Membrane function alterations in erythrocytes from mood disorder patients

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ABSTRACT

Objectives: To examine erythrocyte membrane functions in mood disorder patients and to establish possible diagnostic marker parameter(s).
Design: Collection of blood samples from mood disorder patients and age-matched control volunteers.Preparation of erythrocyte membranes for the proposed studies.
Setting: Out patients / in patients, psychiatry ward, Civil Hospital, Ahmedabad, Gujarat, India, Department of Biochemistry, Faculty of Science, M.S.University of Baroda, Vadodara, Gujarat, India.
Subjects: Unipolar and bipolar subjects. Control subjects (randomly selected volunteers).
Results: The most significant results were a duration dependent decrease in the TPL/CHL ratio (mole:mole),changes in both the substrate and temperature kinetics properties of AChE and elevated plasma BChE activity in the mood disorder patients.
Conclusion: The results suggest that the altered lipid profiles and the TPL/CHL (mole: mole) ratio and the altered temperature-dependent activity coefficients of erythrocyte membrane AChE and elevated plasma BChE activities could serve as useful diagnostic pointers for mood disorders.

Keywords:

Membrane function; Erythrocytes; Mood disorder

Abbreviations used:

ACh - Acetylcholinesterase; *ACTI* - Acetylthiocholineiodide; *BChE* - Butyrylcholinesterase; *BCTI* -Butyrylthiocholineiodide; *CHL* - Cholesterol; *DPH* - 1,6-Diphenyl –1,3,5-hexatriene; *DTNB* - 5,5' – Dithiobis(2-nitrobenzoic acid); *ETPZ.HCl* - Ethopropazine hydrochloride; Lyso, Lysophospholipid; *PA* - Phosphatidic acid; *PC -* Phosphatidylcholine; *PE* - Phosphatidylethanolamine; *PC* - Phosphatidylinositol; *PS* - Phosphatidylserine; *SPM* - Sphingomyelin; *SDS* - Sodium dodecyl sulfate; *TPL* - Total phospholipid.

INTRODUCTION

Mood fluctuation is a common and normal component of human behavior and involves both depression and anxiety. However, when these emotional states become uncontrollable, they lead to behavioral disorders known as mood disorders.[1] The diagnostic statistical manual IV (DSM – IV) has classified the mood disorder conditions based on the form and frequency of episodes, as well as the duration.[1,2] The mood disorders (also known as affective disorders) fall in two broad groups i.e. major depressive disorder and bipolar disorder (Type 1); the two conditions are also commonly known as unipolar and bipolar conditions. Erythrocyte membrane abnormalities such as decreased methylation activity resulting in decreased PC levels, decreased erythrocyte membrane Na^+, K^+-and Mg^{2+} AT-Pase activity, altered intra-erythrocytic cationic concentrations and changes in AChE activity in mood disorders have been reported.[3-12] Alterations in platelet membranes have been studied to relate these

with possible abnormalities in the brain.[13-16] However, the reports in the literature are conflicting, contradictory and equivocal.[4,5,8-12,16-18] No clear-cut pointer or a diagnostic biochemical parameter has been identified thus far.

The incidence of affective disorders is very high. It has been reported that in the United States about 15% of the population suffers from mood disorders.[1] This results in considerable loss of productivity. The World Health Organization (WHO) in its 1997 annual health report indicated that most persons with severe activity limitation suffered from mood disorders; the estimated number was 146 million.[19]

In the light of the above, it is highly desirable to search for a parameter which can be useful for the diagnosis, prognosis and for monitoring the recovery of mood disordered patients undergoing treatment. It is also desirable that the procedure should be minimally invasive. With these objectives in mind we decided to quantitate characteristics of the erythrocyte membranes from unipolar and bipolar patients in comparison with age and sex matched controls.

METHODS
Patient selection

Diagnoses were made by the committee comprising the head, psychiatry department, other doctors attached to the ward and RMOs

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in the panel, of the Civil Hospital, Ahmedabad, Gujarat, India. The patients were diagnosed through clinical interview (DSM IV criteria). Collateral information was obtained from their family members. All the subjects were married, none were single, divorced or widow(er) and they all belonged to the middle income group. None of them were suffering from any other diseases.

Sample collection

Blood samples were collected from mood disorder patients and age-matched control volunteers with informed consent in heparinized vials. The unipolar and bipolar subjects were either from the Out Patients Department of Psychiatry Ward, Civil Hospital, Ahmedabad, Gujarat, India, or those who were admitted in the ward for treatment. Control subjects were randomly selected volunteers from the Department of Biochemistry, Faculty of Science, M.S.University of Baroda, Vadodara, Gujarat, India.

Isolation of erythrocyte membranes

Erythrocyte membranes were isolated essentially according to the method of Hanahan and Ekholm.[20.21]

Briefly, the blood samples were centrifuged at 475 X g for 8 min to separate the cells and the plasma. The plasma was carefully decanted and was used as the source for the measurement of BChE activity. The buffy coat overlaying the RBC pellet was discarded and the pellet was washed twice with 0.9 % NaCl. The washed RBCs were then subjected to hypotonic lysis in 14 mM Tris HCl buffer pH 7.4 at 0-4°C. The lysate was centrifuged at 30,000 X g for 35 min and the supernatant was discarded. The pellet was washed repeatedly with the same buffer to obtain hemoglobin-free membranes. The final pellet was resuspended in the same buffer at a concentration of about 1mg protein/ ml.

Lipid analysis

Extraction of total lipids, separation of phospholipid classes by thin layer chromatography and estimations of cholesterol and total phospholipid phosphorous were according to the methods cited.[22-26]

Membrane fluidity

The fluidity of erythrocyte membranes was determined at 25 °C using DPH as the probe, in a RF 5000 Shimadzu spectrofluoriphotometer.[27]

Enzyme assays
ATPase

The Na^+, K^+ ATPase activity was measured in the medium (total volume: 0.4 ml) containing 50 mM Tris HCl buffer pH 7.4, 100 mM NaCl, 10 mM KCl and 4 mM $MgCl_2$. 50-100 ìg of erythrocyte membrane protein was used as the source of the enzyme. After pre-incubation at 37°C for 5 min the reaction was initiated by adding 1.25 mM ATP. The reaction was carried out for 90-120 min and terminated by adding 0.1 ml 5 % (w/v) of SDS.[22] Estimation of liberated inorganic phosphorus was according to the method of Fiske and Subba Row.[28]

AChE and BChE activity

The activities were measured essentially according to the procedure of Ellman et al. as described previously.[22,29]

Thus for the measurement of AChE activity, the assay system (total volume: 1 ml) contained 100 mM potassium phosphate buffer pH 8.0, 0.32 mM DTNB, 0.1 mM ETPZ.HCl and 10-50 mg of the erythrocyte membrane protein as the source of the enzyme. After

pre-incubation at 37 °C for 1-2 min the reaction was initiated by adding the substrate ACTI and the linear rate of increase in absorbance at 412 nm was recorded over a period of 60 to 90 seconds.

For determination of BChE activity the assay system was essentially the same as that for AChE except that the buffer was 50 mM Tris HCl pH 8.0 and ETPZ.HCl was omitted; BCTI was the substrate. 20 ml of 1:10 diluted plasma was used as the source of the enzyme.

For substrate kinetics analysis the concentration of substrates (ACTI/ BCTI in the two assay systems respectively) was varied from 25 ìM to 10 mM.

For temperature kinetics studies the enzyme activity was determined over a temperature range of 5 to 53 °C with an increment of 4 °C at each step. Measurements were carried out at substrate (ACTI/ BCTI) concentration of 5 mM.

The data for substrate kinetics were analyzed by the Lineweaver-Burk, Eadie-Hofstee and Eisenthal and Cornish-Bowden methods for determination of Km and Vmax.[30] The values of Km and Vmax obtained by the three methods were in close agreement and were averaged. The results are given as mean ± SEM of the averaged values. The ranges for individual mean value are indicated in the parentheses. The data on temperature kinetics were analyzed for determination of energies of activation in the high and low temperature ranges (E_1 and E_2 respectively) and phase transition temperature (Tt) .[31]

All the kinetics data were computer analyzed employing Sigma plot version 5.0.[21,22,30]

Protein estimation was according to the method of Lowry et al. with bovine serum albumin used as the standard.[32]

Chemicals

ACTI, BCTI, ETPZ.HCl and DPH were purchased from Sigma Chemical Co. U.S.A. Sodium salt of vanadium free ATP and DTNB were purchased from SRL, India. Silica Gel G was from E. Merck, Germany. All other chemicals were of analytical re-agent grade and were purchased locally.

Statistics

Statistical evaluation of the data was by Student's t-test. Comparisons among the groups were by analysis of variance (ANOVA).

RESULTS

The details regarding the age, sex, duration of mood disorder and treatment for the 9 unipolar patients and 12 bipolar I patients are given in Table I. Of the patients listed in the unipolar group # 5 was certified by the psychiatrists as "cured", since this patient had been asymptomatic for the last one and half year. The patient suffered from headache and the family members advised him to visit the psychiatry ward (where he had been admitted two years ago) rather than going to a general practitioner. [We met this patient by chance and he agreed to participate in the study.]

The patients were either admitted in the hospital or were visiting the OPD for further treatment. Thus most of these patients (except patients # 1 and # 2 in bipolar group and #1 in unipolar group) were on drug therapy. The unipolar patients received treatment with tricyclic anti-depressants e.g. amitriptyline or benzodiazepam e.g. lorazepam. The patients in bipolar group received lithium treatment. Patient # 11 received megitol and largacril.

Mean age of the patients in the unipolar group was 34.8± 3.5 years whereas that of patients in the bipolar group was 33.1±3.4 years. The mean age of the control subjects was 32.0± 3.0 years.

Table I. Case histories of unipolar/ bipolar patients.					
Group	Patient number	Age (Y)	Sex	Duration	
				First Symptom observed/diagnosed	Drug taken
Unipolar	1	35	F	20 Days	—
	2	32	F	1 Months	4 Days
	3	27	M	5 Years	5 Years
	4	32	F	17Months	17 Months
	5	57	M	2 Years	2 Years
	6	21	F	6 Years	6 Years
	7	34	F	3 Years	3 Years
	8	28	M	1 Year	1 Year
	9	52	M	6 Years	6 Years
	10	30	F	3 ° Years	3 ° Years
Bipolar	1	22	F	—	—
	2	25	M	2 Months	—
	3	34	F	4 Months	4 Months
	4	40	M	6 Months	6 Months
	5	55	F	8 Months	8 Months
	6	52	F	1 Year	1 Year
	7	39	M	3 Years	3 Years
	8	20	F	3 Years	3 Years
	9	36	M	4 Years	4 Years
	10	25	M	5 Years	5 Years
	11	21	F	6 Years	6 Years
	12	28	M	13Years	13 Years

The mean duration from first symptoms to study entry was 2.8±0.7 years and 3.3±1.1 years for unipolar and bipolar I groups respectively with the mean duration of treatment 3.1±0.7 and 3.6±1.2 years respectively for the two groups.

The unipolar group included 4 males and 6 females while the bipolar group consisted of 6 males and 6 females. Control groups comprised of 11 males and 5 females.

In view of the large variations in the duration of the mood disorder and of drug treatment (e.g. see Table I) the final results of analysis are given for a group as a whole and the values are represented as mean ± SEM.

The data in Table II show TPL and CHL content of erythrocyte membranes and the molar ratios of TPL/CHL. From the data in Table II it can be noted that the TPL content increased significantly in the unipolar patients. A similar trend was seen even in the bipolar group. However, the increase was of a lesser magnitude and the increase was not statistically significant. The CHL content was significantly high (2.3 and 2 folds higher respectively) in the unipolar and bipolar patients. Once again the magnitude of the increase was much higher in the unipolar group. Consequently TPL/CHL (mole: mole) ratio was significantly low, more so in the unipolar group. {It may be mentioned here that the data for patient # 5 in unipolar group who was certified as " cured" are not included in Table II and also that in the patient #5 TPL/CHL ratio was normalized to 1.0} Thus disproportionate increase in membrane TPL and CHL, and significantly decreased TPL/CHL ratio seem to be the characteristic feature of unipolar patients. For the bipolar patients increase only in CHL content and decreased TPL/CHL (mole: mole) ratio may be a distinguishing feature. Analysis of variance revealed that the differences in the groups with respect to the three parameters, TPL, CHL and their molar ratios were highly significant.

It has been reported that the erythrocytes have a characteristic methylase, which is responsible for conversion of PE to PC.[33,34] It has been further reported that the methylase activity decreased in mood disorders thereby resulting in decreased PC content. In the

Table II. Erythrocyte membrane TPL, CHL and TPL/CHL (mole: mole) ratio in mood disorder patients.

	TPL	CHL	TPL/CHL
	(mg /mg protein)		(mole: mole)
Control (12)	516.17±28.50 (376.55-723.71)	238.72±12.83 (191.1-303.11)	1.082±0.039 (0.927-1.314)
Unipolar (8)	832.00±58.99*** (563.51-1062.01)	545.96±52.91*** (396.00-766.56)	0.775±0.039*** (0.688-0.794)
Bipolar (10)	691.44±149.60 (301.34-988.98)	480.03±112.53* (167.12-460.53)	0.866±0.043** (0.645-0.992)
Significance by ANOVA	p< 0.01	p< 0.01	p< 0.01

The experimental details are as given in the text. The results are given as mean ±SEM of the number of observations indicated in the parentheses.
* p < 0.05, ** p < 0.002 and *** p < 0.001 compared to control.

light of this observation it was of interest to find out if the membrane phospholipid composition had altered in these affective disorders. These data are shown in Table III. It is clear that there was no change composition-wise in any of the phospholipid class except for PA, which increased in both unipolar and bipolar patients. Comparison of groups by ANOVA showed that the difference was highly significant. It is possible that the increased PA contents in the two mood disorder groups may relate to altered phospholipid turnover.

Since CHL is one of the determinants of the membrane fluidity/rigidity[35] it was of interest to find out if the membrane fluidity is altered in mood disorders. The data in Table IV show that indeed the membranes were somewhat more rigid in the two affective disorders although the values in the unipolar group did not reach a significance level by Student's t-test. However, the group analysis showed that the difference amongst the groups was significant at p< 0.05.

We next examined the erythrocyte membrane Na+, K+ ATPase and AChE, and plasma BChE activities. The results are given in Table V. As can be noted, the Na+, K+ ATPase activity was unchanged. Even analysis by ANOVA showed that the difference among the groups was not significant. Therefore Na+, K±-ATPase may not be a good marker. Our results are thus consistent with the observation of earlier researchers.[4,17] The AChE activity was high in both unipolar as well as bipolar groups. However, the increase was statistically

Table III. Phospholipid composition of erythrocyte membranes in mood disorder patients.

	Phospholipid composition (% of total)		
	Control (10)	Unipolar (9)	Bipolar (11)
Lyso	2.87 ±0.78	3.45 ±0.57	3.25 ±0.81
SPM	23.44 ±1.06	22.15 ±1.55	23.88 ±1.19
PC	30.99 ±1.19	30.10 ±1.01	29.85 ±1.01
PI	5.05 ±0.77	5.13 ±0.39	3.76 ±0.76
PS	6.93 ±0.84'	5.07 ±0.69	4.93 ±0.92
PE	25.28 ±1.11	25.15 ±2.14	24.55 ±1.07
PA	5.32 ±0.83	8.22 ±1.28	9.50 ±1.3* [a]

The experimental details are as given in the text. The results are given as mean ±SEM of the number of observations indicated in the parentheses.
* p < 0.02 compared to control.
[a] Significance by ANOVA p< 0.01

Table IV. Fluidity parameters of erythrocyte membranes in mood disorders.

	Fluidity parameter			
	Fluorescence polarization, p	Fluorescence anisotropy, r	Limited hindered anisotropy, ra	Order parameter, s
Control (16)	0.350 ± 0.005 (0.304-0.382)	0.264 ± 0.004 (0.226-0.296)	0.253±0.006 (0.201-0.295)	0.799±0.009 (0.713-0.864)
Unipolar (9)	0.377±0.022 (0.296-0.478)	0.288±0.02 (0.219-0.379)	0.285±0.026 (0.192-0.405)	0.842±0.039 (0.697-1.013)
Bipolar (12)	0.375±0.006* (0.332-0.399)	0.286±0.007** (0.249-0.307)	0.282±0.007* (0.232-0.309)	0.844±0.010*** (0.766-0.885)
Significance by ANOVA	p< 0.05	p< 0.05	p< 0.05	p< 0.05

The experimental details are as given in the text. The results are given as mean ±SEM of the number of observations indicated in the parentheses.
* p < 0.01, ** p < 0.002 and *** p < 0.001 compared to control.

Table V. Erythrocyte membrane Na^+, K^+ ATPase and AChE and plasma BChE activities in mood disorder patients.

	Na^+, K^+ ATPase (mmole Pi/hr/mg protein)	AChE (mmole ACTI hydrolyzed/ min /mg protein)	BChE (mmole BCTI hydrolyzed /min /ml plasma)
Control	211.00±28.34 (122.29-376.80)	0.662±0.082 (0.223-0.996)	4.98±0.22 (3.80-6.67)
Unipolar	247.0±50.24 (80.09-425.08)	1.08±0.062** (0.840-1.343)	6.28±0.40* (4.87-8.28)
Bipolar	287.3±50.00 (108.86-494.9)	0.90±0.100 (0.588-1.273)	6.24±0.60 (4.21-7.11)
Significance by ANOVA	N.S	p< 0.01	p< 0.01

The experimental details are as given in the text. The results are given as mean ±SEM of 8-12 independent observations.
* p < 0.01 and ** p< 0.002 compared to control.

been reported that its kinetic properties change after binding to membrane.[36]

In view of the altered TPL and CHL contents in the erythrocyte membranes (Table II) it was of interest to find out whether these alterations influenced the temperature kinetics of AChE. The typical plots depicting the temperature-dependent changes in AChE activity are shown in Fig. 2. The corresponding Arrhenius plots are also included. It is self evident that the patterns for the two mood disorder groups differ considerably from that of the control. Thus one notes that in the case of the control group AChE activity shows a sharp rise after around 40°C as compared to a progressive steady increase in the two mood disorder groups; the changes are also reflected in the corresponding Arrhenius plots. Thus in the control groups the Arrhenius plot is biphasic and the values of E_1 and E_2 were 72.6 and 37.4 KJ/mole and the phase transition temperature was around 40°C. This agrees well with our previously reported values.[21] By contrast, in the two affective disorders the Arrhenius plots were monophasic and the phase transition was abolished. The energies of activation were 37.4 and 45.6 KJ/mole respectively in the two groups (Table VII). The values of energies of activation were comparable to E_2 in the control groups, and this value in the bipolar group was significantly high. Comparison of groups by ANOVA reveled that the differences were significant. Obviously the changes correlate with and reflect the membrane lipid alterations. However, in case of the bipolar patients # 3 and # 4 who were suffering from this disorder for 4-6 months the Arrhenius plots were biphasic (data not shown). This would suggest that the membrane
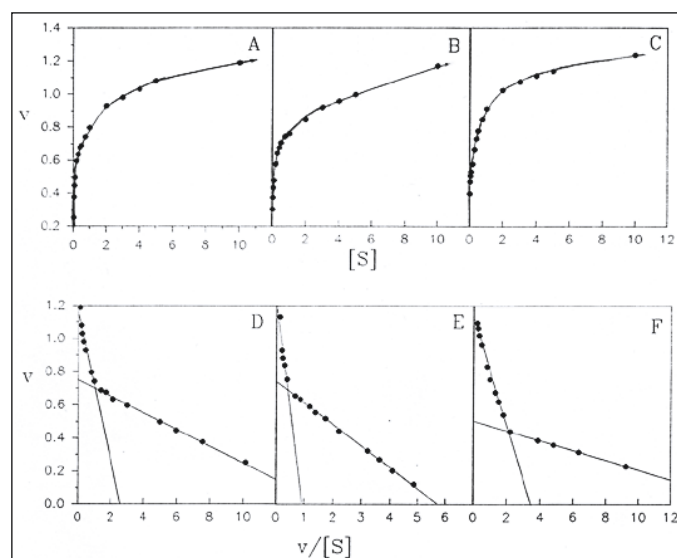
significant only for the unipolar group. The differences in the groups were highly significant when evaluated by ANOVA.

Likewise the plasma BChE activity also increased significantly in the unipolar group. A similar increase was noted even for the bipolar group, although the difference was not statistically significant compared to the controls. Comparison of groups by ANOVA showed that the difference was highly significant.

We have previously reported that the human erythrocyte membrane AChE has two kinetically distinguishable components while plasma BChE has three components.[21] It was therefore of interest to find out if the observed changes i.e. increase in the erythrocyte membrane AChE and the plasma BChE in both the mood disorders could be traced to the individual components. To elucidate this possibility we examined the substrate saturation kinetics.

The typical substrate saturation curves for the AChE are shown in Fig. 1. The corresponding Eadie-Hofstee plots are also included. The values for the Km for the two components (Km_1 and Km_2) and the corresponding Vmax (V max_1 and V max_2) values are given in Table VI. As can be noted, in the unipolar patients the value of Km_2 increased almost by 2.5 folds whereas in the bipolar group the V max_1 had decreased by 40 %. As evaluated by ANOVA, difference among the groups were highly significant. The observed changes may relate to altered membrane compositions (e.g. see Tables II and III). AChE is known to be a membrane-bound enzyme and it has

Fig.1. Typical substrate saturation curves for human erythrocyte membrane AChE in (a) control, (b) unipolar and (c) bipolar patients. The respective Eadie-Hofstee plots are shown in (d), (e) and (f). Experimental details are as given in the text. For determination of substrate kinetics of AChE, ACTI was used as a substrate over a concentration range of 0.025 to 10mM. The abscissa represents the reaction velocity v, while the ordinate represents [S] and v/[S] ratio for substrate saturation curves and the Eadie-Hofstee plots respectively. Reaction velocity v = mmol of ACTI hydrolyzed min^{-1} mg protein^{-1}. V/[S]= reaction velocity divided by the corresponding substrate concentration.

Table VI. Substrate kinetics properties of erythrocyte membrane AChE in mood disorders.

	Component I		Component II	
	Km1	Vmax 1	Km 2	Vmax 2
Control(10)	0.073±0.008 (0.041-0.126)	0.693±0.069 (0.406-1.015)	0.490±0.074 (0.206-0.826)	1.275±0.125 (0.772-1.847)
Unipolar (8)	0.089±0.017 (0.038-0.163)	0.685±0.052 (0.490-0.914)	1.212±0.198** (0.450-1.550)	1.150±0.043 (0.940-1.270)
Bipolar (10)	0.073±0.009 (0.026-0.125)	0.412±0.057* (0.141-0.699)	0.415±0.078 (0.319-0.516)	1.029±0.129 (0.471-1.690)
Significance by ANOVA	N.S	p< 0.01	p< 0.01	N.S

The experimental details are as given in the text. The results are given as mean ±SEM of the number of observations indicated in the parentheses. Substrate kinetics measurements of RBC membrane AChE were carried out using ACTI as the substrate over the concentration range of 0.025 to 10 mM.
Units: Km= mM, Vmax = m mol of ACTI hydrolyzed min-1 mg protein-1.
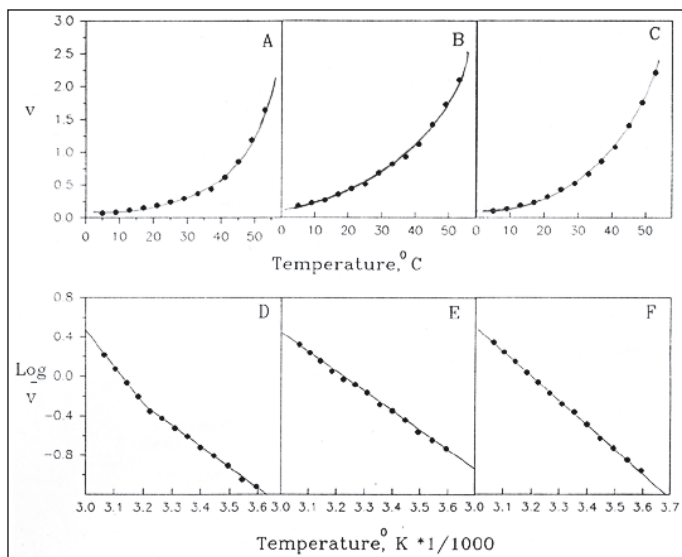* p < 0.02, ** p < 0.01 compared to the corresponding controls.

Fig. 2. Typical temperature curves for AChE from human erythrocyte in (a) control, (b) unipolar and (c) bipolar patients. The respective Arrhennius plots are shown in (d), (e) and (f). Experimental details are as given in the text. The AChE activity in RBC membranes was determined with 5 mM ACTI. The abscissa represents the log of reaction velocity v, while the ordinate represents reciprocal of absolute temperature T*1000. Reaction velocity v = m mol of ACTI hydrolyzed min⁻¹ mg protein⁻¹. Absolute temperature T= °Kelvin.

Table VII. Arrhennius kinetics analysis of erythrocyte membrane AChE in mood disorders.

	(Energy of activation, KJ/mole) E1	E2	Phase transition temperature, Tt (°C)
Control (12)	72.55 ±5.43 (59.63-101.30)	37.36 ±3.36 (20.55-44.39)	39.9 ±1.09 (36.25-43.59)
Unipolar (8)	—	37.40 ±3.44 (22.18-53.97)	—
Bipolar (10)	—	45.64 ±3.83* (25.99-69.70)	—
Significance by ANOVA		p< 0.05	

The results are given as mean ± SEM of the number of observations indicated in the parentheses.
Enzyme activities were determined with 5 mM ACTI concentration.
* p < 0.05 compared with control.

alterations occur at later stages and are sustained despite drug treatment for long duration.

Since the pattern for the temperature-dependent-changes in AChE activity differed significantly in the two mood disorders (Fig.2), we decided to compare the activities of the AChE at 25°C, 37°C and 53°C the latter being the highest temperature we have employed in our studies. These data are shown in Table VIII. We then decided to evaluate the data by taking the activity coefficient ratios of two temperature groups i.e. 53°C/25°C and 53°C/37°C. These values are also included in Table VIII. As can be noted, the values of the activity coefficients were 6.88 and 3.92 respectively in the control group. Interestingly both the values decreased significantly in the unipolar group whereas activity coefficient corresponding to the latter value only had decreased in the bipolar patients. The observed differences were also found to be highly significant also by ANOVA. Our results therefore suggest that determination of AChE activity at three given temperatures and the activity coefficient ratios could serve as good diagnostic pointers.

The typical substrate saturation curves and the corresponding Eadie-Hofstee plots for plasma BChE are shown in Fig. 3. As is evident, consistent with our earlier observation three kinetically different and distinguishable components of BChE are noted in the plasma of control group. A similar pattern is seen also for the two mood disorder groups. The Km values for the three components were comparable in all the groups. The Vmax values tended to be higher in the mood disorder groups. However, these latter differences were not statistically significant. ANOVA revealed that the groups differed only with respect to V max$_2$ (Table IX).

We then determined the temperature dependence of BChE activity. The Typical plots are shown in Fig. 4. The corresponding Arrhenius plots are also included. It is evident that in the mood disorder patients, at any given temperature, the BChE activity was higher than that in the control group. The Arrhenius plots were monophasic straight lines and the energies of activation were 22.58±0.87, 23.21±1.36 and 25.22±0.73 KJ/mole respectively for control and the two mood disorder groups. A slight variation in the energies of activation as observed in the two mood disorder groups may be attributed to the possible compositional changes (discussed below).

DISCUSSION

The present studies were undertaken to examine erythrocyte membrane functions in mood disorders in comparison with those from normal volunteers. The objective of these studies was to look for a suitable biochemical diagnostic marker. Indeed our studies have brought forth the subtle differences in erythrocyte membrane function in the two mood disorder groups. Thus, the TPL content increased only in the unipolar group while CHL content increased in both the groups. Consequently the TPL/CHL molar ratio was always low in the two mood disorder groups compared with the controls. Most interestingly, in patient # 5 in unipolar group (who was certified as cured) the TPL/CHL molar ratio returned to normal value.

Earlier, it has been reported that in mood disorder the PC content decreased.[3] However, we could not detect any change in the PC component. This may possibly be attributed to the fact that the drug treatment for 2 weeks is re-

Table VIII. Erythrocyte membrane AChE activities at 25°C, 37°C and 53°C in mood disorder patients.

	Activity (m mol of ACTI hydrolyzed min^{-1} mg protein^{-1}) 25°C	37 °C	53°C	Activity coefficient ratio (53°C/25°C)	[53°C/37°C]
Control (11)	0.267±0.042	0.572±0.092	1.849±0.301	6.877±0.452	3.915±0.178
Unipolar (8)	0.525±0.045****	0.823±0.067	1.908±0.176*	3.785±0.363****	2.365±0.172****
Bipolar (12)	0.430±0.047**	0.869±0.115	2.621±0.419	6.463±0.685	2.909±0.197***
Significance by ANOVA	p< 0.01	p< 0.01	p< 0.01	p< 0.01	p< 0.01

The results are given as mean ±SEM of the number of observations indicated in the parentheses.
Enzyme activities were determined with 5 mM ACTI concentration.
* p < 0.05, ** p<0.01, *** p<0.002and **** p<0.001 compared with control.

ported to normalize the PC content in the membranes.[37] However, we did observe increased PA content which is suggestive of altered phospholipid turnover in mood disorders. We also found that the RBC membranes in the mood disorder groups especially the bipolar group had become more rigid.

Consistent with the reports of earlier workers[4,17] the erythrocyte membrane Na$^+$, K$^+$, ATPase activity was unchanged but the AChE activity tended to be higher.[9,10] Of particular interest was the decreased Vmax of
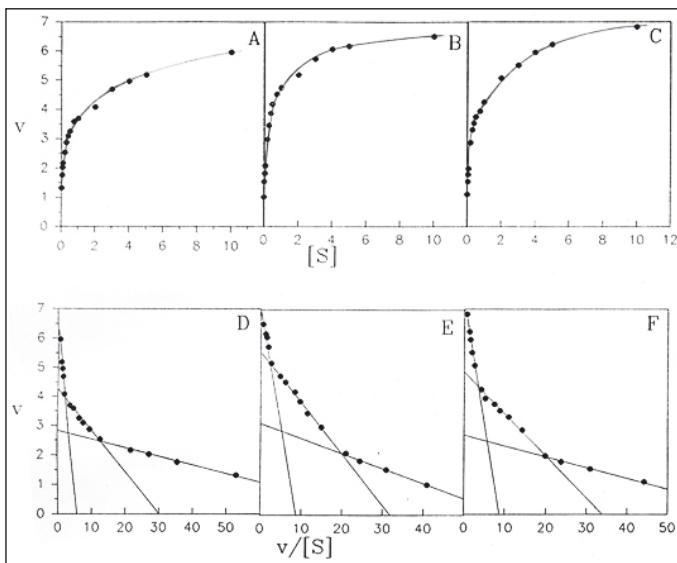

Fig. 3. Typical substrate saturation curves for human plasma BChE in (a) control, (b) unipolar and (c) bipolar patients. The respective Eadie-Hofstee plots are also shown in (d), (e) and (f). Experimental details are as given in the text. For determination of substrate kinetics of BChE, BCTI was used as a substrate over a concentration range of 0.025 to 10mM. The abscissa represents the reaction velocity v, while the ordinate represents [S] and v/[S] ratio for substrate saturation curves and the Eadie-Hofstee plots respectively. Reaction velocity v = mmol of BCTI hydrolyzed min^{-1} ml plasma^{-1}. V/[S]= reaction velocity divided by the corresponding substrate concentration.
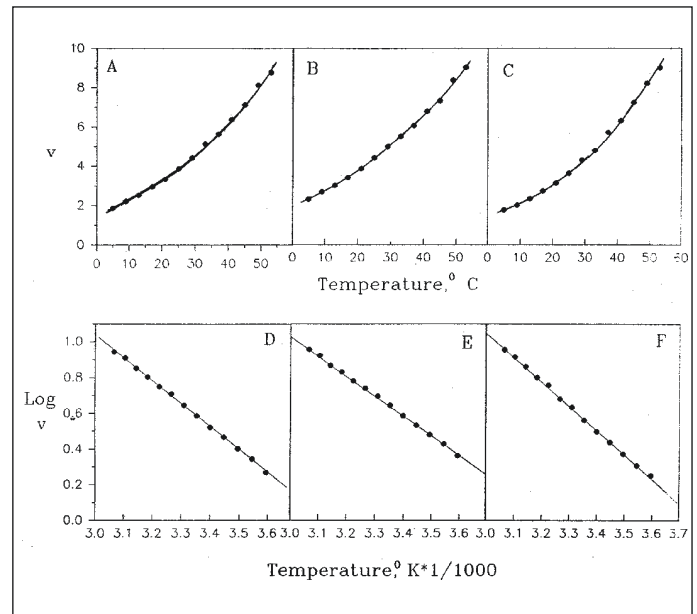

Fig. 4. Typical temperature curves for human plasma BChE in (a) control, (b) unipolar and (c) bipolar patients. The respective Arrhenius plots are shown in (d), (e) and (f). Experimental details are as given in the text. The BChE activity in plasma was determined with 5 mM BCTI. The abscissa represents the log of reaction velocity v, while the ordinate represents reciprocal of absolute temperature T*1000. Reaction velocity v = m mol of BCTI hydrolyzed min^{-1} ml plasma^{-1}. Absolute temperature T= °Kelvin.

Table IX. Substrate kinetics properties of plasma BChE in mood disorders

	Component I		Component II		Component III	
	Km 1	V max 1	Km 2	V max 2	Km 3	V max 3
Control (13)	0.037±0.005 (0.017-0.079)	2.33±0.186 (1.43-3.56)	0.188±0.012 (0.123-0.249)	3.78±0.202 (2.28-4.98)	1.104±0.110 (0.608-1.910)	6.32±0.377 (4.49-9.46)
Unipolar (8)	0.037±0.007 (0.014-0.064)	2.26±0.405 (1.02-4.23)	0.198±0.018 (0.111-0.276)	4.65±0.393 (3.00-6.22)	0.920±0.094 (0.634-1.170)	7.23±0.488 (5.46-9.25)
Bipolar (10)	0.044±0.004 (0.027-0.062)	2.67±0.442 (1.12-5.52)	0.203±0.052 (0.090-0.651)	5.69±0.761 (2.81-11.71)	0.844±0.101 (0.333-1.440)	7.19±0.546 (5.35-13.80)
Significance by ANOVA	N.S	N.S	N.S	p< 0.01	N.S	N.S

The experimental details are as given in the text. The results are given as mean ±SEM of the number of observations indicated in the parentheses. The substrate used for kinetics determination of the plasma BChE was BCTI over the concentration range of 0.025 to 10 mM.
Units: Km= mM, Vmax = m mol of BCTI hydrolyzed min-1 ml plasma-1.

component I of AChE in the bipolar group and increased Km of component II in the unipolar group which are unique features not reported so far. Also consistent with the changes in the membrane lipids the temperature kinetics was significantly altered in the two affective disorders. Thus the Arrhenius plots for AChE became monophasic straight lines in both the mood disordered groups. Once again the bipolar group exhibited a difference in that the energy of activation was higher than that in the controls (Table VII). In the unipolar group the energy of activation was comparable to E$_2$ com-

ponent of controls but the distinguishing feature was a monophasic plot (Table VII, Fig 2). Of particular importance was the temperature dependent activity coefficient ratios i.e. the ratios of AChE activity at 53°C/25°C and 53°C/37°C decreased significantly in the unipolar group; in the bipolar group this decrease was seen only in 53°C/37°C ratio. Therefore our result suggest that the analysis of AChE activity at these different specified temperatures and the ratios of activities can distinguish between unipolar and bipolar groups.

Interestingly we also found that the plasma BChE activity was significantly elevated in both the mood disorders and the elevated activity could be traced to Vmax of component II; possibly Vmax of component III also contributed (Table IX). However, the Km values of the three components were not changed (Table IX). The results therefore suggest that possibly compositional changes occurred in plasma BChE. Thus there were no qualitative changes in kinetic properties i.e. Km values did not change but the proportion of component II and possibly component III seems to have altered. BChE is synthesized in the liver and is then secreted in the plasma.[38] Hence it may be suggested that mood disorders might effect even the liver metabolism. We have earlier reported that even in epilepsy the plasma BChE profile is altered.[39]

Although we carried out the present studies with erythrocyte membranes, in principle lipid analyses can be carried out even on washed RBCs especially if one is interested in finding out the lipid-related parameters. Similarly the enzyme assays can be performed using RBCs for which methods are available.[39] The analyses thus carried out, could, it is hoped, be of value in diagnosis, prognosis and for monitoring the progress of mood disorder patients receiving treatments.

One limitation of the study was that the sample size was small. However, there was a genuine difficulty in collecting samples from the hospitals and patients. For clinical and diagnostic purposes a large sample size is desirable. Future work in this direction with large sample size may substantiate these findings. Nevertheless, it was felt that the data was significant and could be useful for diagnostic purposes.

CONCLUSION

The findings have demonstrated that specific membrane alterations in terms of TPL and CHL content and the molar ratios thereof occur in mood disorders and the latter parameter is restored to normality in those patients who are "cured" (e.g. patient # 5 in unipolar group). Besides, changes in the activity and kinetic parameters of AChE and BChE are also noted and the observed changes such as temperature-dependent activity coefficient for AChE and increased BChE activities could serve as a useful pointer for diagnosis.

References

1. Kandle ER. Disorders of mood: Depression, Mania and Anxiety disorders. In Principles of Neuroscience. ER Kandle, JH Schwartz and TM Jessell.(eds) McGraw-Hill,2000; 1209-1226.
2. Gabbard GO. Psychodyanamic psychiatry in clinical practice: The DSM-IV edition. American Psychtric Press, Washington.,1994. 317-391.
3. Hitzemann R, Mark C, Hirschowitz J, Garver D. Characteristics of phospholipid methylation in human erythrocyte ghosts: relationship(s) to the psychoses and affective disorders. Biol Psychiatry 1985; 20:397-407.
4. Thakar JH, Lapierre YD, Waters BG. Erythrocyte membrane sodium-potassium and magnesium ATPase in primary affective disorder. Biol Psychiatry 1985; 20:734-740.
5. Durosinmi MA, Ojo JO, Oluwole AF, Ononye AF, Akanle OA, Spyrou NM. Elemental concentrations in nigerians with affective disorders using proton-induced X-ray emission. Biol Trace Elem Res 1994;43-45:357-361.
6. Rybakowski JK, Lehmann W. Decreased activity of erythrocyte membrane ATPases in depression and schizophrenia. Neuropsychobiology 1994;30:11-14.
7. Joffe RT, Blank DW, Berrettini WH, Post RM. Erythrocyte sodium and potassium in affective illness. Acta Psychiatr Scand 1986;73:416-419.
8. Frazer A, Ramsey TA, Swann A, Bowden C, Brunswick D, Garver D, Secunda S. Plasma and erythrocyte electrolytes in affective disorders. J Affect Disord 1983;5:103-113.
9. Jope RS, Walter-Ryan WG, Alarcon RD, Lally KM. Cholinergic processes in blood samples from patients with major psychiatric disorders. Biol Psychiatry 1985;20:1258-1266.
10. Thakar JH, Lapierre YD, Waters BG. Cholinesterases in primary affective disorders. Clin Biochem 1985;18:308-310.
11. Looney SW, el-Mallakh RS. Meta-analysis of erythrocyte Na,K-ATPase activity in bipolar illness. Depress Anxiety 1997;5:53-65.
12. El-Mallakh RS. The Na,K-ATPase hypothesis for manic-depression. I. General considerations. Med Hypotheses 1983;12:253-268.
13. Soares JC, Mallinger AG, Dippold CS, Frank E, Kupfer DJ. Platelet membrane phospholipids in euthymic bipolar disorder patients: are they affected by lithium treatment? Biol Psychiatry 1999;45:453-457.
14. Soares JC, Mallinger AG. Intracellular phosphatidylinositol pathway abnormalities in bipolar disorder patients. Psychopharmacol Bull 1997;33:685-691.
15. Soares JC, Krishnan KR, Keshavan MS. Nuclear magnetic resonance spectroscopy: new insights into the pathophysiology of mood disorders. Depression 1996;4:14-30.
16. Soares JC, Dippold CS, Wells KF, Frank E, Kupfer DJ, Mallinger AG. Increased platelet membrane phosphatidylinositol-4,5-bisphosphate in drug-free depressed bipolar patients. Neurosci Lett 2001;299:150-152.
17. Fieve RR, Go R, Dunner DL, Elston R. Search for biological/genetic markers in a long-term epidemiological and morbid risk study of affective disorders. J Psychiatr Res 1984;18:425-445.
18. Reddy PL, Khanna S, Subhash MN, Channabasavanna SM, Rao BS. Erythrocyte membrane sodium-potassium adenosine triphosphatase activity in affective disorders. J Neural Transm Gen Sect 1992;89: 209 -218.
19. World Health Report 1997 -executive summary Conquering suffering, enriching humanity.(http://www.who.int/whr/1997/exsum97e.htm)
20. Hanahan DJ, Ekholm JF. The expression of optimum ATPase activities in human erythrocytes, A comparison of different lytic procedures. Arch. Biochem. Biophys 1978;187,170-179.
21. Dave KR, Syal AR, Katyare SS. Tissue cholinesterases. A comparative study of their kinetic properties. Z Naturforsch 2000;C 55:100-8.
22. Patel HG, Aras RV, Dave KR, Katyare SS. Kinetic attributes of Na^+, K^+ ATPase and lipid/phospholipid profiles of rat and human erythrocyte membrane. Z Naturforsch 2000;C 55: 770-777.
23. Folch J, Lees M, Sloane-Stanley GH. A simple method for isolation and purification of total phospholipids from animal tissues. J Biol Chem 1957;226: 497-509.
24. Skipski VP, Barclay M, Barclay RK, Fetzer VA, Good JJ, Archibald FM. Lipid composition of human serum lipoprotein. Biochem J 1967;104: 340-361.
25. Zlatkis A, Zak B, Boyle JA. A new method for the determination of serum cholesterol. J. Lab. Clin Med 1951;41: 486-492.
26. Bartlett GR. Phosphorous assay in column chromatography. J Biol Chem 1954;234: 466-468.
27. Bangur CS, Howland JL, Katyare SS. Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria. Biochem J 1995;305 :29-32.
28. Fiske CH, Subba Row Y. Colorometric determination of phosphorous. J. Biol. Chem 1925;66: 375-400.
29. Ellman GL, Courtny KD, Anders V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol 1961;7:88-95.
30. Dixon M, Webb EC. Enzymes. Dixon M, Webb EC, Thorne C Jr., Tipton KF.(eds) 3rd edn.1979. Longman, London.
31. Kaushal R, Dave KR, Katyare SS. Paracetamol hepatotoxicity and microsomal function. Environ. Toxicol. Pharmacol 1999;1: 67-74.
32. Lowry OH, Rosebrough NJ, Farr A L, Randall RJ. Protein measurement with Folin phenol reagent. J. Biol. Chem 1951;193: 265-275.
33. Hirata F, Axelrod J. Enzymatic synthesis and rapid translocation of phosphatidylcholine by two methyltransferases in erythrocyte membranes. Proc Natl Acad Sci U S A 1978;75:2348-2352.
34. Hirata F, Viveros OH, Diliberto EJ Jr, Axelrod J. Identification and properties of two methyltransferases in conversion of phosphatidylethanolamine to phosphatidylcholine.Proc Natl Acad Sci U S A 1978;75:1718-

1721.

35. Zubay J. Biochemistry, 3rd edn. WmC Brown Publishers, Dubuque, Iowa. 1993,pp. 189-191.

36. Khandkar MA, Mukherjee E, Parmar DV, Katyare SS. Alloxan-diabetes alters kinetic properties of the membrane-bound form, but not of the soluble form, of acetylcholinesterase in rat brain. Biochem J 1995;307:647-649.

37. Smythies JR, Alarcon RD, Morere D, Monti JA, Steele M, Tolbert LC, Walter-Ryan WG. Abnormalities of one-carbon metabolism in psychiat-

ric disorders: study of methionine adenosyltransferase kinetics and lipid composition of erythrocyte membranes. Biol Psychiatry 1986;21:1391-1398.

38. Jbilo O, Bartels CF, Chatonnet A, Toutant J-P, Lockridge O. Tissue distribution of human acetylcholinesterase and butyrylcholinesterase messenger RNA. Toxicon 1994; 32:1445-1457.

39. Shah SK. Studies on erythrocyte and erythrocyte membrane functions in young epileptic patients. M.Sc Dissertation, 2000, M S University of Baroda. India.

Commentary

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Numerous reports on alterations in circulating red cell and platelet membrane and intracellular events in patients with mood disorders have appeared in the scientific literature. Despite very encouraging data, some findings have nevertheless been contradictory and equivocal. Studies on cellular changes in circulating cells in patients with mood disorders were originally conducted to attempt to evaluate whether circulating cells could be used as a surrogate for neuronal cell activity in patients with mood disorders. The assumption is that the intrinsic cellular defect in mood disorders would be reflected in all cells. Although this assumption is not necessarily true, as the defect may exist at a neuronal network level rather than at a cellular level, some very intriguing data have emerged from studies conducted in circulating cells from patients with mood disorders.

Findings of altered cellular constituents and events in circulating cells in patients with mood disorders have prompted some investigators to attempt to identify a cellular change in circulating cells that could have diagnostic, prognostic or therapeutic implications. Indeed there is a need to identify more sensitive markers to support decisions regarding the diagnosis and prognosis of mood disorders, and to attempt to improve on the monitoring of patient's progress.

In the present study Katewa et al. have attempted to identify an unequivocal red cell membrane change in patients with either major depressive or bipolar I disorder. The authors were able to show that in patients with either a unipolar or a bipolar disorder there was a considerable decrease in the ratio between total phospholipids and cholesterol in the red cell membrane, mainly because of an increase in membrane cholesterol concentrations.

Modification in cell membrane lipid composition is thought to both influence the activity of cell surface receptors and pumps by affecting the fluidity of the membrane, as well as determine the activity of membrane bound enzymes that influence synaptic concentrations of neurotransmitter substances (e.g. acetylcholinesterase). If the same membrane lipid changes identified in red cell membranes occurred in neural tissue, an altered activity of receptors, pumps, proteins and enzymes could ultimately influence neuronal transmission and synaptic function.

Although the membrane lipid changes noted in the study by Katewa et al. were associated with alterations in red cell membrane fluidity (rigidity), alterations in fluidity failed to translate into changes in the activity of a membrane pump that is largely responsible for maintaining resting membrane potentials, the Na^+-K^+ ATPase pump. This does not imply that membrane fluidity changes could not have affected the activity of receptors or other membrane pumps and enzymes. Indeed, membrane lipid changes in patients with mood disorders evaluated by Katewa et al. were associated with an increased activity of red cell membrane acetylcholinesterase (AChE) and plasma butyrylcholinesterase (BChE) activity. Importantly, Katewa et al. were able to show that the alterations in red cell membrane AChE activities were temperature-dependent and hence could be explained by changes in the lipid composition of the membrane. The authors have therefore provided evidence to link the membrane lipid change to alterations in enzyme activity.

The study by Katewa et al. was designed to identify an unambiguous red cell membrane change associated with mood disorders. If their data are confirmed in larger studies conducted by other centers, the altered composition of red cell membrane lipids and temperature-dependent red cell membrane AChE activity may offer sensitive tests to either confirm diagnoses, or to predict therapeutic responses. However, there are a number of issues that need to be resolved.

First, are the changes noted in the study by Katewa et al. due to therapy or through primary changes associated with mood disorders? In their study, the authors evaluated these changes in treated patients. Would the same changes be noted in newly diagnosed patients whilst still off therapy? Second, what is the sensitivity and specificity of the tests advocated? To assess this question the authors would have to show that the majority of data points obtained in patients with mood disorders are above the 95% confidence intervals for controls. Third, if the red cell membrane changes do indeed represent a primary cellular alteration in mood disorders, what is the mechanism that mediates these changes? Alterations in cell membrane fluidity and lipid content are sensitive to modifications in dietary fat. Do the present data therefore indicate that dietary lipids may play a role in the pathogenesis of mood disorders, or are these membrane lipid changes genetically encoded? Fourth, why do these cellular changes produce mood disorders? Perhaps the study by Katewa et al. supports the cholinergic hypothesis of mood disorders! Fifth, is it only alterations in AChE activity, or alternative molecules that influence neuronal activity that could be mediated by changes in cell membrane fluidity? Clearly the work by Katewa et al. and many others in this exciting field could ultimately lead to the identification of the cellular basis of mood disorders.

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