrane Na⁺-K⁺ ATPase activity and serum magnesium levels were decreased while HMG CoA reductase activity and serum digoxin levels were increased in myalgic encephalomyelitis (ME). There were increased levels of tryptophan catabolites—nicotine, strychnine, quinolinic acid, and serotonin—and decreased levels of tyrosine catabolites—dopamine, noradrenaline, and morphine in ME. There was an increase in dolichol levels, carbohydrate residues of glycoproteins, glycolipids, total/individual GAG fractions, and lysosomal enzymes in ME. Reduced levels of ubiquinone, reduced glutathione, and free-radical scavenging enzymes, as well as increased lipid peroxidation products and nitric oxide, were noticed in ME. The biochemical patterns in ME correlated with those obtained in right hemispheric chemical dominance. The role of hypothalamic digoxin and neurotrans-

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mitter induced immune activation, altered glycoconjugate metabolism, and resultant defective viral antigen presentation, NMDA excitotoxicity and cognitive dysfunction, and mitochondrial dysfunction related myalgia in the pathogenesis of ME is stressed. ME occurs in individuals with right hemispheric chemical dominance.

Keywords cerebral chemical dominance, digoxin, myalgic encephalomyelitis

Several theories have been put forward with respect to chronic fatigue syndrome or myalgic encephalomyelitis (Shafraan, 1991; Wessely, 1991; Kennard, 1990). Persistent viral infections, especially the Epstein barr virus and enteroviruses, have been described in ME. Several immune system abnormalities have been described. Deficiencies in the amounts of IgG1 and IgG3, and decreased amounts of IgA, have been noticed. Elevated levels of alpha interferon in the spinal fluid and increased levels of interleukin-2 have been reported by some groups. T4 cells have been reported to not function as effectively as normal when stimulated with phytohaemagglutinin in ME. T8 suppressor cells changes have also been reported. Delayed type of hypersensitivity skin testing is abnormal in 80% of patients. Physical and mental stress have been reported to predispose to ME. Muscle fatigue, myalgia, and muscle twitchings are noticed in ME. Mitochondrial abnormalities have also been reported in the muscle in ME. Altered brain function has been reported in ME including loss of concentration and loss of recent memory.

The isoprenoid pathway is an important pathway crucial in cellular regulation. It produces important metabolites—digoxin, dolichol, ubiquinone, and cholesterol (Goldstein & Brown, 1990). Digoxin is an endogenous membrane $\text{Na}^+\text{-K}^+$ ATPase inhibitor secreted by the human hypothalamus (Haupt, 1989). Dolichol is important in N-glycosylation of proteins and protein processing. Ubiquinone is an important component of the mitochondrial electron transport chain. Cholesterol is an important component of cellular membranes. It was therefore considered pertinent to assess the isoprenoid pathway in ME. Since endogenous digoxin can regulate multiple neurotransmitter systems it could possibly play a role in the genesis of hemispheric dominance. The pathway was also assessed in individuals of differing hemispheric dominance to find out the role of hemispheric dominance in predisposition to ME.

MATERIALS AND METHODS

Informed consent was obtained from the patients/normal individuals included in the study. The permission of the ethics committee of the institute was also obtained, and the work was carried out in accordance with the guidelines of the Helsinki declaration. Fifteen cases of myalgic encephalomyelitis (30–40 years of age—8 males and 7 females) were chosen for the study from the medicine and neurology wards of Medical College, Trivandrum. This group was chosen at random from the ME patients admitted to the wards. The criteria given by Wessely (1991) was chosen for the purpose. None of the subjects studied was under medication at the time of removal of blood. Fasting blood was removed from each of the patients for various estimations. An equal number of age- and sex-matched healthy subjects served as controls. Fifteen cases (30–40 years—8 males and 7 females) of right hemispheric dominant, left hemispheric dominant, and bihemispheric dominant normal healthy individuals chosen by the dichotic listening test were also included in the study. They were chosen at random from the general population of Trivandrum. RBCs were separated within 1 h of collection of blood for the estimation of membrane $\text{Na}^+\text{-K}^+$ ATPase. Serum was used for the estimation of HMG CoA reductase activity. Plasma/serum was used for the estimation of the other parameters. All biochemicals used in this study were obtained from M/s. Sigma Chemicals, USA. Activity of HMG CoA reductase of the plasma was determined using the method of Rao and Ramakrishnan (1975) by determining the ratio of HMG CoA to mevalonate. For the determination of the $\text{Na}^+\text{-K}^+$ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat (1966) was used. Digoxin in the plasma was determined by the procedure described by Arun, Ravikumar, Lellamma, and Kurup (1998a). For estimation of ubiquinone and dolichol in the plasma, the procedure described by Palmer, Maureen, and Robert (1984) was used. Mg^{++} in the plasma was estimated by atomic absorption spectrophotometry (Price, 1985). Tryptophan was estimated by the method of Bloxam and Warren (1974) and tyrosine by the method of Wang, O'Flynn, and Inouye (1964). Serotonin was estimated by the method of Curzon and Green (1970) and catecholamines

by the method of Well-Malherbe (1971). Quinolinic acid content of plasma were estimated by HPLC (C_{18} column micro Bondapak™ 4.6×150 mm), solvent system 0.01 M acetate buffer (pH 3.0), and methanol (6:4), flow rate 1.0 ml/min and detection UV 250-nm). Morphine, strychnine, and nicotine were estimated by the method described by Arun, Ravikumar, Leelamma, and Kurup (1998b). Details of the procedures used for the estimation of total and individual GAG, carbohydrate components of glycoproteins, activity of enzymes involved in the degradation of GAG (beta glucuronidase, beta N-acetyl hexosaminadase, hyaluronidase, and cathepsin D) and activity of glycohydrolases (beta galactosidase, beta fucosidase, and beta glucosidase) have been described before (Manoj & Kurup, 1998). Serum glycolipids (gangliosides, glycosyl diglycerides, cerebrosides, and sulphatides) were estimated as described in Methods in Enzymology (Lowenstein, 1969). Cholesterol was estimated by using commercial kits supplied by Sigma chemicals, USA. SOD was assayed by the method of Nishikimi et al. as modified by Kakkar, Das, and Viswanathan (1984). Catalase activity was estimated by the method of Maehly and Chance (1954), glutathione peroxidase by the method of Paglia and Valentine (1967), and glutathione reductase by the method of Horn and Burns (1978). MDA was estimated by the method of Will (1969) and conjugated dienes and hydroperoxides by the procedure of Brien (1969). Reduced glutathione was estimated by the method of Beutler, Duran, and Kelley (1963). Extraction of erythrocytes for vitamin E was carried out and vitamin E was estimated in the extract by HPLC (Waters HPLC, Nova-Pak C_8 column (4.6×150 mm). Solvent-acetonitrile:methanol:water (63:33:4), flow rate—2 ml/min, detection—UV 280 nm). For vitamin E, retention time was 3.5 min under these conditions (Rammel, Cunliffe, & Keiboom, 1983). Nitric oxide was estimated in the plasma by the method of Gabor and Allon (1994). Iron binding capacity in the plasma was estimated by the method of Wootton (1964), and ceruloplasmin by the method of Henry, Chiamori, Jacobs, and Segalov (1960). Serum albumin was estimated by the method of Spencer and Price (1977). Free fatty acid was estimated by the method of Falholt, Lund, and Falholt (1973). Statistical analysis was done by ANOVA.

RESULTS

1. The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in ME. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na⁺-K⁺ ATPase and serum magnesium were decreased (Table 1).
2. The concentration of serum tryptophan, quinolinic acid, and serotonin was increased in the plasma, while that of tyrosine, dopamine, and noradrenaline was decreased in ME (Table 2).
3. Nicotine (1.07 µg/100 ml) and strychnine (9.54 µg/dL) were detected in the plasma of patients with ME but were not detectable in control serum. Morphine was not detected in the plasma of ME patients (Table 3).
4. The concentration of total glycosaminoglycans (GAG) increased in the serum of ME patients. The concentration of heparan sulphate (HS) heparin (H), dermatan sulphate (DS), chondroitin sulphates (ChS), and hyaluronic acid (HA) was increased. The concentration total hexose, fucose, and sialic acid was increased in the glycoproteins of the serum in these patients. The concentration of gangliosides, glycosyl-diglycerides, cerebroside, and sulphatide showed significant increase in the serum in these patients (Table 4).
5. The activity of glycosaminoglycan (GAG) degrading enzymes-beta glucuronidase, beta N-acetyl hexoseaminidase, hyaluronidase, and cathepsin-D-was increased in ME when compared to the controls.

TABLE 1. Concentration of serum digoxin, dolichol, magnesium, ubiquinone, and RBC membrane Na⁺-K⁺ ATPase activity in ME

Groups	HMG CoA reductase (ratio of HMG CoA/ mevalonate)	Digoxin (ng/dl)	Dolichol (µg/dl)	Ubiquinone (µg/dl)	Na ⁺ -K ⁺ ATPase (µg/p/mg protein)	Magnesium (mg/dl)
1. Control	1.15 ± 0.12	12.80 ± 1.09	39.1 ± 2.36	144.2 ± 8.65	5.04 ± 0.221	2.40 ± 0.24
2. ME	0.746 ± 0.06**	2154 ± 1.47**	69.8 ± 4.19**	101.6 ± 8.13**	1.94 ± 0.18**	2.16 ± 0.22**

***p* less than .01.

TABLE 2. Tyrosine and tryptophan catabolic patterns in ME

Group	Tryptophan (mg/dl)	Tyrosine (mg/dl)	5 HT (μ g/dl)	Dop (ng/dl)	Norepi (ng/dl)	QA (ng/ml)
1. Control	1.11 \pm 0.08	1.14 \pm 0.09	20.9 \pm 1.9	12.89 \pm 0.67	45.15 \pm 2.35	370.60 \pm 21.07
2. ME	2.05 \pm 0.07**	1.01 \pm 0.07**	48.9 \pm 3.9**	8.43 \pm 0.44**	33.54 \pm 1.78**	655.73 \pm 48.8**

***p* less than .01.

5 HT = serotonin, Dop = dopamine, Norepi = norepinephrine, QA = quinolinic acid.

TABLE 3. Tryptophan and tyrosine derived alkaloids in ME

Groups	Morphine (μ g/dl)	Strychnine (μ g/dl)	Nicotine (μ g/dl)
1. Control	ND	ND	ND
2. ME	ND	8.44 \pm 0.3	4.56 \pm 0.2

Note. Values are mean \pm SD of 15 cases in each group.

ND = not detectable.

TABLE 4. Concentration of plasma glycoconjugates in ME

	1 Control	2 ME
Total GAG (mg uronic acid/dl)	4.57 \pm 0.408	8.38 \pm 0.73**
HA (mg uronic acid/dl)	0.525 \pm 0.41	1.76 \pm 0.112**
HS (mg uronic acid/dl)	0.318 \pm 0.022	2.105 \pm 0.112**
H (mg uronic acid/dl)	0.284 \pm 0.019	1.803 \pm 0.038**
DS (mg uronic acid/dl)	2.83 \pm 0.232	3.42 \pm 0.229**
ChS (mg uronic acid/dl)	0.587 \pm 0.043	2.46 \pm 0.146**
Hexose (mg/g protein)	13.55 \pm 1.26	27.5 \pm 2.48**
Fucose (mg/g protein)	1.65 \pm 0.149	2.51 \pm 0.206**
Sialic acid (mg/g protein)	6.85 \pm 0.617	10.27 \pm 0.822**
Gangloside (μ g/dl)	26.5 \pm 1.2	35.58 \pm 2.1**
Glycosyl diglyceride (μ g/dl)	12.5 \pm 0.72	20.22 \pm 1.66**
Cerebrosides (μ g/dl)	16.25 \pm 1.10	22.52 \pm 1.4**
Sulphatides (μ g/dl)	5.25 \pm 0.61	6.94 \pm 0.628**

***p* less than .01.

TABLE 5. Lysosomal enzymes in ME

	1 Control	2 ME
Beta glucuronidase (μg p-nitrophenol/h/g protein)	59.52 \pm 5.26	112.29 \pm 2.20**
Beta N-acetyl hexosaminidase (μg p-nitrophenol/h/g protein)	22273 \pm 78.6	3209 \pm 79.50**
Hyaluronidase (μg N-acetyl glucosamine/h/g protein)	62.9 \pm 4.1	220 \pm 6.8**
Cathepsin-D (μg tyrosine/h/g protein)	90.9 \pm 8.9	313.4 \pm 9.8**
Beta galactosidase (μg p-nitrophenol/h/mg protein)	52.8 \pm 3.75	95.39 \pm 4.77**
Beta fucosidase (μg p-nitrophenol/h/mg protein)	23.63 \pm 1.65	33.38 \pm 2.87**
Beta glucosidase (μg p-nitrophenol/h/mg protein)	27.36 \pm 2.46	35.2 \pm 2.78**

***p* less than .01.

The activity of beta galactosidase, beta fucosidase, and beta glucosidase increased in ME (Table 5).

- The concentration of total GAG and hexose and fucose residues of glycoproteins in the RBC membrane decreased significantly in ME. The concentration of RBC membrane cholesterol increased in ME while that of phospholipid decreased. The ratio of RBC membrane cholesterol:phospholipids increased in ME (Table 6).
- The activity of superoxide dismutase (SOD), catalase, glutathione reductase, and glutathione peroxidase in the erythrocytes decreased significantly in ME. In ME the concentration of MDA, hydroperoxides, conjugated dienes, and NO increased significantly. The

TABLE 6. RBC membrane composition in ME

	1 Control	2 ME
GAG ($\mu\text{g}/\text{mg}$ protein)	6.62 \pm 0.71	3.53 \pm 0.29**
Hexose ($\mu\text{g}/\text{mg}$ protein)	145.09 \pm 11.85	80.40 \pm 6.77**
Fucose ($\mu\text{g}/\text{mg}$ protein)	63.33 \pm 4.60	47.5 \pm 2.5**
Cholesterol (nmol/mg protein)	704.33 \pm 63.09	676.31 \pm 42.27**
Phospholipid (nmol/mg protein)	717.57 \pm 67.36	550.2 \pm 48.97**
Cholesterol:phospholipid	0.982 \pm 0.095	1.229 \pm 0.12**

***p* less than .01.

TABLE 7. Free radical metabolism in ME

	1 Control	2 ME
MDA ($\mu\text{m/ml RBC}$)	10.830 \pm 0.432	11.633 \pm 0.291**
Hydroperoxide ($\mu\text{m/ml RBC}$)	253.60 \pm 10.18	269.88 \pm 8.23**
Conjugated dienes ($\mu\text{m/ml RBC}$)	49.33 \pm 2.53	56.94 \pm 4.81**
Nitric oxide ($\mu\text{m/g protein}$)	2.835 \pm 0.207	3.320 \pm 0.161**
Glutathione ($\mu\text{g/ml RBC}$)	256.60 \pm 10.96	243.23 \pm 13.99**
Superoxide dismutase (units/mg protein)	43.14 \pm 1.94	40.43 \pm 1.45**
Catalase $\times 10^{-2}$ (units/mg protein)	3.486 \pm 0.117	3.115 \pm 0.086**
GSH peroxidase (units/g protein)	48.10 \pm 1.64	45.20 \pm 1.32**
GSH reductase (units/g protein)	8.370 \pm 0.87	7.653 \pm 0.322**

***p* less than .01.

concentration of glutathione decreased while that of alpha tocopherol was unchanged in ME. Iron binding capacity, ceruloplasmin, and albumin decreased significantly in ME (Tables 7 and 8).

8. The results showed that HMG CoA reductase activity, serum digoxin, and dolichol were increased and ubiquinone reduced in left-handed/right hemispheric dominant individuals. The results also showed that HMG CoA reductase activity, serum digoxin, and dolichol were decreased and ubiquinone 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 also showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine, and nicotine was found to be lower in the

TABLE 8. Concentration of alpha tocopherol acetate, iron binding capacity, ceruloplasmin, and albumin in ME

	1 Control	2 ME
Alpha tocopherol acetate ($\mu\text{g/ml RBC}$)	5.253 \pm 0.328	5.215 \pm 0.309
Iron binding capacity ($\mu\text{g/dl}$)	261.80 \pm 16.00	229.41 \pm 14.52**
Ceruloplasmin (mg/dl)	35.81 \pm 1.53	32.20 \pm 1.80**
Albumin ($\mu\text{g/dl}$)	4.78 \pm 0.05	3.09 \pm 0.04**

***p* less than .01.

TABLE 9. Concentration of serum digoxin, dolichol, magnesium ubiquinone, and RBC membrane Na⁺-K⁺ ATPase activity/hemispheric dominance

Groups	HMG CoA reductase (ratio of HMG CoA/ mevalonate)	Digoxin (ng/dl)	Dolichol (μg/dl)	Ubiqui- none (μg/dl)	Na ⁺ -K ⁺ ATPase (μg/p _i /mg protein)	Magnesium (mg/dl)
1. LH Dom.	1.13 ± 0.12**	7.80 ± 0.06**	36.1 ± 2.36**	142.1 ± 8.65**	5.02 ± 0.220**	2.90 ± 0.24**
2. Bihem. Dom.	0.82 ± 0.065	14.80 ± 1.01	63.8 ± 2.96	86.40 ± 5.91	3.01 ± 0.18	1.72 ± 0.13
3. RH Dom.	0.46 ± 0.07**	30.95 ± 2.19**	90.2 ± 3.63**	42.8 ± 2.12**	1.01 ± 0.120**	1.06 ± 0.11**

***p* less than .01.

LH Dom. = left hemispheric dominant, Bihem. Dom. = bihemispheric dominant, RH Dom. = right hemispheric dominant.

plasma of right-handed/left hemispheric dominant individuals while that of tyrosine, dopamine, morphine, and norepinephrine was higher (Tables 9, 10, and 11).

DISCUSSION

The increase in the activity of HMG CoA reductase in ME suggests an upregulation of the isoprenoid pathway. There is a marked

TABLE 10. Tyrosine and tryptophan catabolic patterns/hemispheric dominance

Group	Tryptophan (mg/dl)	Tyrosine (mg/dl)	5 HT (μg/dl)	Dop (ng/dl)	Norepi (ng/dl)	QA (ng/ml)
1. LH Dom.	1.13 ± 0.09**	1.15 ± 0.08**	17.9 ± 1.8**	11.72 ± 0.62**	42.10 ± 2.30**	362.28 ± 51.63**
2. Bihem. Dom.	2.02 ± 0.05	0.840 ± 0.06	43.9 ± 1.9	8.72 ± 0.42	30.56 ± 1.32	632.52 ± 49.42
3. RH Dom.	2.96 ± 0.08**	0.142 ± 0.06**	52.66 ± 2.2**	4.92 ± 0.42**	21.19 ± 1.32**	690.28 ± 41.32**

***p* less than .01.

5 HT = serotonin, Dop = dopamine, Norepi = norepinephrine, QA = quinolinic acid, Bihem. Dom. = bihemispheric dominant, LH Dom. = left hemispheric dominant, RH Dom. = right hemispheric dominant.

TABLE 11. Tryptophan and tyrosine derived alkaloids/hemispheric dominance

<i>Groups</i>	<i>Morphine</i> ($\mu\text{g}/\text{dl}$)	<i>Strychnine</i> ($\mu\text{g}/\text{dl}$)	<i>Nicotine</i> ($\mu\text{g}/\text{dl}$)
1. Left hemispheric dominant	7.56 \pm 0.56	ND	ND
2. Bi-hemispheric dominant	ND	ND	ND
3. Right hemispheric dominant	ND	0.92 \pm 0.02	6.28 \pm 0.24

Mean of the values from 15 samples \pm SD.

ND = not detectable.

increase in plasma digoxin and dolichol and this increase may be a consequence of increased channeling of intermediates of the isoprenoid pathway for their biosynthesis. In this connection, incorporation of ^{14}C -acetate into digoxin in rat brain has been shown by us indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also (Ravikumar, Jyothi, & Kurup, 2001). The increase in endogenous digoxin, a potent inhibitor of membrane $\text{Na}^+\text{-K}^+$ ATPase, can decrease this enzyme activity (Hauptert, 1989). In ME there was significant inhibition of the RBC membrane $\text{Na}^+\text{-K}^+$ ATPase activity. The inhibition of $\text{Na}^+\text{-K}^+$ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased $\text{Na}^+\text{-Ca}^{++}$ 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 (Haga, 1992). This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation, which along with low magnesium, can cause further inhibition of $\text{Na}^+\text{-K}^+$ ATPase, since ATP-magnesium complex is the actual substrate for this reaction (Haga, 1992). Cytosolic free calcium normally buffered by two mechanisms: ATP-dependent calcium extrusion from cells 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 $\text{Na}^+\text{-K}^+$ ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to $\text{Na}^+\text{-K}^+$ ATPase inhibition appears to be crucial to the pathophysiology of ME. Serum magnesium was assessed in ME and was found to be reduced.

Increased intracellular calcium activates the calcium-dependent calcineurin signal transduction pathway that can produce T cell activation and secretion of Interleukin 3, 4, 5, 6, and TNF alpha (Tumour necrosis factor alpha) (Finkel, 1991). TNF alpha binds to its receptor TNFRI and activates the transcription factors NF- κ B and AP-I leading to the induction of proinflammatory and immunomodulatory genes (Ashkenazi & Dixit, 1998). This can explain the immune activation in ME. Polyclonal B cell activation and proliferation have been described in ME. Membrane Na⁺-K⁺ ATPase inhibition can produce immune activation and is reported to increase CD₄/CD₈ ratios as exemplified by the action of lithium.

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 (Hisaka, Kasamatu, & Takenaga, 1990). 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 (Arun et al., 1998b). The results showed that the concentration of tryptophan, quinolinic acid, nicotine, strychnine, and serotonin was found to be higher in the plasma of patients with ME while that of tyrosine, morphine, dopamine, and norepinephrine was lower. Thus, there is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the patient's serum. This could be due to the fact digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine (Hisaka et al., 1990). The decrease in membrane Na⁺-K⁺ ATPase activity in ME could be due to the fact that the hyperpolarizing neurotransmitters (dopamine, morphine, and noradrenaline) are reduced and the depolarizing neuroactive compounds (serotonin, strychnine, nicotine, and quinolinic acid) are increased (Wyllie, 1996).

The schizoid neurotransmitter pattern of reduced dopamine, noradrenaline, and morphine, and increased serotonin, strychnine, and nicotine is common to ME and schizoid state (Carpenter & Buchanan, 1994). This could be the basis of the schizophreniform psychosis described in ME. Quinolinic acid, an NMDA agonist, can contribute

to NMDA excitotoxicity reported in schizoid state. Strychnine, by blocking glycinergic transmission, can contribute to the decreased inhibitory transmission in schizoid state. Recent data suggest that the initial abnormality in schizoid state involves a hypodopaminergic state, and the low dopamine levels now observed agrees with this (Carpenter & Buchanan, 1994). Nicotine, by interacting with nicotinic receptors, can facilitate the release of dopamine, promoting the dopaminergic transmission in the brain. This can explain the increased dopaminergic transmission in the brain in the setting of decreased dopamine synthesis. The increased serotonergic activity and reduced noradrenergic outflow from locus coeruleus reported earlier in schizoid state agrees with our finding of elevated serotonin and reduced noradrenaline levels in ME and schizophrenia (Carpenter & Buchanan, 1994). Quinolinic acid has been implicated in immune activation in other autoimmune diseases like SLE and could contribute to the same in ME (Saito, Crowley, Markey, & Heyes, 1993). Serotonin, dopamine, and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation serotonin is increased with a corresponding reduction in dopamine and noradrenaline in the brainstem monoaminergic nuclei (Felton, Cohen, & Ader, 1991). Thus, elevated serotonin and reduced noradrenaline and dopamine could contribute to the immune activation in ME. Endogenous morphine deficiency is found in patients with ME. Morphine has an immunosuppressive effect and its deficiency can contribute to immune activation in ME (Frichione, Mendoza, & Stefano, 1994). Quinolinic acid, as well as neurotransmitter-induced immune activation, can promote ME. Thus, a schizoid neurotransmitter pattern can predispose to ME.

In the presence of hypomagnesemia, the Mg^{++} block on the NMDA receptor is removed leading to NMDA excitotoxicity (Greenamyre & Poter, 1994). The increased presynaptic neuronal Ca^{++} can produce cyclic AMP dependent phosphorylation of synapsins resulting in increased neurotransmitter release into the synaptic junction and vesicular recycling (Greenamyre & Poter, 1994). Increased intracellular Ca^{++} in the post synaptic neuron can also activate the Ca^{++} -dependent NMDA signal transduction. The plasma membrane neurotransmitter (on the surface of the glial cell and presynaptic neuron) is coupled to a Na^{+} gradient that is disrupted by the inhibition of

$\text{Na}^+\text{-K}^+$ ATPase, resulting in decreased clearance of glutamate by presynaptic and glial uptake at the end of synaptic transmission (Greenamyre & Poter, 1994). By these mechanisms, inhibition of $\text{Na}^+\text{-K}^+$ ATPase can promote glutamatergic transmission. The elevated levels of quinolinic acid and serotonin can also contribute to NMDA excitotoxicity. Quinolinic acid and serotonin are positive modulators of the NMDA receptor (Greenamyre & Poter, 1994). Strychnine 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 NMDA excitatory transmission (Greenamyre & Poter, 1994). NMDA excitotoxicity has been implicated in neuronal degeneration and could contribute to altered brain function including loss of concentration and memory in ME (Lipton & Rosenberg, 1994).

The Mg^{2+} depletion can affect the metabolism of glycosaminoglycans, glycoproteins, and glycolipids (Jaya & Kurup, 1986). The elevation in the level of dolichol may suggest its increased availability for N-glycosylation of proteins. Magnesium deficiency can lead to increased cerebroside and ganglioside synthesis. In Mg^{++} deficiency, the glycolysis, citric acid cycle, and oxidative phosphorylation are blocked and more of glucose-6-phosphate is channeled for the synthesis of glycosaminoglycans (GAG). The results show an increase in the concentration of serum total and differential GAG fractions, glycolipids, and carbohydrate components of glycoproteins in ME. The increase in the carbohydrate components—total hexose, fucose, and sialic acid—in ME was not to the same extent suggesting qualitative change in glycoprotein structure. The activity of GAG degrading enzymes and that of glycohydrolases showed significant increase in the serum in ME patients. Intracellular Mg^{++} deficiency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires Mg^{++} for its function (Monia, Ecker, & Crooke, 1990). The increase in the activity of glycohydrolases and GAG degrading enzymes could be due to reduced lysosomal stability and consequent leakage of lysosomal enzymes into the serum. The increase in the concentration of carbohydrate components of glycoproteins and GAG in spite of increased activity of glycohydrolases may be due to their possible resistance

to cleavage by glycohydrolases consequent to qualitative change in their structure. Proteoglycan complexes formed in the presence of altered calcium/magnesium ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate.

The protein processing defect can result in defective glycosylation of exogenous viral glycoprotein antigens with consequent defective formation of MHC-glycoprotein antigen complex (Ploegh, 1998). The MHC linked peptide transporter, a P-glycoprotein that transports MHC-antigen complex to the antigen presenting cell surface, has an ATP binding site. The peptide transporter is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class I—viral glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD₄ or CD₈ cell. Defective presentation of exogenous viral antigens can produce immune evasion by the virus in ME and viral persistence. This could be the reason for the persistence of enterovirus and EB virus in ME. A number of fucose and sialic acid containing natural ligands are involved in adhesion of the lymphocyte, producing leukocyte trafficking and extravasation in to the perivascular space and the same phenomena could contribute to the pathology of ME (Linstinsky, Siegal, & Linstinsky, 1998).

The alteration in the isoprenoid pathway specifically, cholesterol as well as changes in glycoproteins and GAG, can affect cellular membranes. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipases A₂ and D. The cholesterol:phospholipid ratio of the RBC membrane was increased in ME. The concentration of total GAG, hexose, and fucose of glycoprotein decreased in the RBC membrane, and increased in the serum, suggesting their reduced incorporation into the membrane and defective membrane formation. The glycoproteins, GAG, and glycolipids of cellular membrane are formed in the endoplasmic reticulum, which is then budded off as a vesicle that 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 that are crucially dependent on magnesium and are defective

in magnesium deficiency (Wiedemann & Cockcroft, 1998). The change in membrane structure produced by alteration in glycoconjugates and cholesterol:phospholipid ratio can produce changes in the conformation of sodium-potassium ATP resulting in further membrane $\text{Na}^+\text{-K}^+$ ATPase inhibition. The same changes can affect the structure of organallae membrane. This results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum. Defective peroxisomal membranes lead to catalase dysfunction which has been documented in ME.

The concentration of ubiquinone decreased significantly in ME, which may be the result of low tyrosine levels consequent to digoxin's effect in preferentially promoting tryptophan transport over tyrosine (Hisaka et al., 1990). 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 up the mitochondrial PT pore causing a collapse of the hydrogen gradient across the inner membrane and uncoupling of the respiratory chain (Green & Reed, 1998). Intracellular magnesium deficiency can lead to a defect in the function of ATP synthase. All of this leads to defects in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen, and generation of superoxide ion that 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 that combines with superoxide radical to form peroxynitrite (Olanow & Arendash, 1994). 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 damage which can further inactivate $\text{Na}^+\text{-K}^+$ ATPase, triggering the cycle of free radical generation once again. Magnesium deficiency can affect glutathione synthase and glutathione reductase function. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with increased intracellular calcium related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective owing

to membrane $\text{Na}^+\text{-K}^+$ ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. There was an 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 ME. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase is decreased in ME suggesting reduced free radical scavenging. Mitochondrial dysfunction related free radical generation could be implicated in the pathogenesis of ME (Shafran, 1991; Wessely, 1991; Kennard, 1990). The mitochondrial dysfunction could account for the muscle pain and fatigability described in ME.

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 (Green & Reed, 1998). The outer membrane of the mitochondria ruptures and releases 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 (Ashkenazi & Dixit, 1998). Increased apoptosis could also contribute to pathogenesis of ME.

Retroviruses have been related to the pathogenesis of ME (Shafran, 1991; Wessely, 1991). The retroviral genome is probably integrated into the genome of mammals including humans as vertically transmitted endogenous proviruses (Stoye, 1997). This retroviral sequences are transposable, and are kept silenced by DNA methylation (Lie, Beard, & Jaenisch, 1993). Increased secretion of hypothalamic digoxin contributes to an intracellular magnesium deficiency, which leads to a DNA methylation defect. DNA methylation requires abundant supply of S-adenosyl methionine, which requires magnesium for its generation. In the presence of hyperdigoxinemia, DNA methylation is defective and the retroviral transposons are activated and expressed. This leads to transcription of reteroviral proteins and assembly of the virus. This leads to retroviral persistence and ME.

Thus, the isoprenoid pathway and endogenous $\text{Na}^+\text{-K}^+$ ATPase inhibition can play a role in the genesis of the ME.

The neurotransmitter patterns of reduced dopamine, morphine,

and noradrenaline, and increased serotonin, strychnine, and nicotine are associated with right hemispheric chemical dominance. The digoxin and dolichol synthesis is also increased and ubiquinone levels low in right hemispheric chemically dominant individuals. The membrane $\text{Na}^+\text{-K}^+$ ATPase activity is inhibited, and serum magnesium depleted in right hemispheric chemical dominance. Right hemispheric chemically dominant individuals may have an increased predilection for ME. Left hemispheric chemically dominant individuals have reduced digoxin and dolichol levels, increased ubiquinone levels, upregulated RBC membrane $\text{Na}^+\text{-K}^+$ ATPase activity, serum hypermagnesemia, increased levels of serum dopamine, noradrenaline, and morphine, and reduced levels of serum strychnine, nicotine, and serotonin. This neurotransmitter patterns and hypodigoxinemia could protect against ME. Thus, myalgic encephalomyelitis may be a reflection of right hemispheric chemical dominance and the neurotransmitter and immune changes related to it.

1. NMDA excitotoxicity due to (a) membrane $\text{Na}^+\text{-K}^+$ ATPase inhibition-related hypomagnesemia; (b) presence of NMDA agonists like quinolinic acid, strychnine, and serotonin.
2. Digoxin-induced hypomagnesemia and elevated dolichol-related protein processing defects, and defective presentation of viral glycoprotein antigen leading on to immune evasion by the virus and viral persistence.
3. Mitochondrial dysfunction due to low ubiquinone levels, digoxin-induced alteration in intracellular calcium/magnesium ratios, and increased ceramide levels leading to (a) apoptosis and (b) free radical generation.
4. DNA methylation defects due to digoxin-induced hypomagnesemia and retroviral transposon expression
5. ME occurs in the right hemispheric dominant state.

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