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A cell membrane correlate of tardive dyskinesia in patients treated with phenothiazines

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Abstract. Phenothiazine administration to psychiatric patients is associated with an increase in the "structural order" of platelet membranes as determined by steady-state fluorescence polarization measurements with 1,6-diphenyl-1,3,5-hexatriene (DPH), a fluorescent probe that localizes preferentially in the hydrocarbon region of cell membranes (Zubenko and Cohen 1984, 1985a, b). In this study, platelet membranes prepared from a group of psychiatric patients who developed tardive dyskinesia following chronic treatment with phenothiazines exhibited a significant elevation in DPH fluorescence polarization when compared to similar preparations from an otherwise matched group of patients who had no symptoms or history of tardive dyskinesia. The distribution of polarization values obtained for the tardive dyskinesia group displayed minimal overlap with that of an unmedicated, psychiatrically-healthy control group matched for age and gender. The fluorescence polarization of DPH-labelled platelet membranes was not significantly correlated with phenothiazine daily dose or serum cholesterol concentration in the phenothiazine-treated patient groups, or with dyskinesia severity (AIMS rating) in the tardive dyskinesia group. Patient gender and the presence of an affective disorder did not significantly correlate with DPH fluorescence polarization. The potential physiological and clinical significance of these findings is discussed.

Key Words: Tardive dyskinesia – Fluorescence polarization – Phenothiazines – Platelet membranes

An analysis of the physical state of a biological membrane, which is dependent on membrane composition and organization, can be performed with the use of fluorescent probes that partition into particular regions of the membrane (Waggoner and Stryer 1970; Radda 1971; Yguarabide and Stryer 1971; Radda and Vanderkooi 1972; Hawkes et al. 1976; Podo and Blasie 1977; Shinitzky and Barenholz 1978; Chen et al. 1978; Thulborn and Sawyer 1978; Thulborn et al. 1978). The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene, which preferentially localizes within the hydrocarbon region of synthetic and biological membranes (Shinitzky and Barenholz 1978), has been used extensively in studies of the acute and chronic effects of ethanol and other sedative-hypnotic agents on the structural organization of biological membranes (Heron et al. 1980a; Johnson et al. 1980; Harris and Schroeder 1982; Crews et al. 1983; Harris et al. 1984). Steady-state fluorescence polarization measurements obtained from cell membranes labelled with DPH provide an index of the "structural order" of the membrane (Van Blitterswijk et al. 1981; Pottel et al. 1983). Even small changes in this cell membrane characteristic can have considerable effects on cell functions as diverse as signal recognition and transduction, ion transport, regulation of enzyme activities, and possibly even gene expression (Mavis and Vagelos 1972; Farias et al. 1975; Kimelberg 1975; Alivisatos et al. 1977; Hanski and Levitzky 1978; Insel et al. 1978; Klein et al. 1978; Pang et al. 1979; Heron et al. 1980a, b; Hirata and Axelrod 1980; Loh and Hitzemann 1981; Ueno and Kuriyama 1981; Crews 1982).

Exposure of normal human platelet membranes in vitro to clinically relevant concentrations of representative phenothiazine antipsychotic drugs bearing aliphatic, piperidine, or piperazine side chains results in a significant increase in membrane order as determined by steady-state fluorescence polarization studies employing DPH (Zubenko and Cohen 1984; 1985b; Boudet et al. 1985). Moreover, phenothiazine administration is associated with an increase in the fluorescence polarization of DPH-labelled platelet membranes prepared from psychiatric patients receiving treatment with therapeutic doses of these agents (Zubenko and Cohen 1985a). It seems likely that pharmacotherapy with phenothiazine antipsychotic agents is accompanied in vivo by similar effects on the physical properties of membranes of other cells, including those in the central nervous system, since both acute and chronic administration of phenothiazines to Sprague-Dawley rats produces an increase in the steady-state fluorescence polarization of brain cell membranes labelled with DPH ex vivo (Cohen and Zubenko 1985).

While the effect of phenothiazines on the biophysical properties of cell membranes does not appear to be essential for antipsychotic activity (Zubenko and Cohen 1984; 1985a, b), drug-induced changes in these membrane properties, as well as possible attendant compensatory responses, may modify other drug effects and may be important in long-term exposure or at times of withdrawal. Tardive dyskinesia, for example, is a debilitating and often irreversible movement disorder associated with chronic phenothiazine treatment, whose onset may parallel the accumulation of these lipophilic agents in tissue stores (Simpson et al. 1978; APA Task Force Report 1980). Considerable pharmaco-

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logic evidence suggests that tardive dyskinesia may result, at least in part, from dopamine receptor supersensitivity as a consequence of dopamine receptor blockade (APA Task Force Report 1980), but it remains unclear why some patients exposed to phenothiazines develop this side effect and some do not. Based upon (1) the observation that phenothiazine administration is associated with an increase in the DPH fluorescence polarization of labelled human platelet and rat brain cell membranes, (2) the dependence of neurotransmitter signal recognition and transduction on this parameter (Heron et al. 1980a; Hirata and Axelrod 1980; Crews 1982), and (3) the reported therapeutic effect of lecithin, an agent that decreases this membrane characteristic in vitro (Heron et al. 1980a, b), on tardive dyskinesia (Growdon et al. 1978; Gelenberg et al. 1979), we have investigated the relationship of phenothiazine-induced increases in the DPH fluorescence polarization of platelet membranes to the development of this iatrogenic movement disorder.

Materials and methods

Patient selection. All research subjects were male or female Caucasians between the ages of 18 and 53 years. The diagnosis of tardive dyskinesia was based upon clinical appearance, a history of neuroleptic use, and the absence of an alternative neurologic diagnosis to account for the dyskinesia. The study population consisted of three groups, including (1) psychiatric patients who had received chronic ($>2y$) treatment with phenothiazines and had developed tardive dyskinesia, (2) psychiatric patients who had received chronic ($>2y$) treatment with phenothiazines but did not exhibit signs and had no history of tardive dyskinesia, and (3) unmedicated, non-psychiatric controls with no evidence of dyskinesia. The group of patients with phenothiazine-related tardive dyskinesia consisted of ten patients from the Mental Health Clinical Research Center (MHCRC)-affiliated units at McLean Hospital or from Metropolitan State Hospital. The group of phenothiazine-treated patients without tardive dyskinesia consisted of 23 individuals from the MHCRC-affiliated units at McLean Hospital or from Metropolitan State Hospital. The spectrum of phenothiazines received by patients in this study included chlorpromazine, mesoridazine, thioridazine, perphenazine, trifluoperazine, and fluphenazine. Patients receiving additional medications were included in these groups only if the medications were among those agents previously shown to have no significant effect on the DPH fluorescence polarization of platelet membranes in vitro at clinically relevant concentrations, as determined under conditions previously described (Zubenko and Cohen 1984, 1985a, b). These medications include haloperidol, thiothixene, imipramine, lithium, carbamazepine, chlordiazepoxide, pentobarbital, benztropine mesylate, and trihexyphenidyl. Both groups of psychiatric patients were diagnostically heterogeneous and included patients with chronic paranoid or undifferentiated schizophrenia, schizoaffective disorder, bipolar or atypical bipolar disorder, borderline personality disorder, mental retardation, and organic brain syndromes. Psychiatric diagnoses were made according to *DSM-III* criteria. These disorders do not appear to be associated with abnormalities in the biophysical properties of platelet membranes as reflected by DPH fluorescence polarization (Zubenko and Cohen 1985a). Seventeen unmedicated, non-psychiatric controls were recruited from clinical staff and blood bank donors. This study was approved by the Institutional Review Boards of the McLean Hospital and Metropolitan State Hospital. Written informed consent was obtained from all research candidates before admission to the study.

Dyskinesia rating. Patients with tardive dyskinesia were rated within 1 day of blood drawing by one of the investigators (GSZ) with the use of the Abnormal Involuntary Movements Scale (AIMS) (Guy 1976). The comparison group of psychiatric patients without tardive dyskinesia exhibited no evidence of this disorder.

Preparation of platelet membranes. Platelet membranes were prepared immediately after blood drawing by a minor modification of the method of Raisman et al. (1981) as previously described (Zubenko and Cohen 1984, 1985a, b). Blood was withdrawn by antecubital venepuncture into prefilled vacutainer tubes (Beckton-Dickenson) containing anticoagulant citrate dextrose (ACD). Platelet-rich plasma was prepared by centrifugation at 300 g for 20 min at 4° C and titrated to pH 6.5 by the addition of ACD. Platelets were obtained from platelet-rich plasma by centrifugation at 16,000 g for 10 min at 4° C. Platelets were resuspended in an equal volume of buffer (50 mM Tris HCl, 110 mM NaCl, 10 mM EDTA, pH 6.3) and subjected to an identical centrifugation. Membranes were prepared by hypotonic lysis (5 mM Tris HCl, 5 mM EDTA, pH 8.0), homogenization (Polytron PT 10, setting 5.0, 30 s), and centrifugation at 39,000 g for 10 min at 4° C. The pellet was resuspended in phosphate-buffered saline (PBS). Suspensions were stored frozen at −20° C prior to use. Storage at −20° C did not significantly affect the DPH fluorescence polarization of platelet membranes.

Determination of steady-state fluorescence polarization and membrane order. Platelet membrane suspensions were thawed and diluted in PBS to a final optical density of 0.03 at 600 nm. The resulting suspensions were labelled by exposure to DPH (Sigma) at a final concentration of 2 μM (Shinitzky and Barenholz 1978; Whitkin et al. 1982), except that the labelling was performed for 30 min at 37° C as previously described (Zubenko and Cohen 1984, 1985a, b). Assuming a protein/phospholipid ratio of 1/1, the estimated DPH/phospholipid ratio was approximately 1/100. Aqueous probe did not contribute significantly to the total fluorescence signal of the labelled suspensions and all unlabelled samples were devoid of fluorescence. No contribution of scattered excitation light to the emission signal was detectable.

DPH was excited at 360 nm and emitted light was measured at 37° C through cutoff filters (Corning no. 3–75). Steady-state polarization (P_s) was calculated from observed relative fluorescence intensities (I) by the following equation (Lakowicz 1983):

$$P_s = \frac{(I_{vv}/I_{vh})/(I_{hv}/I_{hh}) - 1}{(I_{vv}/I_{vh})/(I_{hv}/I_{hh}) + 1}$$

First and second subscripts refer to the vertical (v) or horizontal (h) positions of the excitation and emission polarizers, respectively. Reported polarization values are the means calculated from triplicate determinations. The coefficient of variance for replicate determinations was less than 0.01.

The fluorescence polarization of DPH in labelled membrane is related to the membrane order parameter, S, by rearrangement of the equation of Van Blitterswijk et al. (1981) as previously described (Zubenko and Cohen 1985a):

$$S^2 = \frac{20P_s - 0.25}{3(3\text{-}P_s)}$$

This equation assumes a value of 0.4 for the limiting anisotropy of DPH in the absence of probe rotation. Experimental values of 0.362 (Shinitzky and Barenholz 1974), 0.390 (Kawato et al. 1977), and 0.395 (Lakowicz et al. 1979) have been reported for this parameter. This semi-empirical relationship of the order parameter (S) and DPH steady-state polarization (P_s) is reliable over a range of P_s values from 0.18 to 0.37 (Van Blitterswijk et al. 1981; Pottel et al. 1984). All P_s values reported in this study fall within this range.

Statistical analysis. Mean steady-state fluorescence polarization values and standard errors were calculated for each study group. Comparison of values for subject cohorts were made by the non-parametric, Mann-Whitney test. Correlations between variables were investigated with the use of the Spearman correlation coefficient.

Instrumentation. All fluorescence studies were performed at 37° C on an SLM 4800 spectrofluorometer equipped with Glan-Thompson calcite prism polarizers and organized in a T-format that permits simultaneous ratiometric polarization measurements. Cuvette temperature was maintained by means of a jacketed cuvette holder coupled to a circulating water bath (Forma).

Results

Steady-state polarization values were determined for DPH-labelled platelet membranes from ten psychiatric patients who developed tardive dyskinesia following chronic treatment with phenothiazines, 23 psychiatric patients who exhibited no signs and had no history of tardive dyskinesia following a comparable period of phenothiazine exposure, and 17 unmedicated, psychiatrically-healthy controls (Fig. 1). Neither patient group included individuals receiving medications, other than phenothiazines, which significantly alter this platelet membrane parameter in vitro at clinically relevant concentrations, as previously described (Zubenko and Cohen 1984, 1985b).

The steady-state polarization of DPH-labelled platelet membranes (and hence the membrane order parameter), exhibits a significant positive correlation with patient age (Cohen and Zubenko 1985a). A similar finding has been reported for lymphocyte membranes (Rivnay et al. 1980). Therefore, we selected a limited age range for subjects in the three study groups and checked to see that the mean and distribution of ages were similar in each group. The mean ages of the tardive dyskinesia group, the dyskinesia-free group, and the psychiatrically-healthy control group were closely matched at 31.1 ± SE 3.9 years, 32.1 ± SE 2.1 years and 29.5 ± SE 1.8 years, respectively. The sex ratios of the three groups were also similar (4F/6M, 7F/16M, 3F/14M; no significant difference by Chi-square); however, patient gender does not appear to affect the platelet membrane parameter studied (Zubenko and Cohen 1985a).

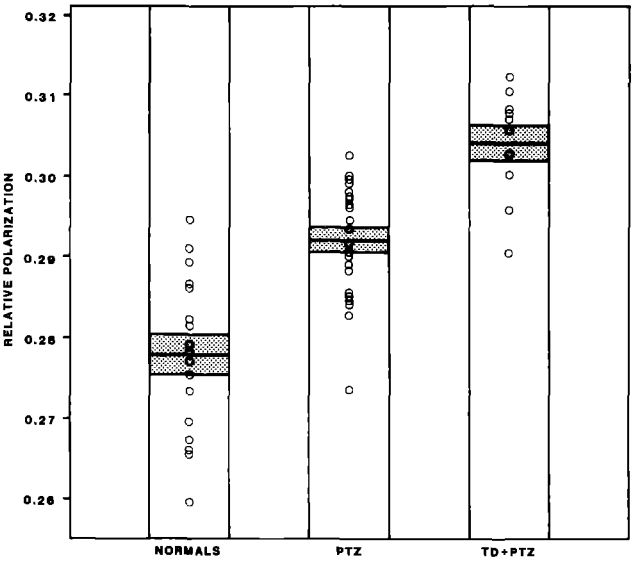

Fig. 1. Fluorescence polarization of DPH-labelled platelet membranes from unmedicated, psychiatrically-healthy controls (*NORMALS*), psychiatric patients without tardive dyskinesia who were treated chronically with phenothiazines (*PTZ*), and psychiatric patients who developed tardive dyskinesia after chronic phenothiazine administration (*TD+PTZ*). The three groups were similar in age and sex distribution; the two groups of psychiatric patients were diagnostically heterogeneous. Mean values and standard errors are indicated by *solid central bars* and *shaded regions*, respectively

Both groups of psychiatric patients included individuals from McLean and Metropolitan State Hospital. Because the means and disributions of the DPH polarization values for the platelet membranes from the McLean and Metropolitan State patients in the tardive dyskinesia or dyskinesia-free groups were similar, combining them seemed justified. The mean polarization values from the McLean and Metropolitan State patients in the tardive dyskinesia group were 0.3062 ± SE 0.0018 and 0.2955 ± SE 0.0049, while the means for the McLean and Metropolitan State patients in the dyskinesia-free groups were 0.2848 ± SE 0.0030 and 0.2930 ± SE 0.0016, respectively.

The mean polarization value for the DPH-labelled platelet membranes from the ten patients with tardive dyskinesia (0.3041 ± SE 0.0068) was significantly higher than that of the dyskinesia-free patient group (0.2920 ± SE 0.0015) ($P = 1.8 \times 10^{-4}$, Mann-Whitney). Consistent with our previous report (Zubenko and Cohen 1985a), phenothiazine administration was associated with a significant increase in the fluorescence polarization of DPH-labelled platelet membranes. The mean fluorescence polarization values of the tardive dyskinesia and dyskinesia-free patient groups were both significantly higher than that of the unmedicated, psychiatrically-healthy control group (0.2778 ± SE 0.0024, $P = 1.5 \times 10^{-5}$ and 3.4×10^{-5}, respectively; Mann-Whitney). While there was considerable overlap in the distribution of the polarization data from the dyskinesia-free patient group with those of the tardive dyskinesia and unmedicated, psychiatrically-healthy groups, nine of the ten patients with tardive dyskinesia had polarization values above the range of the unmedicated, psychiatrically healthy control group.

Since the platelet membranes prepared from patients who developed tardive dyskinesia following chronic treatment with phenothiazines exhibited a larger mean increase in DPH fluorescence polarization than those from others who did not develop this disorder, the relationship of this parameter to dyskinesia severity was investigated in the tardive dyskinesia group. The polarization values along with the corresponding AIMS ratings are shown in Fig. 2. Although a statistically significant correlation was not observed ($r_s = 0.01$, P = n.s.), the fact that seven of the ten patients had AIMS ratings between 15 and 18 limited the sensitivity of this analysis.

Based upon the suggestion that patients with affective disorders may be predisposed to the development of tardive dyskinesia (Davis et al. 1976; Rosenbaum et al. 1977; Yassa et al. 1983), the mean DPH fluorescence polarization value for the platelet membranes from patients with affective disorders (bipolar or atypical bipolar disorder) was compared to that of patients who had non-affective disorders in each of the two patient groups. The mean polarization values for the patients from the tardive dyskinesia group with or without affective disorders were $0.3033 \pm$SE 0.026 and $0.3048 \pm$SE 0.0037, respectively. The mean polarization values for the patients from the dyskinesia-free group with or without affective disorders were $0.2928 \pm$SE 0.0023 and $0.2916 \pm$SE 0.0019, respectively. Therefore, the presence of bipolar or atypical bipolar disorder (for patients who were treated chronically with phenothiazines) was not associated with an observable difference in the fluorescence polarization of DPH-labelled platelet membranes.

The magnitude of the increases seen in the DPH fluorescence polarization of platelet membranes following exposure to phenothiazines in vitro appears to reflect the membrane phenothiazine concentration (Zubenko and Cohen 1985b). Based on this observation, the relationship of daily phenothiazine dose to the corresponding fluorescence polarization of DPH-labelled platelet membranes from patients receiving treatment with these agents was examined. The absence of a statistically significant positive correlation of these variables ($r_s = +0.21$, P = n.s.) does not contradict the in vitro observation of a relationship between phenothiazine concentration and membrane order, since there is considerable variation in the baseline polarization values from unmedicated controls (Fig. 1), the platelets from patients receiving phenothiazines would have been exposed to variable concentration ratios of parent compound and a host of metabolites of unestablished effect on DPH fluorescence polarization (Cohen 1983), the blood concentrations of these agents achieved in response to particular oral doses of phenothiazines vary widely from patient to patient (Cohen 1983), and the membrane concentration of a particular phenothiazine will be a function of the membrane/solvent partition coefficient which itself varies approximately ten-fold across this class of agents (Despopoulos 1970; Seeman 1972). These factors may have obscured any relationship of membrane drug concentration to drug-induced increase in DPH fluorescence polarization in vivo. Moreover, drug-induced perturbations in the biophysical characteristics of platelet membranes in vivo may be normalized, at least in part, by homeostatic mechanisms as has been reported in a variety of cell types including mammalian neurons (Sinensky 1974; Chin and Goldstein 1977; Heron et al. 1980; Hirata and Axelrod 1980; Crews et al. 1983; Harris et al. 1984).

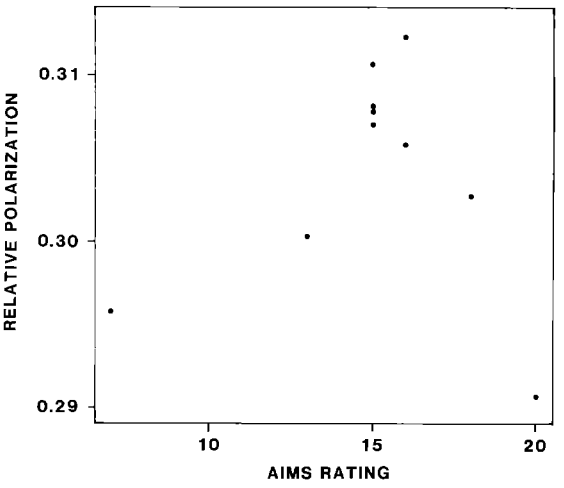

Fig. 2. Relationship of AIMS ratings to the fluorescence polarization of DPH-labelled platelet membranes from patients with tardive dyskinesia

Cholesterol exposure has been reported to increase the fluorescence polarization of DPH-labelled biological membranes in vitro (Heron et al. 1980a, b), and the fluorescence polarization of lymphocyte membranes labelled with this probe has been reported to be positively correlated with serum cholesterol concentration (Rivnay et al. 1980). Differences in serum cholesterol were not responsible for the observed mean increase in the fluorescence polarization of DPH-labelled platelet membranes associated with the development of tardive dyskinesia, since the mean serum cholesterol concentrations for the dyskinesia and dyskinesia-free patient groups, $204 \pm$SE 9 mg/dl and $203 \pm$SE 13 mg/dl, were very similar. Moreover, differences in the DPH fluorescence polarization of platelet membranes among individuals within these groups were not related to variations in serum cholesterol concentration. Specifically, no significant correlation of serum cholesterol concentration with DPH fluorescence polarization was found in either of the patient groups, with ($r_s = -0.08$, P = n.s.) or without ($r_s = -0.07$, P = n.s.) dyskinesia.

The effect of gender on platelet membrane order was determined for each of the study groups. In all cases, the mean polarization values for the female subjects did not differ significantly from those of the males.

Discussion

These results are consistent with our previous report that the administration of phenothiazines to psychiatric patients is associated with an increase in the "structural order" of platelet membranes as determined by the steady-state fluorescence polarization of DPH (Zubenko and Cohen 1985a). The observed alteration in platelet membranes cannot simply be attributed to the psychiatric disorders of the patients since we found no significant change in the fluorescence polarization of DPH-labelled platelet membranes from a diagnostically-heterogeneous group of psychiatric patients who were not taking phenothiazines when compared to an age and sex matched group of psychiatrically-healthy, drug-free controls (Zubenko and Cohen 1984, 1985a). Based on the observation that phenothiazine exposure results in an increase in the fluorescence polarization of DPH-labelled membrane fragments in vitro (Zubenko and Cohen 1984,

1985b), the elevation in the fluorescence polarization of similarly labelled platelet membranes prepared from patients receiving treatment with phenothiazines is likely to arise from the direct actions of these agents on platelet membranes (Zubenko and Cohen 1985a). While this explanation seems the most parsimonious, it is possible that the effects of phenothiazine administration on the membrane characteristics of circulating platelets may be mediated by less direct mechanisms.

It is likely that the administration of phenothiazines to patients is accompanied by a similar increase in the DPH fluorescence polarization of membranes from other cells, including those in the central nervous system. Both acute and chronic administration of phenothiazines to Sprague-Dawley rats produces an increase in the fluorescence polarization of brain cell membranes labelled with DPH ex vivo (Cohen and Zubenko 1985). Therefore, phenothiazine-induced increases in the DPH fluorescence polarization of platelet membranes may be reflective of similar changes that occur in the brain cell membranes of patients receiving treatment with these agents.

The results of this study also indicate that DPH-labelled platelet membranes from patients with tardive dyskinesia treated with phenothiazines exhibit an increase in mean fluorescence polarization relative to those prepared from an otherwise comparable group of patients who did not develop this movement disorder. One interpretation of this finding is that the patients with tardive dyskinesia had higher tissue levels of phenothiazines than the dyskinesia-free patients and that high tissue levels of phenothiazines is a factor that predisposes patients to the development of tardive dyskinesia. This is consistent with evidence from epidemiological studies suggesting that prolonged neuroleptic exposure or large total amounts of ingested drug may be contributing factors to the development of tardive dyskinesia (APA Task Force Report 1980). This interpretation is also consistent with studies suggesting that patients with neuroleptic-related tardive dyskinesia may develop higher blood levels of neuroleptics than similarly treated patients who are dyskinesia-free (Jeste et al. 1979; Jeste et al. 1982), although other studies do not support this finding (Jeste et al. 1981; Widerlov et al. 1982; Csernansky et al. 1983). A possibility consistent with all of these findings is that patients who develop tardive dyskinesia may have higher concentrations of phenothiazines at the membrane sites where they presumably act and that blood levels of phenothiazine may not reliably reflect this. Patients who develop tardive dyskinesia may have been exposed to relatively high tissue levels of drug or preferentially concentrate the drug at the sites responsible for the development of tardive dyskinesia.

An alternative interpretation of the results relies upon the hypothesis that phenothiazine-related alterations in the biophysical properties of cell membranes (presumably within the CNS) contribute to the development of tardive dyskinesia. Platelet membranes from the tardive dyskinesia group revealed a 9–10% increase in DPH fluorescence polarization compared to the unmedicated, non-psychiatric control group. Comparable increases in the DPH fluorescence polarization of both crude mouse brain membrane and synaptosomal membrane preparations have been reported to be accompanied by an approximate tripling of the density (B_{max}) of serotonin receptors and a doubling of the density of opiate receptors in mouse brain (Heron et al. 1980a, b). Should a similar relationship exist for dopamine receptors, a phenothiazine-related alteration in the biophysical properties of brain cell membranes might contribute directly to the development of dopamine receptor supersensitivity (by increasing B_{max}), a state thought to be related to the pathogenesis of tardive dyskinesia (APA Task Force Report 1980). This hypothesis is also consistent with reports that the administration of lecithin, an agent that decreases the fluorescence polarization of DPH-labelled cell membranes in vitro (Heron et al. 1980a, b), may result in a reduction of the symptoms of tardive dyskinesia (Growdon et al. 1978; Gelenberg et al. 1979). The fact that non-phenothiazine neuroleptics without significant effects on this platelet membrane characteristic in vitro, such as haloperidol and thiothixene, are also associated with the development of tardive dyskinesia does not contradict this alternative interpretation, since a likely common stimulus for the production of dopamine receptor supersensitivity shared by phenothiazine and non-phenothiazine neuroleptics is dopamine receptor blockade (Seeman 1977). If confirmed, this interpretation of the results could have considerable basic and clinical ramifications for the pharmacologic treatment of psychotic disorders as well as tardive dyskinesia.

Alterations in the structural order of cell membranes, as reflected by DPH fluorescence polarization, have been reported to affect a wide range of membrane properties and functions (Mavis and Vagelos 1972; Farias et al. 1975; Kimbelberg 1975; Alivisatos et al. 1977; Hanski and Levitzky 1978; Insel et al. 1978; Klein et al. 1978; Heron et al. 1980a; Hirata and Axelrod 1980; Loh and Hitzemann 1981; Ueno and Kuriyama 1981; Crews 1982). Several lines of evidence suggest that the phenothiazine-induced increases in DPH fluorescence polarization that accompany treatment with these agents are likely to be physiologically significant. In addition to the apparent dependence of synaptosomal serotonin and opiate receptors on this membrane parameter, as already described, the density of beta-adrenergic receptors on rat reticulocytes has been shown to increase by over 30% in response to the reduction of membrane order produced by phospholipid methylation. The efficiency of beta receptor-adenyl cyclase coupling also increases in response to this treatment (Hirata and Axelrod 1978, 1980; Strittmatter et al. 1979). Hence, significant effects on these and, most likely, other cell membrane receptors are associated with changes in cell membrane characteristics of the magnitude produced by phenothiazine treatment. Moreover, the alterations in cell membrane order that are associated with ethanol intoxication and tolerance/dependence (Chin and Goldstein 1977; Heron et al. 1980a; Johnson et al. 1980; Harris and Schroeder 1982; Crews et al. 1983; Harris et al. 1984) are smaller than those associated with chronic phenothiazine administration. Finally, the phenothiazine-related increases in the fluorescence polarization of DPH-labelled human platelet and rat brain cell membranes are similar in direction and magnitude to those that occur in the membranes of human platelets (Cohen and Zubenko 1985b) and lymphocytes (Rivnay et al. 1980) with advancing age. In this regard, it is intriguing that the elderly are more susceptible to the development of tardive dyskinesia (Simpson et al. 1978; Smith et al. 1978; APA Task Force Report 1980) and that the incidence of spontaneous dyskinesias also increases in the elderly (Delwaide and Desseilles 1977; APA Task Force Report 1980).

The effects of phenothiazines on the molecular dynamics of biological and model lipid bilayers are complex, and appear to depend on the membrane system studied, the concentration of phenothiazine relative to other membrane components, temperature, and the membrane region examined (Seeman 1972; Frenzel et al. 1978; Shinitzky and Barenholz 1978; Ogiso et al. 1981; Zimmer 1984). Moreover, interpretations of membrane "structural order" or "fluidity" based upon data from fluorescence spectroscopy, electron spin resonance spectroscopy, nuclear magnetic resonance spectroscopy, and differential scanning calorimetry, while internally consistent, are not always in agreement with one another, even when the same system is examined by multiple methods (Schreier et al. 1978; Ogiso et al. 1981; Perlman and Goldstein 1984; Zimmer 1984). In part, these descrepancies may be related to the use of different reporter molecules and the different time windows over which measurements are made by these techniques. Therefore, we do not feel that our results contradict previous reports describing the actions of phenothiazines on erythrocyte membranes or model lipid bilayers performed by other techniques, over different concentration ranges, and at temperatures other than 37° C (Seeman 1972; Frenzel et al. 1978; Shinitzky and Barenholz 1978; Ogiso et al. 1981; Zimmer 1984). Consistent with our observations (Zubenko and Cohen 1984, 1985a, b), Boudet et al. (1985) have also recently reported that exposure of intact platelets in vitro to concentrations of chlorpromazine in the range 60–90 μM is associated with an increase in the fluorescence polarization of DPH-labelled platelet membranes.

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