Journal of NeuroVirology, 8: 326–334, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290100770

Hypothalamic digoxin-mediated model for subacute sclerosing panencephalitis

Ravi Kumar Kurup[1] and Parameswara Achutha Kurup[2]

[1]*Dept. of Neurology, Medical College, Trivandrum, Kerala, India; and* [2]*Metabolic Disorders Research Center, Trivandrum, Kerala, India*

The isoprenoid pathway including endogenous digoxin was assessed in subacute sclerosing panencephalitis (SSPE). This was also studied for comparison in patients with right hemispheric and left hemispheric dominance. The following parameters were measured in patients with SSPE and in individuals with right hemispheric, left hemispheric and bihemispheric dominance—(a) plasma HMG CoA reductase, digoxin, dolichol, ubiquinone, and magnesium levels; (b) tryptophan/tyrosine catabolic patterns; (c) free-radical metabolism; (d) glycoconjugate metabolism; and (e) membrane composition and RBC membrane Na^+-K^+ ATPase activity. The isoprenoid pathway was upregulated with increased digoxin synthesis in patients with SSPE and in those with right hemispheric dominance. In this group of patients: (a) the tryptophan catabolites were increased and the tyrosine catabolites reduced; (b) the dolichol and glycoconjugate levels were elevated; (c) lysosomal stability was reduced; (d) ubiquinone levels were low and free-radical levels increased; and (e) the membrane cholesterol:phospholipid ratios were increased and membrane glycoconjugates reduced. On the other hand, in patients with left hemispheric dominance the reverse patterns were obtained. The upregulated isoprenoid pathway and hypothalamic digoxin are involved in the pathogenesis of SSPE. SSPE occurs in right hemispheric chemically dominant individuals and a pathogenetic model for SSPE implicating hypothalamic digoxin is proposed. *Journal of NeuroVirology* (2002) **8,** 326–334.

Keywords: digoxin; cerebral dominance; membrane Na^+-K^+ ATPase; ubiquinone; SSPE

Introduction

The hypothalamus produces an endogenous membrane Na^+-K^+ ATPase inhibitor, digoxin, which is a steroidal glycoside (Haupert, 1989). Digoxin is synthesised by the isoprenoid pathway (Ravi Kumar *et al*, 2001). Increased levels of digoxin has been documented in immune diseases such as Kawasaki's disease (Tamura *et al*, 1992). A viral infective theory for Kawasaki's disease has been postulated by several groups of workers. Membrane Na^+-K^+ ATPase inhibition leads to immune stimulation and increased in CD_4/CD_8 ratios as exemplified by the action of lithium (Gorman and Locke, 1989). Digoxin can also modulate amino acid and neurotransmitter transport (Hisaka *et al*, 1990). Saito has reported increased activities of the tryptophan catabolic kynurenine pathway in various tissues following systemic immune stimulation, in conjunction with macrophage infiltration of the affected tissues (Saito *et al*, 1993). These results suggest that kynurenine metabolites may have some connection with immune response. Previous reports have demonstrated induction of indoleamine 2,3-dioxygenase and increased production of quinolinic acid in immune-mediated diseases by the action of interferons (Wallace *et al*, 1996). The isoprenoid pathway produces two other metabolites–ubiquinone and dolichol—important in cellular metabolism (Goldstein and Brown, 1990). Ubiquinone functions as a free-radical scavenger and dolichol is important in *N*-glycosylation of proteins.

Address correspondence to PA Kurup, Gouri Sadan, T.C.4/1525, North of Cliff House, Kattu Road, Kowdiar, Trivandrum, 695011, Kerala, India. E-mail: kvgnair@satyam.net.in

Received 13 February 2002; revised 26 March 2002; accepted 25 April 2002.

Table 1 Concentration of serum digoxin, dolichol, magnesium, and ubiquinone and RBC membrane Na^+-K^+ ATPase activity

Groups	*HMG CoA Reductase ratio of HMG CoA/mevalonate*	*Digoxin (ng/dl)*	*Dolichol (μg/dl)*	*Ubiquinone (μg/dl)*	*Na^+-K^+ ATPase (μg/p_i/mg protein)*	*Magnesium (mg/dl)*
Control (1)	1.15 ± 0.12	14.80 ± 1.09	39.1 ± 2.36	120.2 ± 8.65	3.04 ± 0.221	1.98 ± 0.24
SSPE (2)	0.89 ± 0.06[a]	29.95 ± 2.36[a]	44.2 ± 1.98[a]	86.8 ± 5.6[a]	1.05 ± 0.12[a]	1.03 ± 0.11[a]

Mean of the values of 15 samples ± SD.
Group 2 has been compared with group 1.
[a]$P < 0.01$.

It was therefore considered pertinent to study digoxin status and digoxin synthesis in SSPE (subacute sclerosing panencephalitis). The glycoconjugate metabolism, free-radical metabolism, and RBC membrane composition were also studied in these groups of diseases. These parameters were also studied in patients with right hemispheric and left hemispheric dominance in order to find the correlation between hemispheric dominance and slow viral infection—SSPE. The results are presented here.

Results

Serum HMG CoA reductase activity, serum digoxin, and dolichol were increased in SSPE, indicating upregulation of the isoprenoid pathway but serum ubiquinone, magnesium, and RBC membrane Na^+-K^+ ATPase activity was reduced (see Table 1). Results showed that the concentration of tryptophan, quinolinic acid, serotonin, strychnine, and nicotine was found to be higher in the serum of patients with SSPE while that of tyrosine, dopamine, and norepinephrine was lower. There was no detectable morphine in the serum of SSPE patients as seen in Tables 2 and 3. Lipid peroxidation increased, 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 SSPE. The activity of enzymes involved in free-radical scavenging such as superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase is decreased in SSPE, suggesting reduced free-radical scavenging (see Table 4).

An increase was seen in the concentration of serum total and individual GAG fractions, glycolipids, and carbohydrate components of glycoproteins in SSPE. The activity of GAG-degrading enzymes and that of glycohydrolases showed significant increase in the serum in SSPE as shown in Tables 5 and 6.

The cholesterol:phospholipid ratio of the RBC membrane was increased in SSPE. The concentration of total GAG, hexose, and fucose content of glycoprotein decreased in the RBC membrane and increased in the serum in SSPE, as seen in Table 7.

The results showed that serum HMG CoA reductase activity serum digoxin and dolichol levels were increased and serum ubiquinone, magnesium, and RBC membrane Na^+-K^+ ATPase activity were reduced in left-handed/right-hemispheric dominant individuals. The results also showed that serum HMG CoA reductase activity, serum digoxin, and dolichol levels were decreased, and serum ubiquinone, magnesium, and RBC membrane Na^+-K^+ ATPase activity were 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 serum 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 serum of right-handed/left-hemispheric dominant individuals whereas that of tyrosine, dopamine, morphine, and norepinephrine was higher. The bihemispheric dominant group had intermediate values between the right-hemispheric and left-hemispheric dominant group. The bihemispheric dominant group may have fluctuating dominance with cerebral dominance interchanging between right-hemispheric and left hemispheric. Therefore the biochemical values of the bihemispheric dominant group may also fluctuate between left-hemispheric and right-hemispheric

Table 2 Tyrosine and tryptophan catabolic patterns

Group	*Tryptophan (mg/dl)*	*Tyrosine (mg/dl)*	*5HT (μg/dl)*	*Dop (ng/dl)*	*Norepi (ng/dl)*	*QA (ng/dl)*
Control (1)	1.11 ± 0.08	1.14 ± 0.09	20.9 ± 1.9	12.89 ± 0.67	45.15 ± 2.35	370.60 ± 21.07
SSPE (2)	1.96 ± 0.09[a]	0.883 ± 0.05[a]	59.5 ± 4.6[a]	8.53 ± 0.53[a]	34.18 ± 1.11[a]	659.34 ± 41.21[a]

Mean of the values of 15 samples ± SD.
Group 2 has been compared with group 1.
[a]$P < 0.01$.

Table 3 Tryptophan- and tyrosine-derived alkaloids in serum of patients

Groups	*Morphine (μg/dl)*	*Strychnine (μg/dl)*	*Nicotine (μg/dl)*
Control (1)	ND	ND	ND
SSPE (2)	ND	0.60 ± 0.02^a	5.28 ± 0.21^a

Mean of the values of 15 samples ± SD.
Group 2 has been compared with group 1.
[a]$P < 0.01$.
ND—not detectable.

values. This may be the reason why many of the values for SSPE controls of the bihemispheric dominant type are more similar to LH values in the second study see Tables 8, 9, and 10.

Discussion

The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity in SSPE. There was increased synthesis of digoxin as evidenced by increased HMG CoA reductase activity (Ravi Kumar *et al*, 2001). The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+-Ca^{++} exchange, which displaces magnesium from its binding site and causes a decrease in the functional availability of magnesium (Haga, 1992). This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation that, along with low magnesium, can cause further progressive inhibition of Na^+-K^+ ATPase, because ATP-magnesium complex is the actual substrate for this reaction. Low intracellular magnesium and high intracellular calcium consequent to Na^+-K^+ ATPase inhibition appear to be crucial to the pathophysiology of SSPE. Serum magnesium is reduced in SSPE.

In SSPE, increased intracellular calcium consequent to membrane Na^+-K^+ ATPase inhibition activates the calcium-dependent calcineurin signal transduction pathway, which can produce T cell activation and secretion of interleukin 3, 4, 5, 6, and TNF alpha (tumor necrosis factor alpha) (Finkel, 1991; Ashkenazi and Dixit, 1998). This immune activation can contribute to the genesis of SSPE.

There is a increase in tryptophan and its catabolites and reduction in tyrosine and its catabolites in the serum of patients with SSPE. 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). In the presence of hypomagnesmia, the magnesium block on the NMDA receptor is removed leading to NMDA excitotoxicity (Greenamyre and Poter, 1994). The elevated levels of quinolinic acid, strychnine, and serotonin can also contribute to NMDA excitotoxicity as they are positive modulators of the NMDA receptor. NMDA excitotoxic mechanisms have been postulated to contribute to neuronal death in SSPE. Quinolinic acid has been implicated in immune activation in immune-mediated diseases and could contribute to the same in SSPE (Felton *et al*, 1991). 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 and this can contribute to the immune activation in SSPE (Carpenter and Buchanan, 1994). The schizoid neurotransmitter pattern of reduced dopamine, noradrenaline, and morphine and increased serotonin, strychnine, and nicotine is common to SSPE and could predispose to its development (Carpenter and Buchanan, 1994). A schizoid type of personality could predispose to the development of SSPE. SSPE can also have a neuropsychiatric presentation.

The elevation in the level of dolichol in SSPE may suggest its increased availability for *N*-glycosylation of proteins. Magnesium deficiency can lead on to increased glycolipid and glycosaminoglycan synthesis (Jaya and Kurup, 1986). Intracellular magnesium deficiency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires magnesium for its function (Monia *et al*, 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 many glycohydrolases may be due to their possible resistance to cleavage by glycohydrolases/GAG-degrading enzymes consequent to qualitative change in their structure. The protein-processing defect can result in defective glycosylation of endogenous neuronal glycoprotein antigens and exogenous viral glycoprotein antigens with consequent defective formation of MHC-antigen complex (Ploegh, 1998). The MHC-linked peptide transporter, a P-glycoprotein that transports MHC-antigen complex to the antigen presenting cell surface, has a ATP binding site. The peptide transporter is dysfunctional in the presence of magnesium deficiency.

This results in defective transport of MHC class-1 glycoprotein antigen complex to the antigen-presenting cell surface for recognition by CD_4 or CD_8 cell. Defective presentation of exogenous viral antigens can produce immune evasion by the virus as in SSPE. A number of fucose- and sialic acid-containing natural ligands are involved in trafficking of leukocytes and similar breaches in blood–brain barrier and resultant adhesion and trafficking of the lymphocyte and extravasation in to the perivascular space have been described in the brain in SSPE (Linstinsky *et al*, 1998). The upregulation of isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid

Table 4 Free-radical metabolism

Groups	*MDA*[*]	*Hydroperoxide*[*]	*Conjugated dienes*[*]	*NO*[**]	*Glutathione*[***]	*Superoxide dismutase*[****]	*Catalase*[*****]	*GSH peroxidase*[******]	*GSH reductase*[******]
Control (1)	10.830 ± 0.432	253.60 ± 10.18	49.33 ± 2.53	2.835 ± 0.207	256.60 ± 10.96	43.14 ± 1.94	3.486 ± 0.117	48.10 ± 1.64	8.370 ± 0.487
SSPE (2)	13.421 ± 0.326[a]	276.84 ± 8.18[a]	62.10 ± 4.16[a]	3.926 ± 0.156[a]	210.62 ± 10.24[a]	27.53 ± 1.42[a]	2.109 ± 0.084[a]	43.18 ± 1.29[a]	7.213 ± 0.664[a]

[*] μm/ml RBC.
[**] μm/gm protein.
[***] μg/ml RBC.
[****] Units/mg protein.
[*****] $\times 10^{-2}$ units/mg protein.
[******] Units/g protein.
Mean of the values of 15 samples ± SD.
Group 2 has been compared with group 1.
[a] $P < 0.01$.

Table 5 Concentration of plasma glycoconjugates

Groups	*Total GAG*[*]	*HA*[*]	*HS*[*]	*H*[*]	*DS*[*]	*ChS*[*]	*Hexose*[**]	*Fucose*[**]	*Sialic acid*[**]	*Ganglioside*[***]	*Glycosyl diglycerine*[***]	*Cerebrosides*[***]	*Sulphatides*[***]
Control (1)	4.57 ± 0.408	0.525 ± 0.041	0.318 ± 0.022	0.284 ± 0.019	2.83 ± 0.232	0.587 ± 0.043	13.55 ± 1.26	1.65 ± 0.149	6.85 ± 0.617	26.5 ± 1.2	12.5 ± 0.72	16.25 ± 1.10	5.25 ± 0.61
SSPE (2)	10.23 ± 0.938[a]	0.855 ± 0.011[a]	0.557 ± 0.049[a]	0.519 ± 0.07[a]	8.66 ± 0.815[a]	1.337 ± 0.02[a]	22.32 ± 1.79[a]	1.94 ± 0.173[b]	11.29 ± 0.903[a]	36.5 ± 2.20[a]	21.5 ± 1.84[a]	18.5 ± 1.65[a]	7.125 ± 0.79[a]

[*] Values expressed as mg uronic acid/dl of plasma.
[**] Values expressed as mg/g protein.
[***] Values expressed as μg/dl plasma.
Group 2 has been compared with group 1.
[a] $P < 0.01$.
[b] $0.05 \leq P \leq 0.01$.
Mean of the values of 15 samples ± SD.

Table 6 Lysosomal enzymes

Groups	*β glucuronidase**	*β N-acetyl hexosaminidase**	*Hyaluronidase***	*Cathepsin-D****	*β galactosidase*****	*β fucosidase*****	*β glucosidase*****
Control (1)	59.52 ± 5.26	2273 ± 78.6	62.9 ± 4.1	90.9 ± 8.9	52.8 ± 3.75	23.63 ± 1.65	27.36 ± 2.46
SSPE (2)	117.46 ± 11.12[a]	3209 ± 79.50[a]	241 ± 6.89[a]	313.4 ± 9.8[a]	96.09 ± 7.98[a]	31.98 ± 1.08[a]	39.85 ± 2.39[a]

*μg p-nitrophenol/hr/g protein.
**μg *N*-acetyl glucosamine/hr/g protein.
***μg tyrosine/hr/g protein.
****Values are expressed as μg *p*-nitrophenol/hr/mg protein.
Mean of the values of 15 samples in each group ± SD.
Group 2 has been compared with group 1.
[a]$P < 0.01$.

Table 7 RBC membrane composition

Groups	*GAG**	*Hexose**	*Fucose**	*Cholesterol***	*Phospholipid***	*Cholesterol: phospholipid*
Control (1)	6.62 ± 0.71	145.09 ± 11.85	63.33 ± 4.60	704.33 ± 63.09	717.57 ± 67.36	0.982 ± 0.095
SSPE (2)	5.03 ± 0.48[a]	50.65 ± 4.38[a]	28.03 ± 2.37[a]	824.47 ± 33.06[b]	604.96 ± 73.30[a]	1.26 ± 0.061[a]

*μg/mg protein.
**nmol/mg protein.
Mean of the values from 15 samples in each group ± SD.
Group 2 has compared with group 1.
[a]$P < 0.01$.
[b]$0.05 \leq P \leq 0.01$.

Table 8 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)*	*Ubiquinone (μg/dl)*	*Na^+-K^+ ATPase (μg/p_i/mg protein)*	*Magnesium (mg/dl)*
LH Dom (1)	2.12 ± 0.12[a]	7.80 ± 0.06[a]	36.1 ± 2.36[a]	142.1 ± 8.65[a]	5.02 ± 0.220[a]	3.16 ± 0.24[a]
Bihem Dom (2)	1.14 ± 0.08	14.80 ± 1.01	63.8 ± 2.96	86.40 ± 5.91	3.01 ± 0.18	1.92 ± 0.13
RH Dom (3)	0.68 ± 0.07[a]	30.95 ± 2.19[a]	90.2 ± 3.63[a]	42.8 ± 2.12[a]	1.06 ± 0.120[a]	1.06 ± 0.11[a]

Mean of the values from 15 samples ± SD.
Groups 1 and 3 have been compared with group 2.
[a]$P < 0.01$
LH Dom—Left hemispheric dominant.
RH Dom—Right hemispheric dominant.
Bihem Dom—Bihemispheric dominant.

Table 9 Tyrosine and tryptophan catabolic patterns—hemispheric dominance

Group	*Tryptophan (mg/dl)*	*Tyrosine (mg/dl)*	*5HT (μg/dl)*	*Dop (ng/dl)*	*Norepi (ng/dl)*	*QA (ng/dl)*
LH Dom (1)	1.13 ± 0.09[a]	1.15 ± 0.08[a]	17.9 ± 1.8[a]	11.72 ± 0.62[a]	42.10 ± 2.30[a]	362.28 ± 51.63[a]
Bihem Dom (2)	2.02 ± 0.05	0.840 ± 0.06	43.9 ± 1.9	8.72 ± 0.42	30.56 ± 1.32	632.52 ± 49.42
RH Dom (3)	3.96 ± 0.08[a]	0.142 ± 0.06[a]	52.66 ± 2.2[a]	4.92 ± 0.42[a]	21.19 ± 1.32[a]	790.28 ± 41.32[a]

Mean of the values from 15 samples ± SD.
Groups 1 and 3 has been compared with group 2.
[a]$P < 0.01$.
5 HT–Serotonin, Dop–Dopamine, Norepi–Norepinephrine, QA–Quinolinic acid.

Table 10 Tryptophan- and tyrosine-derived alkaloids—hemispheric dominance

Groups	*Morphine (μg/dl)*	*Strychnine (μg/dl)*	*Nicotine (μg/dl)*
LH Dom (1)	7.56 ± 0.56[a]	ND	ND
Bihem Dom (2)	ND	ND	ND
RH Dom (3)	ND	0.92 ± 0.02[a]	6.28 ± 0.24[a]

Values are mean ± SD of 15 cases in each group.
ND–not detectable.
Mean of the values of samples ± SD.
Groups 1 and 3 have been compared with group 2.
[a]$P < 0.01$.

synthesis in SSPE. Phospholipid degradation is increased due to an increase in intracellular calcium activating phospholipase A_2 and D. The cholesterol:phospholipid ratio of the RBC membrane was increased in SSPE. 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. This trafficking of the glycoconjugates and lipids that are synthesised in the endoplasmic reticulum–golgi complex to the cell membrane depends upon GTPases and lipid kinases that are crucially dependent on magnesium and are defective in magnesium deficiency (Wiedemann and Cockcroft, 1998). The change in membrane structure produced by alteration in glycoconjugates and cholesterol:phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase, resulting in further membrane Na^+-K^+ ATPase inhibition. The same changes can affect the structure of lysosomal membrane. The results in defective lysosomal stability and leakage of glycohydrolases and GAG degrading enzymes into the serum.

The concentration of ubiquinone decreased significantly in SSPE, which may be the result of low tyrosine levels, reported in most of the disorders, 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 a important component of the mitochondrial electron transport chain, is a membrane antioxidant and contributes to free-radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore, causing a collapse of the hydrogen gradient across the inner membrane and uncoupling of the respiratory chain (Green and Reed, 1998). Intracellular magnesium deficiency can lead to a defect in the function of ATP synthase. All this leads to defects in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen, and generation of superoxide, which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free-radical scavenging. The increase in intracellular calcium may lead to increased generation of NO by inducing the enzyme nitric oxide synthase that combines with superoxide radical to form peroxynitrite. Increased intracellular calcium also can activate phospholipase A_2, resulting in increased generation of arachidonic acid that can undergo increased lipid peroxidation. Increased generation of free radicals such as the superoxide ion and hydroxyl radical can produce lipid peroxidation and cell membrane damage that can further inactivate Na^+-K^+ ATPase, triggering the cycle of free-radical generation once again. Magnesium deficiency can affect glutathione synthetase and glutathione reductase function. The mitochondrial superoxide dismutase leaks out and becomes dysfunctional with calcium-related opening of the mitochondrial PT pore and outer membrane rupture. The peroxisomal membrane is defective due to membrane Na^+-K^+ ATPase inhibition-related defect in membrane formation and leads to reduced catalase activity. Mitochondrial dysfunction-related free-radical generation has been implicated in the pathogenesis of immune-mediated diseases such as SSPE (Jacob, 1994; Olanow and Arendash, 1994). The increased intracellular calcium- and ceramide-related opening of the mitochondrial PT pore also leads to volume dysregulation of the mitochondria, causing hyperosmolality of the matrix and expansion of the matrix space. The outer membrane of the mitochondria ruptures and releases apoptosis inducing factor and cytochrome C into the cytoplasm. This results in activation of caspase-9, which can produce apoptosis of the cell (Green and Reed, 1998). Apoptosis has been implicated in the genesis of cell death in neuronal degeneration and probably also in SSPE.

The hemispheric dominance part of the study was done separately. The biochemical patterns observed in SSPE correlates with those obtained in right-hemispheric dominance. Right-hemispheric dominant individuals had elevated HMG CoA reductase activity and increased digoxin and dolichol levels. They also had reduced plasma magnesium levels, ubiquinone levels, and RBC membrane Na^+-K^+ ATPase activity. The tryptophan catabolites were increased and tyrosine catabolites reduced. The left-hemispheric dominant individuals had the opposite biochemical patterns. The bihemispheric dominant group had intermediate values between the right-hemispheric and left-hemispheric dominant group. The bihemispheric dominant group may have fluctuating dominance with cerebral dominance interchanging between right hemispheric and left hemispheric. This may be the reason why many of the values for SSPE controls of the bihemispheric dominant type are more similar to LH values in the second study. The hemispheric dominance part of the study was done separately and independent of the SSPE study. All the 15 SSPE patients were right-handed and left-hemispheric dominant by the dichotic listening test, but all of them had biochemical patterns similar to right hemispheric dominance. Therefore, right-hemispheric and left-hemispheric chemical dominance may not correlate

with handedness and the findings of the dichotic listening test.

The type of chemical hemispheric dominance is important in the regulation of immunity. Thus the immune mechanisms and the response to an invading bacteria/virus differ in the hypo- and hyperdigoxinemic state. The hypodigoxinemic state is associated with immunosuppression, but there is no viral persistence in the hypodigoxinemic state. The hyperdigoxinemic state is associated with immunoactivation and viral persistence as in the case of SSPE. Elevated levels of plasma digoxin levels and an upregulated isoprenoid pathway have been demonstrated in autoimmune diseases such as CNS-lupus by our group (Ravi Kumar et al, 1998). The hyperdigoxinemic state is an immune dysregulatory state with immune activation, viral persistence, and autoimmunity. Similarly, hyperdigoxinemia has been described by our group in degenerative disorders like Parkinson's disease. Digoxin administration has also been demonstrated to lead to neuronal degeneration in experimental rats. Therefore the increase in hypothalamic digoxin could be the cause and not the effect of apoptosis (Ravi Kumar et al, 2001). Geschwind and Behan (1982) postulated a relationship between cerebral lateralization and immune function. They observed a high frequency of left-handedness in patients with immune disorders. Bardos *et al* (1981) demonstrated that lesions of the left neocortex in mice depress T-cell immunity, whereas lesions of right neocortex enhance T-cell immunity. These earlier reports are in agreement with our studies. Hypothalamic digoxin and hemispheric dominance may regulate immune function.

Materials and methods

The cerebral dominance study and the SSPE study were done separately. The following groups were included in the study (1) 15 cases of SSPE—8 males and 7 females between the ages of 10–20 years (CSF measles antibody-positive/characteristic EEG); (2) 15 patients each with left-handed/right-hemispheric dominance, right-handed/left-hemispheric dominance, and ambidextrous/bihemispheric dominance, respectively, detected by the dichotic listening test—8 males and 7 females in each group between the ages of 10–20 years. The dichotic listening test was performed by using headphones to transmit 2 different auditory signals to the right and left ear at the same time. The individuals are asked to indicate which definitive sound he or she has perceived. If the sound in the left ear alone is perceived, the individual is right-hemispheric dominant. If the sound in the right ear alone is perceived, the individual is left-hemispheric dominant. If both the sound in the right and left ear are perceived simultaneously, the individual is bihemispheric dominant. The dichotic listening test was chosen for the purpose as it was done as a screening test in the general healthy population. The dichotic listening test has a sensitivity of nearly 75% when combined with data regarding handedness of the person (Ketz, 1985). All 15 patients with SSPE were right-handed and left-hemispheric dominant by the dichotic listening test; (3) Each patient with SSPE had an age- and sex-matched bihemispheric dominant healthy control. The bihemispheric dominant group may have fluctuating dominance with cerebral dominance interchanging between right hemispheric and left hemispheric.

Permission was obtained from the Ethics Committee of the institute as well as informed consent from the patients/relatives for the study. None of the subjects studied was under medication at the time of removal of blood. It is possible that medications such as anticonvulsants may have an effect on the various metabolic parameters studied including membrane Na^+-K^+ ATPase. This was the reason why freshly detected patients were selected for the study before treatment modalities were initiated. Fasting blood was removed in citrate tubes from each of the number of patients mentioned previously. RBCs were separated within 1 h of collection of blood for the estimation of membrane Na^+-K^+ ATPase. Serum/plasma was used for the analysis of various parameters. Each biochemical analysis was performed twice in a single patient. The mean of the values of 15 samples $\pm$ SD was calculated from the data.

The methodology used in the study were as follows: All biochemicals used in this study were obtained from Sigma Chemicals, USA. Activity of HMG CoA reductase of the serum was determined by the method of Rao and Ramakrishnan (1975) by determining the ratio of HMG CoA to mevalonate. For the determination of the RBC Na^+-K^+ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamath (1966) was used. Digoxin in the serum was determined by the procedure described by Arun *et al* (1998a). For estimation of ubiquinone and dolichol in the serum, the procedure described by Palmer *et al* (1984) was used. Magnesium in the serum 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 Wong *et al* (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 serum was estimated by HPLC (C_{18} column micro Bondapak 4.6 $\times$ 140 mm), solvent system 0.01 M acetate buffer (pH 3.0) and methanol (6:4), flow rate 1.0 ml/minute and detection UV 250 nm). Morphine, strychnine, and nicotine were estimated by the method described by Arun *et al* (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, and activity of glycohydrolases were described before (Manoj and Kurup, 1998).

Serum glycolipids 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 *et al* (1984). Catalase activity was estimated by the method of Maehly and Chance (1971), 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 *et al* (1963). Nitric oxide was estimated in the plasma by the method of Gabor and Allon (1994). Statistical analysis was done by ANOVA.

References

Arun P, Ravi Kumar A, Leelamma S, Kurup PA (1998a). Identification and estimation of endogenous digoxin in biological fluids and tissues by TLC and HPLC. *Indian J Biochem Biophys* **35:** 308–312.

Arun P, Ravi Kumar A, Leelamma S, Kurup PA (1998b). Endogenous alkaloids in the brain of rats loaded with tyrosine/tryptophan in the serum of patients of neurodegenerative and psychiatric disorders. *Ind J Med Res* **107:** 231–238.

Ashkenazi A, Dixit VM (1998). Death receptors signalling and modulation. *Science* **281:** 1305–1308.

Bardos P, Degenne D, Lebranchu Y, Biziere K, Renoux G (1981). Neocortical lateralization in NK activity in mice. *Scand J Immunol* **13:** 609–611.

Beutler E, Duran O, Kelley BM (1963). Modified procedure for the estimation reduced glutathione. *J Lab Clin Med* **61:** 882–886.

Bloxam DL, Warren WH (1974). Error in the determination of tryptophan by the method of Denkala and Dewey. A revised procedure. *Anal Biochem* **60:** 621–625.

Brien PJO (1969). Estimation of conjugated dienes and hydroperoxide. *Can J Biochem* **47:** 485–487.

Carpenter WT Jr, Buchanan RW (1994). Medical progress in schizophrenia. *N Engl J Med* **30**(10): 681–690.

Curzon G, Green AR (1970). Rapid method for the determination of 5-hydroxy tryptamine and 5-hydroxy indoleacetic acid in certain regions of rat brain. *Br J Pharmacol* **39:** 653–655.

Felton D, Cohen N, Ader R (1991). *Psychoneuroimmunology*. Academic Press: New York.

Finkel TH (1991). T-cell development and transmembrane signalling. Changing biological responses through a unchanging receptor. *Immunol Today* **12:** 79–86.

Gabor G, Allon N (1994). Spectrofluorometric method for NO determination. *Anal Bioch* **220:** 16–23.

Geschwind N, Behan P (1982). Left handedness: Association with immune diseases migraine, and developmental learning disorders. *Proc Natl Acad Sci USA* **79:** 5097–5100.

Goldstein JL, Brown MS (1990). Regulation of the mevalonate pathway. *Nature* **343:** 425–430.

Gorman JR, Locke S (1989). *Comprehensive textbook of psychiatry*. Williams and Wilkins: Baltimore.

Green DR, Reed JC (1998). Mitochondria and apoptosis. *Science* **281:** 1309–1316.

Greenamyre JT, Poter RHP (1994). Anatomy and physiology of glutamate in CNS. *Neurology* **44**(8)**:** S7–S13.

Haga H (1992). Effects of dietary magnesium supplementation on diurnal variation of BP and plasma Na^+-K^+ ATPase activity in essential hypertension. *Jpn Heart J* **33**(6)**:** 785–798.

Haupert GT (1989). Sodium pump regulation by endogenous inhibition. *Top Membr Transport* **34:** 345–348.

Hisaka A, Kasamatu S, Takenaga N (1990). Absorption of a novel prodrug of DOPA. *Drug-Metab Disposal* **18:** 621–625.

Horn HD, Burns FH (1978). *Methods of enzymatic analysis*. Academic Press: New York.

Jacob RA (1994). Nutrition, health and antioxidants. *INFORM* **5**(11)**:** 1271–1275.

Jaya P, Kurup PA (1986). Effect of magnesium deficiency on the metabolism of glycosaminoglycans in rats. *J Biosci* **10:** 487–497.

Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of SOD. *Indian J Biochem Biophys* **21:** 130.

Ketz J (1985). *Handbook of clinical audiology*. 3rd ed. Academic Press: New York.

Linstinsky JL, Siegal GP, Linstinsky MC (1998). Alpha-L-fucose: A potentially critical molecule in pathologic process including neoplasia. *Am J Clin Pathol* **110:** 425–440.

Lowenstein JM (1969). *Methods in enzymology, Vol 25*. Academic Press: New York.

Maehly AC, Chance B (1971). *Methods of biochemical analysis*. InterScience: New York.

Manoj AJ, Kurup PA (1998). Changes in the glycosaminoglycans and glycoproteins in the rat brain during protein calorie malnutrition. *J Clin Biochem Nutr* **25:** 149–157.

Monia BP, Ecke J, Crooks ST (1990). Ubiquitination enzymes. *Biotechnology* **8:** 209–215.

Olanow WC, Arendash GW (1994). Metals and free radicals in neurodegenerative disorders. *Curr Opin Neurol* **7:** 548–558.

Paglia DE, Valentine WN (1967). Studies on quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70:** 158–162.

Palmer DN, Maureen AA, Robert DJ (1984). Separation of some neutral lipids by normal phase high performance liquid chromatography on a cyanopropyl column: Ubiquinone, dolichol and cholesterol levels in sheep liver. *Anal Biochem* **140:** 315–319.

Ploegh HL (1998). Viral strategies for immune evasion. *Science* **280**(10): 248–253.

Price WJ (1985). *Spectrochemical analysis by atomic absorption*. Wiley: New York.

Rao AV, Ramakrishnan S (1975). Estimation of HMG CoA reductase activity. *Clin Chem* **21:** 1523–1528.

Ravi Kumar A, Jyothi A, Kurup PA (2001). ^{14}C-acetate incorporation into digoxin in rat rain and effect of digoxin administration. *Ind J Exp Biol* **3:** 420–426.

Ravi Kumar A, Augustine J, Kurup PA (1998). Digoxin—A model for hypothalamic regulation of neuronal transmission endocrine function, immunity and cytodifferentiation. *Neurol India* **46:** 261–267.

Saito K, Crowley JS, Markey SP, Heyes MP (1993). Kynurenine pathway and immune stimulation. *J Biol Chem* **268** (21)**:** 15496–15503.

Tamura H, Shimoyama S, Sunaga Y (1992). Digoxin-like immunoreactive substance in urine of patients with mucocutaneous lymph node syndrome. *Angiology* **10:** 856–865.

Wallace D, Hahn BH, Dubois R (1996). *Lupus erythematosus*. 5th ed, Williams and Wilkins: Baltimore.

Wallach DF, Kamath VB (1966). *Methods in enzymology*, Vol 8. Academic Press: New York.

Well-Malherbe R (1971). *Methods of biochemical analysis*. Wiley InterScience: New York.

Wiedemann C, Cockcroft S (1998). Vesicular transport. *Nature* **394:** 426–428.

Will ED (1969). Lipid peroxide formation in microsomes—General consideration. *Biochem J* **113:** 315–318.

Wong PWK, O'Flynn ME, Inouye ME (1964). Flourimetric method for tyrosine. *Clin Chem* **10:** 1098–1100.