Hypothalamic digoxin – central role in conscious perception, neuroimmunoendocrine integration and coordination of cellular function – relation to hemispheric dominance

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Summary   A family with a high prevalence of Parkinson's disease, schizophrenia, neoplasms, syndrome-X, rheumatoid arthritis and epilepsy has been described. The psychological behavioural patterns of the family were as follows – creativity and high IQ, hypersexual behaviour, reduced appetite and eating behaviour, insomnia and reduced sleep patterns, increased tendency for spirituality, increased tendency for addiction, less of bonding and affectionate behaviour and left handedness. Digoxin, an endogenous Na^+–K^+ ATPase inhibitor secreted by the hypothalamus, was found to be elevated and RBC membrane Na^+–K^+ ATPase activity was found to be reduced in all the disorders and in the indexed family studied. Hypothalamic digoxin can modulate conscious perception and its dysfunction may lead to schizophrenia. Digoxin can also preferentially upregulate tryptophan transport over tyrosine resulting in increased levels of depolarising tryptophan catabolites – serotonin, quinolinic acid, strychnine and nicotine and decreased levels of hyperpolarising tyrosine catabolites dopamine, noradrenaline and morphine contributing to membrane Na^+–K^+ ATPase inhibition in all the above disorders and the indexed family. Digoxin induced membrane Na^+–K^+ ATPase inhibition can result in increased intracellular Ca^{2+} and reduced Mg^{++} levels leading to glutamate excitotoxicity, oncogene activation and immune activation. Digoxin induced altered Ca^{++}/Mg^{++} ratios, reduced ubiquinone and increased dolichol can affect glycoconjugate metabolism, membrane formation and structure and mitochondrial function leading to the diverse disorders described above including those in the indexed family. The isoprenoid pathway and neurotransmitter patterns were compared in right-handed/left hemispheric dominant and left-handed/right hemispheric dominant individuals. The biochemical patterns in the indexed family and the diverse disorders studied correlated with those obtained in right hemispheric dominance. The hyperdigoxinemic state indicates right hemispheric dominance. Hypothalamic digoxin can thus function as the master conductor of the neuroimmunoendocrine orchestra and co-ordinate the functions of various cellular organelles.
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INTRODUCTION

Isoprenoid pathway produces various metabolites that are essential for diverse cellular functions – cholesterol involved in membrane structure, dolichol involved in protein glycosylation, ubiquinone which participates in mitochondrial electron transport and is a membrane

antioxidant and digoxin, an inhibitor of Na^+–K^+ ATPase produced by the hypothalamus. Deficiency of ubiquinone has been documented in Parkinson's disease, epilepsy, schizophrenia and multiple sclerosis (1–4). Increased levels of EDLA (endogenous digoxin like activity) have been reported in epilepsy, bipolar mood disorder, vasculitis and syndrome-X (5–8). Altered levels of dolichol have been observed in Alzheimer's disease (9).

The coexistence of four pathophysiological phenomena – oncogene activation and malignant transformation, neuronal degeneration, psychiatric manifestation and immune activation has been described in motor neuron disease, systemic malignancy, multiple sclerosis and schizophrenia (10,11). Autoantibodies have been demonstrated in multiple sclerosis, motor neuron disease, Alzheimer's disease, Down's syndrome, paraneoplastic syndromes and acquired immunodeficiency syndrome. Psychosis has been documented in multiple sclerosis, Alzheimer's disease, Parkinson's disease, neoplasms and AIDS dementia. Lymphoma has been shown to coexist with multiple sclerosis, motor neuron disease, vasculitis and the acquired immunodeficiency syndrome. Viral persistence as an etiological factor has been documented in multiple sclerosis, Parkinson's disease, neoplasms and schizophrenia. Insulin resistance has been reported in Alzheimer's disease. Immune mediated neuropathies and neoplasms are described in syndrome-X. This interrelationship between these disorders suggests the possibility that a central dysfunction may exist, which could play a part in the pathophysiology of these disorders. The isoprenoid pathway may be a candidate in this respect, in view of many reports on the changes in the concentration of many products of this pathway in many neurological and psychiatric disorders and therefore this pathway was assessed in these disorders. The disorders included in the study are multiple sclerosis, schizophrenia, Parkinson's disease, CNS glioma, syndrome-X with multiple lacunar state, Down's syndrome and acquired immunodeficiency syndrome. The isoprenoid pathway was assessed by plasma HMG CoA reductase activity, serum digoxin, ubiquinone and dolichol levels. Digoxin induced membrane Na^+–K^+ ATPase can produce magnesium depletion and therefore serum magnesium was assessed in all these disorders. Digoxin, apart from affecting cation transport, is also reported to influence the transport of various metabolites across cellular membranes, including aminoacids and various neurotransmitters (12). Two of the aminoacids in this respect are important: tryptophan, a precursor for strychnine and nicotine, and tyrosine, a precursor for morphine. Ongoing studies in our laboratory have shown the 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 (13). In view of these the levels of the following substances were assessed in the serum of the patients of the disorders studied – concentration of tryptophan and its metabolites serotonin, quinolic acid, strychnine and nicotine) and the concentration of tyrosine and its metabolites – (dopamine, norepinephrine and morphine). The Mg^{++} depletion produced by digoxin and membrane Na^+–K^+ ATPase inhibition can affect the metabolism of glycosaminoglycans, glycoproteins and glycolipids (14). The elevation in the level of dolichol may suggest its increased availability of N-glycosylation of proteins. The concentration of glycosaminoglycans, carbohydrate components of glycoprotein, glycolipids and glycohydrolases was studied in the serum of all these disorders. Alteration in ubiquinone levels and altered intracellular calcium/magnesium ratios can affect free radical metabolism. The free radical metabolism was studied in all these orders. The isoprenoid metabolites ubiquinone, dolichol and digoxin can affect membrane structure and function. Therefore RBC membrane composition was studied in all these disorders. A family with coexistence of schizophrenia, Parkinson's disease, neoplasms, rheumatoid arthritis, syndrome-X and increased incidence of left handedness with hyperdigoxinemia has been described previously (15). The isoprenoid pathway related biochemical cascade was also assessed in the family members. The isoprenoid pathway was also assessed in left- and right-handed individuals to find out whether cerebral dominance can affect the operation of the isoprenoid pathway. The results are presented in this paper and a hypothesis discussing the central role of hypothalamic digoxin in conscious perception, neuroimmunoendocrine integration and coordination of cellular functions is presented. The relationship between digoxin status and cerebral dominance is also discussed. The role of these factors in the pathogenesis of these disorders is also highlighted.

MATERIALS AND METHODS

Freshly diagnosed cases of CNS glioma, multiple sclerosis, primary generalised epilepsy, schizophrenia, Parkinson's disease, Down's syndrome, AIDS dementia with neuropsychiatric features, syndrome-X with multiple lacunar state were included in the study. Fifteen cases were included in each group and selected randomly from the patients attending the Department of Medicine, Medical College Hospital, Trivandrum. The freshly diagnosed cases were selected for the study before treatment protocols were initiated. Fifteen members of the indexed family were also included in the study. Each patient had an age and sex matched healthy control. Fifteen cases of healthy right-handed/left hemispheric

dominant and left-handed/right hemispheric dominant individuals between the ages of 20 and 30 years were also chosen for the study. The hemispheric dominance was verified by the dichotic listening test. These normal individuals were free from all the systemic diseases and were selected randomly from general population of Trivandrum city. None of the patients/normal individuals studied were smokers (active or passive). None of the subjects studied were under medication at the time of removal of blood. Fasting blood was removed in citrate tubes from each of the number of patients mentioned above. RBCs were separated with in 1 h of collection of blood for the estimation of membrane Na^+–K^+ ATPase. Plasma was used for the analysis of various parameters.

The methodology used in the study was as follows:

All biochemicals used in this study were obtained from M/s. Sigma Chemicals, USA. Activity of HMG CoA reductase of the plasma that was determined by the method of Rao and Ramakrishnan by determining the ratio of HMG CoA to mevalonate (16). For the determination of the RBC Na^+–K^+ ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used (17). Digoxin in the plasma was determined by the procedure described by Arun et al. (18) was used. For estimation of ubiquinone and dolichol in the plasma, procedure described by Palmer et al. (19) was used. Magnesium in the plasma was estimated by atomic absorption spectrophotometry (20). Tryptophan was estimated by the method of David and William and tyrosine by the method of Wong et al. (21,22). Serotonin was estimated by the method of Curzon et al. and catecholamines by the method of Well-Malherbe et al. (23,24). Quinolinic acid content of plasma was estimated by HPLC (C18 column micro Bondapak™ 4.6 c 140 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 mm). Morphine, strychnine and nicotine were esti-

mated by the method described by Arun et al. (13). 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 (β-glucuronidase, β-N-acetyl hexosaminadase, hyaluronidase and cathepsin D), activity of glycohydrolases (β-galactosidase, β-fucosidase and β-glucosidase) are described before (25). Serum glycolipids (gangliosides, glycosyl diglycerides, cerebrosides and sulphatides) were estimated as described in Methods in Enzymology (26). Cholesterol was estimated by using commercial kits supplied by Sigma Chemicals, USA. SOD was assayed by the method of Nishikini et al. as modified by Kakkar et al. (27). Catalase activity was estimated by the method of Maehly and Chance, glutathione peroxidase by the method of Paglia and Valentine as modified by Lawrence and Burk and glutathione reductase by the method of Horn and Burns (28–30). MDA was estimated by the method of Wills et al. and conjugated dienes and hydroperoxides by the procedure of Brien et al. (31,32). Reduced glutathione was estimated by the method of Beutler et al. (33). Nitric oxide was estimated in the plasma by the method of Gabor and Allon (34).

Statistical analysis was done by Student's 't' test with modified degree of freedom and the Bonferroni correction was applied. In all cases the degree of freedom is 28 only. The degree of freedom is calculated by using the formula $N_1 + N_2 - 2$ (here $15 + 15 - 2$).

RESULTS

1. The results showed that HMG CoA reductase activities of serum digoxin and dolichol were increased in all these disorders and in the family described indicating upregulation of the isoprenoid pathway but serum ubiquinone was reduced in all these disorders and in the indexed family (Table 1).

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)
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. Epilepsy	0.884 ± 0.075[a]	23.05 ± 1.76[a]	77.06 ± 4.6[a]	82.97 ± 6.64[a]	1.48 ± 0.139[a]	2.08 ± 0.11[a]
3. PD	0.810 ± 0.76[a]	20.90 ± 1.41[a]	120.9 ± 9.65[a]	91.40 ± 5.92[a]	1.51 ± 0.142[a]	2.13 ± 0.12[a]
4. Schizophrenia	0.75 ± 0.06[a]	15.13 ± 1.13[a]	67.5 ± 5.4[a]	89.33 ± 5.36[a]	1.24 ± 0.130[a]	1.81 ± 0.12[a]
5. CNS glioma	0.746 ± 0.06[a]	21.54 ± 1.47[a]	69.8 ± 4.19[a]	101.6 ± 8.13[a]	1.94 ± 0.18[a]	2.16 ± 0.22[a]
6. MS	0.705 ± 0.069[a]	29.15 ± 2.19[a]	54.2 ± 3.63[a]	82.85 ± 4.89[a]	0.313 ± 0.023[a]	2.11 ± 0.15[a]
7. HIV infection	0.78 ± 0.07[a]	22.02 ± 1.21[a]	47.9 ± 4.02[a]	94.31 ± 8.27[a]	1.11 ± 0.120[a]	1.56 ± 0.11[a]
8. Syndrome-X	0.89 ± 0.06[a]	29.95 ± 2.36[a]	44.2 ± 1.98[a]	116.8 ± 5.6[a]	1.05 ± 0.12[a]	1.53 ± 0.11[a]
9. Down's syndrome	0.87 ± 0.075[a]	19.05 ± 1.01[a]	43.8 ± 2.98[a]	90.40 ± 5.92[a]	0.349 ± 0.26[a]	1.79 ± 0.12[a]
10. Familial hyperdigoxinemia	0.82 ± 0.065[a]	20.05 ± 1.02[a]	62.8 ± 2.96[a]	87.40 ± 5.91[a]	0.367 ± 0.25[a]	1.69 ± 0.13[a]

Mean of the values from 15 samples ±SD. Groups 2–10 have been compared with group 1.
[a] p less than 0.01.

2. The results showed that the concentration of tryptophan, quinolinic acid and serotonin was found to be higher in the plasma of patients with all these disorders and in the family described while that of tyrosine, dopamine and norepinephrine was lower. Serum of patients with multiple sclerosis showed the presence of morphine. Serum of patients with epilepsy, PD, schizophrenia, multiple sclerosis syndrome-X with multiple lacunar state and the indexed family showed the presence of strychnine. Serum of patients with epilepsy, Parkinson's disease, syndrome-X, schizophrenia CNS glioma patients and indexed family contained detectable amounts of nicotine (Tables 2 and 3).

3. 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 a decrease in ubiquinone and reduced glutathione in most of the disorders and the indexed family studied. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in the above disorders and indexed family studied suggesting reduced free radical scavenging (Table 4).

4. The results show an increase in the concentration of serum total GAG, glycolipids (ganglioside, glycosyldiglyceride, cerebrosides and sulphatides) and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in all the disorders and in the indexed family studied. The increase in the carbohydrate components – total hexose, fucose and sialic acid – in the disorders studied was not to the same extent in all cases suggesting qualitative change in glycoprotein structure. For example in epilepsy, the percentage change in total hexose, fucose and sialic acid when compared to control is 54.3, 20, and 33%, respectively. The pattern of change in individual GAG in the serum was different, however, heparan sulphate (HS) and chondroitin sulphates (ChS) increased in most of the disorders and in the indexed family studied. The activity of GAG degrading enzymes (β-glucuronidase, β-*N*-acetyl hexosaminidase, hyaluronidase and cathepsin-D) and that of glycohydrolases (β-galactosidase, β-fucosidase and β-glucosidase) showed significant increase in the serum in most cases and in the indexed family (Tables 5 and 6).

5. The cholesterol:phospholipid ratio of the RBC membrane was increased in glioma, indexed family and schizophrenia, decreased in MS and PD and not significantly altered in epilepsy. 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 in these disorders and in the indexed family (Table 7).

6. The left-handed/right hemispheric dominant individuals compared to right-handed/left hemispheric

Table 2 Tyrosine and tryptophan catabolic patterns in neuropsychiatric disorders

Group	Tryptophan (mg/dl)	Tyrosine (mg/dl)	5 HT (µg/dl)	Dop (ng/dl)	Norepi (ng/dl)	QA (ng/dl)
1. Control	1.11 ± 0.08	1.14 ± 0.09	20.9 ± 1.9	12.89 ± 0.67	$45.15 + 2.35$	370.60 ± 21.07
2. CNS glioma	2.05 ± 0.07^a	1.01 ± 0.07^a	48.9 ± 3.9^a	8.43 ± 0.44^a	33.54 ± 1.78^a	655.73 ± 48.8^a
3. MS	1.99 ± 0.07^a	0.929 ± 0.06^a	92.1 ± 7.1^a	8.68 ± 0.52^a	31.52 ± 1.38^a	646.92 ± 52.93^a
4. Epilepsy	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	549.34 ± 41.21^a
5. PD	$1.66 + 0.06^a$	$0.901 + 0.06^a$	$77.8 + 5.6^a$	8.45 ± 0.44^a	34.85 ± 1.17^a	645.53 ± 52.64^a
6. Schizophrenia	2.10 ± 0.09^a	0.862 ± 0.05^a	46.6 ± 3.7^a	8.44 ± 0.45^a	37.52 ± 0.83^a	599.28 ± 52.64^a
7. Syndrome-X	1.80 ± 0.06^a	0.935 ± 0.06^a	52.8 ± 4.8^a	8.92 ± 0.51^a	32.26 ± 1.85^a	655.15 ± 44.93^a
8. HIV infection	2.02 ± 0.08^a	0.825 ± 0.03^a	41.8 ± 4.2^a	8.01 ± 0.62^a	36.41 ± 1.74^a	589.50 ± 50.64^a
9. Familial hyperdigoxinemia	2.83 ± 0.05^a	0.842 ± 0.06^a	44.9 ± 3.7^a	8.76 ± 0.42^a	30.51 ± 1.32^a	633.52 ± 49.42^a

Mean of the values from 15 samples $\pm$SD. Groups 2–9 have been compared with group 1. (a) *p* less than 0.01.

Table 3 Tryptophan and tyrosine derived alkaloids in serum of patients with neuropsychiatric disorders

Groups	Morphine (µg/dl)	Strychnione (µg/dl)	Nicotine (µg/dl)
1. Control	ND	ND	ND
2. CNS glioma	ND	ND	4.56 ± 0.2^a
3. MS	9.92 ± 1.21^a	1.02 ± 0.84^a	ND
4. Epilepsy	ND	11.44 ± 0.46^a	1.25 ± 0.04^a
5. PD	ND	9.54 ± 0.38^a	1.07 ± 0.03^a
6. Schizophrenia	ND	0.60 ± 0.02^a	5.28 ± 0.21^a
7. Syndrome-X	ND	2.92 ± 0.12^a	9.72 ± 0.84^a
8. Down's syndrome	ND	1.08 ± 0.12^a	1.24 ± 0.02^a
9. Familial hyperdigoxinemia	ND	2.02 ± 0.10^a	3.19 ± 0.02^a

Mean of the values from 15 samples $\pm$SD. Groups 2–9 have been compared with group 1. (a) *p* less than 0.01; ND – not detectable.

Table 4 Free radical metabolism in neuropsychiatric disorders

	1 Control	2 Epilepsy	3 Schizo	4 PD	5 MS	6 Glioma	7 Familial
MDA (μm/ml RBC)	10.830 ± 0.432	10.845 ± 0.463	11.633 ± 0.291[a]	12.151 ± 0.652[a]	10.98 ± 0.415	11.495 ± 0.331[a]	13.421 ± 0.326[a]
Hydroperoxide (μm/ml RBC)	253.60 ± 10.18	245.80 ± 10.39	269.88 ± 8.23[a]	273.03 ± 7.07[a]	261.95 ± 11.94[a]	266.33 ± 6.30[a]	276.84 ± 8.18[a]
Conjugated dienes (μm/ml RBC)	49.33 ± 2.53	51.37 ± 2.92	56.94 ± 4.81[a]	61.11 ± 4.16[a]	49.09 ± 3.28	53.84 ± 3.28[a]	62.10 ± 4.16[a]
Nitric oxide (μm/g protein)	2.835 ± 0.207	2.973 ± 0.201	3.320 ± 0.161[a]	3.455 ± 0.287[a]	2.99 ± 0.177	3.351 ± 0.159[a]	3.926 ± 0.156[a]
Glutathione (μg/ml RBC)	256.60 ± 10.96	259.53 ± 16.93	243.23 ± 23.99[a]	235.03 ± 10.28[a]	247.43 ± 14.75	242.36 ± 13.93[a]	230.62 ± 10.24[a]
Superoxide dismutase (units/mg protein)	43.14 ± 1.94	42.89 ± 1.61	40.43 ± 1.45[a]	37.63 ± 1.35[a]	42.50 ± 1.20	40.50 ± 1.53[a]	37.53 ± 1.42[a]
Catalase ($\times 10^{-2}$units/mg protein)	3.486 ± 0.117	3.416 ± 0.180	3.115 ± 0.086[a]	3.200 ± 0.149[a]	3.495 ± 0.153	3.305 ± 0.121[a]	2.109 ± 0.084[a]
GSH peroxide (units/g protein)	48.10 ± 1.64	48.26 ± 1.68	45.20 ± 1.32[a]	44.80 ± 1.54[a]	47.63 ± 1.61	45.54 ± 1.30[a]	43.18 ± 1.29[a]
GSH reductase (units/g protein)	8.370 ± 0.487	8.190 ± 0.413	7.653 ± 0.322[a]	7.318 ± 0.662[a]	8.153 ± 0.512	7.140 ± 0.319[a]	7.213 ± 0.664[a]

Groups 2–7 have been compared with group 1. (a) *p* less than 0.01.

Table 5 Concentration of plasma glycoconjugates in neuropsychiatric disorders

	1 Control	2 Family	3 Epilep	4 PD	5 Schizo	6 MS	7 Glioma
Total GAG mg uronic acid/dl	4.57 ± 0.408	16.31 ± 0.99[a]	9.99 ± 0.961[a]	10.23 ± 0.938[a]	11.9 ± 1.07[a]	8.38 ± 0.73[a]	10.63 ± 0.99[a]
HA (mg uronic acid/dl)	0.525 ± 0.041	0.849 ± 0.068[a]	1.45 ± 0.123[a]	0.155 ± 0.011[a]	0.852 ± 0.069[a]	1.76 ± 0.112[a]	1.69 ± 0.114[a]
HS (mg uronic acid/dl)	0.318 ± 0.022	3.18 ± 0.110[a]	1.95 ± 0.174[a]	0.557 ± 0.049[a]	0.813 ± 0.063[a]	2.105 ± 0.112[a]	2.032 ± 0.143[a]
H (mg uronic acid/dl)	0.284 ± 0.019	2.36 ± 0.049[a]	1.60 ± 0.014[a]	0.519 ± 0.07[a]	0.527 ± 0.042[a]	1.803 ± 0.038[a]	1.33 ± 0.052[a]
DS (mg uronic acid/dl)	2.83 ± 0.232	7.63 ± 0.812[a]	2.56 ± 0.215[a]	8.66 ± 0.815[a]	7.27 ± 0.629[a]	2.42 ± 0.229[a]	0.962 ± 0.201[a]
ChS (mg uronic acid/dl)	0.587 ± 0.043	2.32 ± 0.02[a]	2.37 ± 0.02[a]	0.337 ± 0.02[a]	2.46 ± 0.146[a]	0.293 ± 0.029[a]	4.61 ± 0.201[a]
Hexose (mg/g protein)	13.55 ± 1.26	18.18 ± 1.42[a]	20.91 ± 2.01[a]	22.32 ± 1.79[a]	17.20 ± 1.08[a]	27.5 ± 2.48[a]	17.21 ± 1.48[a]
Fucose (mg/g protein)	1.65 ± 0.16	2.48 ± 0.29[a]	1.98 ± 0.186[b]	1.94 ± 0.173[b]	2.23 ± 0.223[a]	2.51 ± 0.206[a]	2.10 ± 0.191[a]
Sialic acid (mg/g protein)	6.85 ± 0.617	8.72 ± 0.819[a]	9.11 ± 0.79[a]	11.29 ± 0.903[a]	8.73 ± 0.804[a]	10.27 ± 0.822[a]	8.4 ± 0.756[a]
Ganglioside (μg/dl)	26.5 ± 1.2	32.25 ± 1.81[a]	43.5 ± 2.81[a]	36.5 ± 2.20[a]	31.5 ± 2.01[a]	35.58 ± 2.1[a]	34.08 ± 1.9[a]
Glycosyl diglyceride (μg/dl)	12.5 ± 0.72	19.45 ± 1.62[a]	22.5 ± 1.60[a]	21.5 ± 1.84[a]	22.25 ± 1.78[a]	20.22 ± 1.66[a]	20.08 ± 1.84[a]
Cerebrosides (μg/dl)	16.25 ± 1.10	24.25 ± 2.01[a]	23.25 ± 2.01[a]	18.5 ± 1.65[a]	25.0 ± 2.02[a]	22.52 ± 1.4[a]	21.42 ± 0.965[a]
Sulphatides (μg/dl)	5.25 ± 0.61	8.32 ± 0.912[a]	7.25 ± 0.82[a]	7.125 ± 0.79[a]	7.36 ± 0.91[a]	6.94 ± 0.628[a]	6.99 ± 0.487[a]

Mean of the values from 15 patients in each group + SD. Groups 2–6 have been compared with group 1. (a) *p* less than 0.01; (b) *p* between 0.01 and 0.05.

dominant individuals had elevated HMG CoA reductase activity, with increased serum digoxin and dolichol levels. The serum ubiquinone, serum magnesium and RBC Na^+–K^+ ATPase activity were reduced in left-handed/right hemispheric dominant individuals. The left-handed/right hemispheric dominant individuals compared to right-handed/left hemispheric dominant individuals had elevated levels of serum tryptophan, quinolinic acid, serotonin, nicotine and strychnine in the serum. The levels of tyrosine, dopamine, noradrenaline and morphine were lower in the left-handed/right hemispheric dominant individuals. The right-handed/left hemispheric dominant individuals had the opposite biochemical patterns (Tables 8, 9a, and 9b).

7. The pattern of incidence of diseases and behavioural patterns in the indexed family is given in Tables 10a, 10b (Fig. 1). It showed a high prevalence of Parkinson's disease (8%), schizophrenia (23%), neoplasms (20%), syndrome-X (33%), rheumatoid arthritis (16%) and epilepsy (6.6%). The psychological behavioural patterns of the family were as follows – creativity and high IQ (60%), hypersexual behaviour (60%), reduced appetite and eating behaviour (60%), insomnia and reduced sleep patterns (60%), increased tendency for spirituality (80%), increased tendency for addiction (50%) and less of bonding and affectionate behaviour (75%). Thirty per cent of the family members were left-handed (Fig. 1, Tables 10a and 10b).

Table 6 Lysosomal enzymes in neuropsychiatric disorders

	1 Control	2 Family	3 Epilep	4 PD	5 Schizo	6 MS	7 Glioma
β-Glucuronidase (μg p-nitrophenol/h/g protein)	59.52 ± 5.26	112.29 ± 2.18[a]	114.29 ± 1.20[a]	117.46 ± 11.12[a]	102.90 ± 9.56[a]	112.29 ± 2.20[a]	100.90 ± 5.26[a]
β-N-Acetyl hexosaminidase (μg p-nitrophenol/h/g protein)	2273 ± 78.6	3592 ± 83.90[a]	3740 ± 85.90[a]	3209 ± 79.50[a]	3102 ± 74.8[a]	3722 ± 80.9[a]	3200 ± 74.5[a]
Hyaluronidase (μg N-acetyl glucosamine/h/g protein)	62.9 ± 4.1	221.0 ± 6.72[a]	190.9 ± 4.3[a]	241 ± 6.89[a]	278 ± 4.8[a]	220 ± 6.8[a]	196.2 ± 4.3[a]
Cathepsin-D (μg tyrosine/h/g protein)	90.9 ± 8.9	178.4 ± 7.24[a]	342.6 ± 8.58[a]	313.4 ± 9.8[a]	205.4 ± 7.1[a]	350.6 ± 8.5[a]	226.6 ± 7.5[a]
β-Galactosidase (μg p-nitrophenol/h/mg protein)	52.8 ± 3.75	93.25 ± 7.91[a]	72.44 ± 5.69[a]	96.09 ± 7.98[a]	64.08 ± 3.20[a]	95.39 ± 4.77[a]	79.23 ± 6.38[a]
β-Fucosidase (μg p-nitrophenol/h/mg protein)	23.63 ± 1.65	29.36 ± 1.67[a]	27.38 ± 1.70[a]	21.98 ± 1.08	33.38 ± 2.87[a]	33.31 ± 2.17[a]	31.68 ± 2.53[a]
β-Glucosidase (μg p-nitrophenol/h/mg protein)	27.36 ± 2.46	34.2 ± 2.71[a]	35.2 ± 2.78[a]	29.85 ± 2.39	29.87 ± 1.49	16.17 ± 1.44[a]	48.96 ± 4.21[a]

Mean of the values from 15 patients in each group ±SD. Groups 2–7 have been compared with group 1. (a) *p* less than 0.01.

Table 7 RBC membrane composition in neuropsychiatric disorders

	1 Control	2 Family	3 Glioma	4 MS	5 Epilep	6 Schizo	7 PD
GAG (μg/mg protein)	6.62 ± 0.71	3.46 ± 0.28[a]	3.53 ± 0.29[a]	5.46 ± 0.34[a]	6.89 ± 0.79	3.78 ± 0.24[a]	5.03 ± 0.48[a]
Hexose (μg/mg protein)	145.09 ± 11.85	61.56 ± 5.82[a]	80.40 ± 6.77[a]	81.19 ± 5.52[a]	66.57 ± 5.82[a]	118.84 ± 8.78[a]	50.65 ± 4.38[a]
Fucose (μg/mg protein)	63.3 ± 4.60	42.6 ± 2.8[a]	47.5 ± 2.5[a]	43.6 ± 2.9[a]	55.24 ± 3.92[b]	13.25 ± 1.28[a]	28.03 ± 2.37[a]
Cholesterol (nmol/mg protein)	704.33 ± 63.09	839.34 ± 49.63[a]	676.31 ± 42.27	684 ± 56.77	510.86 ± 41.18[a]	844.5 ± 53.62[a]	624.47 ± 33.06[b]
Phospholipid (nmol/mg protein)	717.57 ± 67.36	542.21 ± 46.92[a]	550.2 ± 48.97[a]	854 ± 79.42	554.50 ± 56.56[a]	622.92 ± 51.71[b]	904.96 ± 73.30[a]
Cholesterol: phospholipid	0.982 ± 0.095	1.228 ± 0.11[a]	1.229 ± 0.12[a]	0.801 ± 0.054[a]	0.921 ± 0.080	1.36 ± 0.086[b]	0.756 ± 0.061[a]

Mean of the values from 15 patients in each group ±SD. Groups 2–7 have compared with group 1. (a) *p* less than 0.01; (b) *p* between 0.01 and 0.05.

Table 8 Concentration of serum digoxin, dolichol, mangesium and ubiquinone and RBC membrane Na⁺K⁺ ATPase activity in right hemispheric dominance and left 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$_i$/protein mg)	Magnesium (mg/fl)
1. LHD	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. RHD	0.78 ± 0.07[a]	29.95 ± 2.19[a]	54.2 ± 3.63[a]	82.8 ± 4.86[a]	1.11 ± 0.120[a]	1.56 ± 0.11[a]

Mean of the values from 15 samples + SD. Group 2 has been compared with group 1. (a) *p* less than 0.01. RHD, Right hemispheric dominant; LHD, left hemispheric dominant.

Table 9a Tyrosine and tryptophan catabolic patterns in right hemispheric dominance and left hemispheric dominance

Group	Tyrptophan (mg/dl)	Tyrosine (mg/dl)	% HT (μg/dl)	Dop (ng/dl)	Norepi (ng/dl)	QA (ng/dl)
1. LHD	1.11 ± 0.08	1.14 ± 0.09	20.9 ± 1.9	12.89 ± 0.67	45.15 + 2.35	370.60 ± 21.07
2. RHD	2.05 ± 0.07[a]	0.883 ± 0.05[a]	48.9 ± 3.9[a]	8.43 ± 0.44[a]	31.54 ± 1.78[a]	655.73 ± 48.8[a]

Mean of the values from 15 samples ±SD. Group 2 has been compared with group 1. (a) *p* less than 0.01. RHD, Right hemispheric dominant; LHD, left hemispheric dominant.

Table 9b Tryptophan and tyrosine derived alkaloids in right hemispheric dominance and left hemispheric dominance

Groups	Morphine (µg/dl)	Strychnine (µg/dl)	Nicotine (µg/dl)
1. LHD	8.92 ± 1.21	ND	ND
2. RHD	ND	9.52 ± 0.38^a	2.07 ± 0.03^a

Mean of the values from 15 samples $\pm$ SD. Groups 2 has been compared with group 1. (a) *p* less than 0.01. ND, not detectable; RHD, Right hemispheric dominant; LHD, left hemispheric dominant.

Table 10a Description of indexed family

Diseases	Percentage
Syndrome-X	33
Systemic malignancy	20
Degenerative neuronal disorders	16
Rheumatoid arthritis	16
Schizophrenia	23
Primary generalised epilepsy	6.6
Idiopathic basal ganglia calcification (Fahr syndrome)	23
Parkinson's disease	8

Table 10b Psychological profile of family members and handedness

Behaviour	Percentage
Increased spiritual tendency	80
Decreased appetite and eating behaviour	60
High IQ and creativity	60
Decreased bonding and affectionate behaviour	75
Increased addictive behaviour	50
Insomnia and reduced sleep	60
Hypersexual behaviour	60
Left handedness/right hemispheric dominance	40

DISCUSSION

Digoxin and membrane Na⁺–K⁺ ATPase inhibition

The increase in endogenous digoxin, a potent inhibitor or membrane Na^+–K^+ ATPase, can decrease this enzyme activity. In all the disorders studied, there was significant inhibition of the RBC membrane Na^+–K^+ ATPase and this inhibition appears to be a common feature for neuropsychiatric disorders. The inhibition of Na^+–K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+–K^+ 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 Mg^{++} (35). 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–Mg^{++} 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. Low intracellular Mg^{++} and high intracellular Ca^{++} consequent to Na^+–K^+ ATPase inhibition appear to be crucial to the pathophysiology of these disorders. The intracellular positive Ca^{++} signal and negative Mg^{++} signal can regulate diverse cellular process. 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 processess. 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 compartmentalisation as cytosolic calcium signal and nuclear calcium signal. Serum Mg^{++} was assessed in all these disorders and was found to be reduced. Increased intracellular calcium can lead to basal ganglia calcification that was noticed in the family members.

Hypothalamic digoxin and regulation of neurotransmitter synthesis and function (Tables 2 and 3)

There is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the serum of patients with all these disorders. This could be due to the fact digoxin can regulate neutral aminoacid transport system with preferential promotion of tryptophan transport over tyrosine (12). The decrease in membrane Na^+–K^+ ATPase activity in all the disorders studied 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.

The schizoid neutransmitter pattern of reduced dopamine, noradrenaline and morphine and increased serotonin, strychnine and nicotine is common to all the disorders studied and could predispose to their development. Quinolinic acid, an NMDA agonist, can
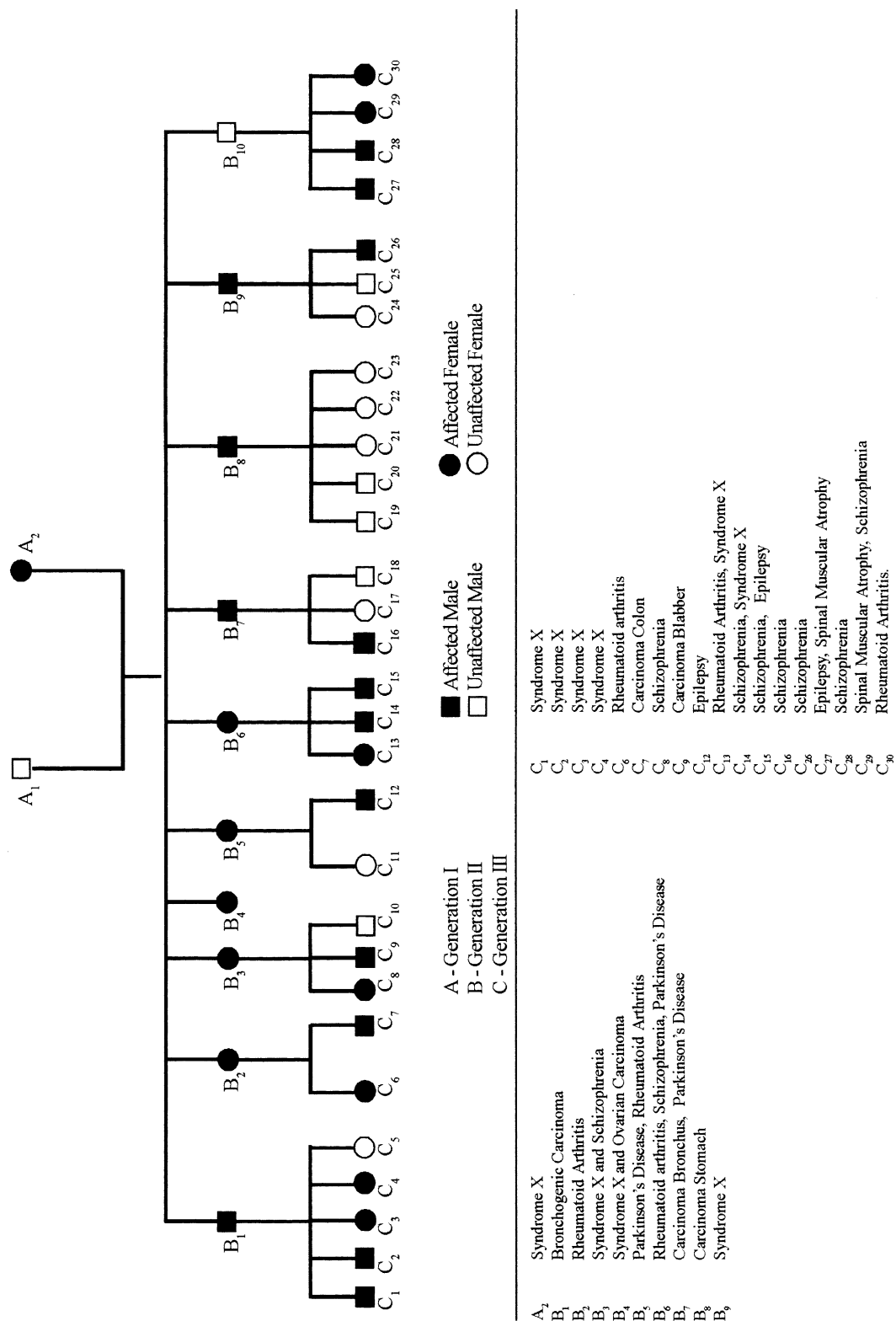
Fig. 1 Family tree.

Legend (right side of figure):

A – Generation I
B – Generation II
C – Generation III

Affected Male ■   Unaffected Male □
Affected Female ●   Unaffected Female ○

A_2 Syndrome X
B_1 Bronchogenic Carcinoma
B_2 Rheumatoid Arthritis
B_3 Syndrome X and Schizophrenia
B_4 Syndrome X and Ovarian Carcinoma
B_5 Parkinson's Disease, Rheumatoid Arthritis
B_6 Rheumatoid arthritis, Schizophrenia, Parkinson's Disease
B_7 Carcinoma Bronchus, Parkinson's Disease
B_8 Carcinoma Stomach
B_9 Syndrome X

C_1 Syndrome X
C_2 Syndrome X
C_3 Syndrome X
C_4 Syndrome X
C_6 Rheumatoid arthritis
C_7 Carcinoma Colon
C_8 Schizophrenia
C_9 Carcinoma Blabber
C_{12} Epilepsy
C_{13} Rheumatoid Arthritis, Syndrome X
C_{14} Schizophrenia, Syndrome X
C_{15} Schizophrenia, Epilepsy
C_{16} Schizophrenia
C_{26} Schizophrenia
C_{27} Epilepsy, Spinal Muscular Atrophy
C_{28} Schizophrenia
C_{29} Spinal Muscular Atrophy, Schizophrenia
C_{30} Rheumatoid Arthritis.

contribute to NMDA excitotoxicity reported in schizophrenia (36). Strychnine by blocking glycinergic transmission can contribute to the decreased inhibitory transmission in schizophrenia (37). Recent data suggest that the initial abnormality in schizophrenia involves a hypodopaminergic state and the low dopamine levels now observed agree with this (38). Nicotine by interacting with nicotine receptors can facilitate the release of dopamine, promoting the dopaminergic transmission in the brain. This can explain the increased dopaminergic transmission in the presence of decreased dopamine levels. The increased serotoninergic activity and reduced noradrenergic outflow from locus coeruleus reported earlier in schizophrenia agree with our finding of elevated serotonin and reduced noradrenaline levels (38).

In the presence of hypomagnesmia, the Mg^{++} block on the NMDA receptor is removed leading to NMDA excitotoxicty. 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. Increased intracellular Ca^{++} in the postsynaptic neuron can also activate the Ca^{++} dependent NMDA signal 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 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 strychinine insensitive site of the NMDA receptor and promote excitatory NMDA transmission. Quinolinic acid and serotonin are also positive modulators of the NMDA receptor. Increased glutamatergic transmission resulting in excitotoxicity has been implicated in neuronal degeneration observed in Parkinson's disease, primary generalised epilepsy, schizophrenia and AIDS dementia (39). Inhibition of Na^+–K^+ ATPase can also result in defective neuronal membrane repolarisation and a paroxysmal depolarisation shift resulting in epileptogenesis (40).

Hyperdigoxinemic state and hemispheric dominance

In left-handed/right hemispheric dominant individuals there was a derangement of the isoprenoid pathway. They had upregulated HMG CoA reductase activity with increased digoxin and dolichol levels and reduced ubiquinone levels. The RBC membrane Na^+–K^+ ATPase activity was reduced and serum magnesium depleted. The isoprenoid pathway metabolites – digoxin, dolichol and ubiquinone, membrane Na^+–K^+ ATPase and serum magnesium levels were normal in right-handed/left hemispheric dominant individuals. The left-handed/right hemispheric dominant individuals had increased levels of tryptophan, serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine, dopamine, noradrenaline and morphine were lower. Thus, an upregulated isoprenoid pathway, increased level of tryptophan and its catabolites and hyperdigoxinemia are suggestive of right hemispheric dominance. Also, in the right hemisphere dominant hyperdigoxinemic state there is upregulated serotoninergic, cholinergic and glutamatergic transmission and downregulated dopaminergic, glycinergic and noradrenergic transmission. Altered right hemispheric function have been described in several of these disorders. In infantile schizophrenia or autism right hemispheric dysfunction is noticed. In Lewy body variant of Parkinson's disease right hemispheric dysfunction is documented. In immune mediated disorders also increased left handedness and right hemispheric dominance has been described. Epileptic individuals have a heightened sense of creativity and dominant right hemispheric function. The disorders described – schizophrenia, neoplasms, degeneration, epilepsy, multiple sclerosis, acquired immunodeficiency syndrome and syndrome-X may have right hemispheric chemical dominance contributing to their pathogenesis. The hyperdigoxinemic state is seen in normal left-handed individuals and may indicate right hemispheric dominance. We had earlier reported a family with hyperdigoxinemia and coexistence of schizophrenia, epilepsy, Parkinson's disease, rheumatoid arthritis, syndrome-X, neoplasms and left handedness. The analysis of the members of the family also showed the following behavioural pattern. There was an increased tendency for spirituality in 75% of the family members. Temporal lobe epileptic phenomena has been described in spiritual individuals. There was an increase in creativity and intelligence and the family members had a very high IQ. Increased glutamatergic transmission is associated with memory and intelligence. They had a tendency towards reduced appetite and eating behaviour. Increased serotoninergic transmission can lead to reduced appetite. There was also hypersexual behaviour in majority of the family members. This could be related to increased production of nitric oxide in hyperdigoxinemic individuals. Nitric oxide has been related to erectile function. There was an increased tendency to addictive behaviour in family members. Endogenous morphine deficiency has been related to addiction. Morphine synthesis is low in members of the indexed family because of low tyrosine levels. There was a tendency to insomnia and

reduced sleep. This could be related to reduced levels of morphine. There was less of bonding and affectionate behaviour. Bonding and affectionate behaviour has been related to dopamine. Dopamine deficiency in hyperdigoxinemic individuals could contribute to less of bonding and affectionate behaviour.

Hypothalamic digoxin and conscious perception

The increase in serum digoxin levels in schizophrenia is significant. It has been postulated that there is an underlying generalised disorder of consciousness or self-awareness that impairs the ability of think with metarepresentations in schizophrenia. Digoxin a membrane Na^+–K^+ ATPase inhibitor may probably regulate conscious perception. The elements of conscious perception include perceptual binding, focussed attention and short-term memory (41). The evidence of increased hypothalamic digoxin points to role for the hypothalamus. The hypothalamus is connected to the thalamus by the mamillothalamic tract and digoxin may play a role in regulating these synapses. There are two way connections between the cerebral cortex and the thalamic nucleus. There are also two-way connections between the cerebral cortex and hypothalamus and digoxin may possibly regulate these synapses also. The hypothalamus–thalamus–cerebral cortex circuit would play a role in mediating conscious perception. Perceptual binding important in consciousness occurs when all the neurons associated with any one object's perceptual map in layer 5 of cerebral cortex fire in bursts and in a synchronised pattern but out of synchrony with those representing other objects. When an object is perceived there is a simultaneous activation of the cerebral cortex–hypothalamic two-way connections and liberation of digoxin from the hypothalamus to stimulate the widely dispersed cerebral cortical neurons receiving the incoming perception and their resultant/synchronised burst firing. Digoxin by the sodium–potassium ATPase inhibition it produces can lead to a paroxysmal depolarisation shift resulting in sustained synchronised burst firing of cerebral cortical neurons. Short-term memory important in conscious perception depends on the hypothalamic–thalamic–cerebral cortex reverberatory circuit as well as the phenomena of sustained synchronised burst firing of neurons in layer 5 of the cerebral cortex. Sustained synchronised burst firing produced by digoxin can temporarily strengthen the relevant synapses so that this particular pattern of firing is recalled quickly – a type of short-term memory. Transient synaptic changes of this type are due to alteration in the presynaptic neuronal calcium produced by digoxin. The thalamic–cerebral cortex reverberatory circuit mediating short-term memory is glutamatergic

and digoxin could amplify the circuit by its inhibitory effect on glial uptake of glutamate and increasing synaptic glutamate content.

All axons that pass either way between the cerebral cortex and thalamic nuclei must go through the thalamic reticular nucleus and all give off collateral excitatory glutamatergic branches that innervate the reticular nucleus. The reticular nucleus in turn provides an inhibitory GABAergic innervation back to the thalamic nucleus that provides the input. Reticular nucleus is involved in mediating selective attention by intensifying or detaching a particular active thalamic input into the cortex. The amplification (or focussing) and detachment of attention occur due to digoxin's effect in promoting glutamatergic transmission in the collaterals to the reticular nucleus by inhibiting the glial uptake of the glutamate and increasing its synaptic content. The back projections from the cerebral cortical perceptual map of external world to hypothalamus decide whether hypothalamic digoxin should act on the glutamatergic collaterals to reticular nucleus and thus focus or detach attention. In schizophrenia, hypersensitivity to perceptual stimulae is noticed as a deficit and patients find it difficult to screen out various stimuli and to focus on one piece of information. The defective stimulus barrier causes difficulty throughout every phase of development. The increased secretion of digoxin produces a hyperconscious state with increased focussed attention, perceptual binding and short-term memory. The altered glycoconjugates in schizophrenia lead to disordered synaptic connectivity in the hypothalamo-thalamo-cerebral cortical circuit leading to disordered conscious perception. Cortical cytoarchitectural disorganisation of the temporolimbic cortex has been reported in schizophrenia. Elevated levels of serum digoxin and schizoid neurochemical pathology are common to all the disorders studied and could predispose to their development. In the hyperdigoxinemic right hemisphere overdominant state conscious perceptive mechanisms are disrupted leading to a schizoid state.

Hypothalamic digoxin and regulation of golgi body/lysosomal function (Tables 5 and 6)

The elevation in the level of dolichol may suggest its increased availability of N-glycosylation of proteins. Magnesium deficiency can lead to defective metabolism of sphinganine producing its accumulation, which may lead to increased cerebroside and ganglioside synthesis. In Mg^{++} deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glucose 6 phosphate is channelled for the synthesis of glycosaminoglycans (GAGs). Intracellular Mg^{++} defi-

ciency also results in defective ubiquitin dependent proteolytic processing of glycoconjugates as it requires Mg^{++} for its function. 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 consequent to qualitative change in their structure. Proteoglycan complexes formed in the presence of altered Ca^{++}/Mg^{++} ratios intracellularly may be structurally abnormal and resistant to lysosomal enzymes and may accumulate.

Previous reports of alteration in glycoproteins in this connection include alteration in α-acid glycoprotein (AAG) and β-amyloid precursor protein in epilepsy and Alzheimer's disease and α-synnuclein in Parkinson's disease (42). Structurally abnormal glycoproteins resist catabolism by lysosomal enzymes and accumulate in neuronal degeneration. Interaction between HS-proteoglycan and ChS-proteoglycan with proteins like β-amyloid, τ-protein, parkin and α-synuclein and reduced proteolytic digestion of these complexes leading to their accumulation in the neurons has been reported in neurodegenerative diseases like Alzheimer's disease and Parkinson's disease. Alteration in sulphated proteoglycan matrix of the synaptic vesicles can alter neurotransmitter release into the synapse and produce a functional disorder like schizophrenia and epilepsy. Membrane Na^+–K^+ ATPase inhibition can lead to defective notch signalling. Notch is a transmembrane protein that acts as a signal receptor and is important in neurogenesis. Neuronal growth by extending neurites and forming connections is regulated by the notch signalling pathway. The notch signalling inhibits extension of neurites and keeps them stable in the mature brain. A notch ligand known as δ regulates neurogenesis by binding to notch in membranes of embryonal cells and prevents them from developing along the neuronal pathway. Notch activation by the ligand causes notch to be cleaved releasing the notch intracellular domain. This then passes into the nucleus and activates transcription as part of DNA binding complex with CSL protein. Intracellular cleavage of the notch is regulated by presenilin and also depends upon the lysosomal protease. In the presence of a lysosomal instability consequent to defective lysosomal membranes notch cleavage by protease is defective leading to functional disorders consequent to defective synaptic connectivity. Defective notch signalling pathway can lead to neuronal degeneration. Altered glycoproteins, glycolipids and GAG of neuronal membrane can also contribute to schizophrenia and epilepsy by producing disordered synaptic connectivity.

The protein processing defect can result in defective glycosylation of endogenous myelin glycoprotein antigens and exogenous viral glycoproteins antigens with consequent defective formation of MHC–antigen complex (43). The MHC linked peptide transporter, a P-glycoprotein which transports MHC–antigen complex to the antigen presenting cell surface, has an ATP binding site. The peptide transporter is dysfunctional in the presence of Mg^{++} deficiency. This results in defective transport of MHC class-1 glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD4 or CD8 cell. Defective presentation of endogenous myelin glycoprotein antigen can explain the immune dysregulation in MS. A CD8 MHC class-I restricted immune dysregulatory defect has been described in MS (44). Defective presentation of exogenous viral antigens can produce immune evasion by the virus as in AIDS dementia. Viral persistence has been implicated in the development of tumours (Ebstein–Barr virus and lymphoma), degenerations (Parkinson's disease and corona virus) and schizophrenia (Borna virus disease). A number of fucose and sialic acid containing natural ligands are involved in trafficking of leukocytes and similar breaches in blood–brain barrier and adhesion of the lymphocyte producing leukocyte trafficking and extravasation into the perivascular space have been described in MS (45). Altered myelin glycoprotein due to defective glycosylation and alteration in GAG of proteoglycans of myelin can affect the structural integrity of myelin leading to demyelination. Abnormally glycosylated tumour antigens can lead to defective tumour antigen presentation and loss of immunosurveillance by the natural killer cells. Altered cell surface glycoproteins, glycolipids and GAG can lead to defective contact inhibition and oncogenesis. A number of fucose and sialic acid containing natural ligands have been implicated in neoplastic transformation and metastasis. The MHC glycoproteins are involved in the formation of synaptic connectivity during neuronal development. Defective formation and presentation of MHC–neuronal glycoprotein complex can lead to disordered synaptic connectivity and functional disorders like schizophrenia and epilepsy.

Thus, in the hyperdigoxinemic right hemisphere dominant state there are reduced lysosomal stability, defective ubiquitin dependent proteolytic processing of proteins and alteration in glycoconjugate structure leading to their defective catabolism and accumulation. There is also a defect in the MHC antigen presenting pathway leading to immunodysregulation and viral persistence.

Hypothalamic digoxin and alteration in membrane structure and membrane formation (Table 7)

The alteration in the isoprenoid pathway specifically, cholesterol as well as changes in glycoproteins and GAG can affect cellular membranes. The upregulation of isoprenoid pathway can lead to increased cholesterol synthesis and Mg^{++} deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipase A_2 and D. The cholesterol:phospholipid ratio of the RBC membrane was increased in glioma, indexed family and schizophrenia, decreased in MS and PD and not significantly altered in epilepsy. 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 of as a vesicle, which fuses with the golgi complex. The glycoconjugates are then transported via the golgi channel and the golgi vesicle fuses with the cell membrane. This trafficking depends upon GTPases and lipid kinases, which are crucially dependent on magnesium and are defective in Mg^{++} deficiency (46). 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 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 these disorders.

Thus, in the hyperdigoxinemic right hemisphere dominant state there are defective membrane formation, membrane structure and function.

Hypothalamic digoxin and mitochondrial dysfunction (Table 4)

The concentration of ubiquinone decreased significantly in most of the cases 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 (12). 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 Ca^{++} can open the mitochondrial PT pore causing a collapse of the H^+ gradient across the inner membrane and uncoupling of the respiratory chain (47). Intracellular Mg^{++} 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 ion, 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, which combines with superoxide radical to form peroxynitrite. Increased calcium also can activate phospholipase A_2 resulting in the 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 Na^+–K^+ ATPase, triggering the cycle of free radical generation once again. Mg^{++} 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 owing 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 the neuronal degeneration oncogenesis and immune mediated disorders (48).

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 and caspase-3. Caspase-9 can produce apoptosis of the cell (47). Apoptosis has been implicated in neuronal degeneration. Apoptosis can produce defective synaptogenesis and synaptic connectivity contributing to functional disorders like schizophrenia and epilepsy. Apoptosis of the CD4 cell can contribute to CD4 depletion in the acquired immunodeficiency syndrome. Oligodendrocyte (the myelin forming cell) apoptosis is crucial to the pathogenesis of MS. Caspase-3 activation can cleave P_{21} that is involved in linking DNA duplication to cell division resulting in a polyploid cell and oncogenesis We have been able to demonstrate neuronal degeneration and apoptosis in digoxin injected rat brain.

Thus, in the hyperdigoxinemic right hemisphere dominant state there is a defect in the mitochondrial function and increased free radical generation and reduced scavenging. There is also increased apoptosis.

Hypothalamic digoxin and immunoregulation

Increased intracellular calcium activates the calcium dependent calcineurin signal transduction pathway, which can produce T-cell activation and secretion of interleukin 3, 4, 5, 6 and TNFα (tumour necrosis factor α) (49). TNFα binds to its receptor TNFR1 and activates the transcription factors HF-κB and AP-1 leading to the induction of proinflammatory and immunomodulatory genes. This can also explain the immune activation in MS. TNFα can also bring about apoptosis of the cell. It binds to its receptor and activates caspase-9, an ICE protease, which converts IL-Iβ precursor to IL-1β. IL-1β produces apoptosis of the neurons (in Alzheimer's disease and AIDS dementia), – the oligodendrocyte myelin forming cell in MS and the CD4 cell in HIV infection. IL-Iβ and TNFα induce HIV protein expression by transcription related mechanism and contributes to the pathogenesis of AIDS dementia. Membrane Na^+–K^+ ATPase inhibition can produce immune activation and is reported to increase CD4/CD8 ratios as exemplified by the action of lithium. The hyperdigoxinemic right hemisphere dominant state results in immune activation.

Hypothalamic digoxin and regulation of cell division, cell proliferation and neoplastic transformation

Intracellular magnesium depletion can 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.

Increased intracellular calcium activates phospholipase Cβ, which results in increased production of diacyglycerol (DAG) with resultant activation of protein kinase C (50). 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 α-subunit of G protein. This results in ras-oncogene activation, as more of the ras is 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 (50). Upregulation of isoprenoid pathway can result in increased production of farnesyl phosphate, which can farnesylate the ras oncogene producing its activation. Ubiquitin system of catabolic processing of proteins is important in DNA repair mechanism (50). 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 are oncogene activation and increased cell proliferation.

Hypothalamic digoxin and the metabolic regulation

Inhibition of Na^+–K^+ ATPase can also explain the pathogenesis of syndrome-X. Increased TNFα as mentioned above consequent to Na^+–K^+ ATPase inhibition related T-cell activation can contribute to insulin resistance in syndrome-X at the receptor level. The decrease in the intracellular magnesium can block the phosphorylation reactions involved in protein tyrosine kinase receptor activity leading to insulin resistance (51). Increase in β-cell calcium can contribute to increased insulin release from β-cells and hyperinsulinemia. Increased intracellular calcium can activate the G-protein coupled signal transduction of the contra insulin hormones (growth hormone and glucagon) leading to hyperglycemia. Decrease in intracellular magnesium can lead to inhibition of glycolysis. Decreased intracellular magnesium can lead to a mitochondrial ATP synthase defect. Increased intracellular calcium can open up the mitochondrial PT pore, disrupt the H^+ gradient across the inner membrane and block mitochondrial oxidative phosphorylation. All this leads to defective glucose utilisation and hyperglycemia. Increase in intracellular calcium can activate G-protein coupled angiotensin receptor producing hypertension and G-protein coupled thrombin receptor and platelet activating factor producing thrombosis observed in syndrome-X. Na^+–K^+ ATPase inhibition related increased smooth muscle calcium and decreased magnesium can contribute to vasospasm and ischaemia observed in stroke and CAD. Na^+–K^+ ATPase inhibition related altered glycoprotein can contribute to microangiopathy and macroangiopathy observed in syndrome-X. Metabolic syndrome-X could be visualised as due to hypothalamic digoxin hypersecretion. In the hyperdigoxinemic right hemisphere dominant state glucose metabolism and utilisation is impaired consequent to insulin resistance as also a tendency for vasospasm and thrombosis.

Hypothalamic digoxin and regulation of the immune response to viral infection

The same biochemical Na^+–K^+ ATPase related cascade described above could contribute to the acquired immunodeficiency syndrome. There is increased incidence of neoplasms like non-Hodgkin's lymphomas and vasculitis in the acquired immunodeficiency syndrome. Neuronal degenerations like AIDS dementia have been related to glutamate excitotoxicity. An AIDS related schizophreniform psychosis has been described. Polyclonal B-cell proliferation and lymphadenopathy have

been described in AIDS. Digoxin induced calcineurin signal transduction mediated T-cell activation and polyclonal B-cell proliferation can contribute to HIV-1 replication. This is because chief among the inducible cellular proteins that promote the growth of HIV-1 is transcription factor NF-κB. HIV-1 has incorporated two such NF-κB binding-enhancer elements into its own genome, which allows the triggering of HIV-1 transcription in the presence of nuclear NF-κB. Digoxin induced protein glycosylation defects can also lead to defective glycosylation of HIV glycoprotein antigens leading to defective formation of HIV glycoprotein antigen–MHC complex for presentation to CD4 cell. This results in immune evasion by the virus and could also contribute to the persistence of herpes virus and Epstein–Barr virus producing Kaposi's sarcoma and non-Hodgkin's lymphoma, respectively. Hypothalamic structural abnormalities have been described in homosexuals predisposed to the development of acquired immunodeficiency syndrome. In the hyperdigoxinemic right hemisphere dominant state there is a tendency for viral persistence consequent to defective processing of viral proteins and defective immune response to the virus.

Hypothalamic digoxin and neuroimmunoendocrine integration

Hypothalamic digoxin can thus integrate multiple brain functions. Digoxin can regulate neuronal transmission and conscious perception in the brain by its effect on neutral aminoacid and neurotransmitter transport. Digoxin can also play a role in endocrine integration. The hypothalamic hormone secretion is regulated by the biogenic amines noradrenaline, dopamine and serotonin. Digoxin by regulating the release and uptake of these neurotransmitters can control hypothalamic hormone secretion. Digoxin, by its lithium like action in modulating G-protein function and by facilitating calcium induced signal transduction by increasing sodium–calcium exchange, can regulate the function of these hormones. Digoxin can act as an immunomodulator owing to its effect on calcineurin signal transduction in the lymphocyte and subsequent immune activation.

Hypothalamic digoxin and integration of cellular function

Digoxin can regulate multiple cellular functions. Digoxin can regulate plasma membrane transport as well as membrane structure and fluidity. It can also regulate the fluidity of organelle membranes. Digoxin by its effects of calcium mediated opening of mitochondrial PT pore and ubiquinone synthesis can regulate mitochondrial func-

tion. The dolichol pathway can regulate protein glycosylation and golgi body function. Digoxin induced hypomagnesemia can regulate the ubiquitin dependent protein catabolic pathway. Digoxin induced change in intracellular magnesium can regulate nuclear function as DNA polymerase, DNA ligase and ribosomes require magnesium for their function. Digoxin by producing magnesium depletion can regulate GAG metabolism and the structure of the cell matrix. Digoxin can regulate cell death or apoptosis by opening up the mitochondrial PT pore consequent to a rise in intracellular calcium it produces. Digoxin by its effect upon ras oncogene can modulate cell proliferation. Digoxin induced hypomagnesemia by producing changes in cell surface glycoproteins and GAG can regulate contact inhibition and cell proliferation. 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 and digoxin induced hypomagnesemia can modulate its function. Intracellular magnesium deficiency can regulate phosphorylation of MAP (microtubule associated proteins) and regulate cytoskeletal structure/function.

Digoxin can thus produce conscious perception, neuroimmunoendocrine integration and integrate the function of multiple cellular organelles. The hyperdigoxinemic state is a right hemisphere dominant state.

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