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COMMENTARY

DOES THE FLUIDITY OF THE LIPID ENVIRONMENT MODULATE MEMBRANE-BOUND ACETYLCHOLINESTERASE?

EFFECTS OF TEMPERATURE, MEMBRANE COMPOSITION AND AMPHIPHILES

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Acetylcholinesterase (acetylcholine hydrolase; EC 3.1.1.7; AChE‡), whose three-dimensional structure has been elucidated recently [1], is a pivotal enzyme in cholinergic transmission, with pronounced structural polymorphism [2–4]. A major distinction is made between asymmetric and globular AChE forms; it appears that whereas asymmetric forms are composed of one or more tetrameric catalytic subunits, connected by a collagen-like fibrous tail to extracellular structures, such as the muscular basal lamina, globular forms include monomeric (G1), dimeric (G2) and tetrameric (G4) aggregates which may exist as either soluble or membrane bound (i.e. detergent-soluble) forms. It is now well established that membrane-bound forms of AChE are not inserted into the lipid matrix through a hydrophobic domain of the polypeptide chain but by alternative modes [3, 4]. Studies developed on the basis of the early observations by Low and Finean [5] eventually led to the demonstration that AChE from various sources inserts into the membrane through a complex glycophosphatidylinositol anchor and can be released into the water-soluble phase following treatment with bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) [3]. Phosphatidylinositol provides a major but not the only anchoring system of AChE: membrane-bound AChE, in fact, may be partly or fully resistant to solubilization by PI-PLC treatment [6]. In the case of the human erythrocyte enzyme, this behaviour is to be ascribed to palmitoylation of the inositol ring [7], whereas in other systems different anchoring modes may be involved. In this connection, Inestrosa *et al.* [8] have demonstrated that membrane anchoring of bovine brain AChE is provided by a 20-kDa hydrophobic polypeptide linked by disulfide bridges to the catalytic subunits. Indeed, it now appears that whereas the latter anchoring system is characteristic of membrane-associated G4 forms, which predominantly occur in the nervous system, anchoring by phosphatidylinositol is typical of G2 forms [2, 3]. The interaction of amphiphilic forms of AChE with detergents, as well as enzyme behaviour after reconstitution in liposomes, has been reviewed extensively [9]. Knowledge of AChE anchoring to membranes is increasing rapidly but an old question is still unsolved: namely, is membrane-bound AChE sensitive to the physical state of the surrounding lipid environment? This question was first addressed when the enzyme was thought to be a "true" integral one, and brings now additional aspects of interest in consideration of its alternative modes of insertion into membranes. Herein we present an overview of the work aimed to answer this question and to assess whether the action of amphiphilic molecules of pharmacological interest on the enzyme is mediated by drug effects on membrane physical properties.

Arrhenius plots studies

Membrane fluidity refers to the motional characteristics of membrane components and is operatively defined on the basis of spectroscopic parameters reflecting the motional freedom of membrane-inserted probes [10, 11]. At constant temperature, the fluidity of biological membranes depends on their composition and, as a general rule, increases with unsaturation of phospholipid fatty acids and decreases with cholesterol enrichment [12]. Temperature is a major physical determinant of membrane fluidity [12]; the temperature-dependence of a membrane enzymatic activity—usually visualized in the form of an Arrhenius plot (i.e. a plot of log V vs $1/T$)—may thus combine the direct effects of temperature on the enzyme and the indirect effects arising from physical changes of the surrounding lipid environment. Membrane-bound AChE from various sources has been reported to display non-linear Arrhenius plots. This phenomenon implicates an abrupt change in the activation energy of the

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‡ Abbreviations: AChE, acetylcholinesterase; PI-PLC, phosphatidylinositol-specific phospholipase C; DMPC, dimyristoyl phosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; and EFA, essential fatty acid.

Table 1. Breakpoint temperatures of native and solubilized AChE

Source of enzyme	Breakpoint (°) Native	Solubilized	Remark	Ref.
Rat erythrocytes	14	14		[13]
Rat erythrocytes	30	30	EFA-suff. pH 7.0	[14]
	20	20	EFA-suff. pH 8.0	
Rat erythrocytes	Linear	30	EFA-def. pH 7.0	[14]
	29	20	EFA-def. pH 8.0	
Torpedo	16	Linear		[13]
Sheep platelets	17	Linear		[15]
Dog brain synaptosomes	23	Linear		[16]
Human erythrocytes, incorporated into egg phosphatidylcholine liposomes	Linear	Linear		[17]
Human erythrocytes, incorporated into DMPC liposomes	22	Linear		[17]
Human erythrocytes	24 & 36	24 & 36		[18]

Table 2. Breakpoint temperatures of AChE in native and cholesterol-enriched membranes

Source of enzyme	Breakpoint (°) Native	Solubilized	Remark	Ref.
Human erythrocytes, incorporated into DMPC liposomes	22	Linear		[17]
Human erythrocytes	24 & 36	24 & 36		[18]
Sheep platelets	17	Linear		[19]
Rat erythrocytes	20	22 & 28	Corn oil supplemented diet	[20]
Rat erythrocytes	20	20	Lard supplemented diet	[20]
Rat erythrocytes	28	19	Hydrogenated fat supplemented diet	[20]

catalytic process at certain critical temperatures and is likely to be driven by conformational modifications of the protein. It has been questioned whether the occurrence of "breaks" in AChE Arrhenius plots is due to a direct effect of temperature on protein conformation or whether it is primarily driven by changes in membrane fluidity: in particular whether it is due to phase transitions of the lipid environment sensed by the enzyme. To deal with this issue, a major strategy has been to compare the temperature-dependence of enzyme activity in the native state with that occurring after manipulation of the composition of its membrane environment and/or solubilization. The work performed is summarized in Tables 1 and 2. Barton *et al.* [13] studied the temperature-dependence of rat erythrocyte AChE in the native state and after solubilization with PI-PLC or papain; it was demonstrated that enzyme solubilization does not modify the Arrhenius plot with respect to that of the native enzyme. A similar observation had been made previously by Bloj *et al.* [14] on red blood cells from rats fed an essential fatty acid (EFA) diet; AChE solubilization, in this case, was carried out with the Triton X-100. Further observations by Bloj *et al.* [14], however, indicated that modification of membrane composition, achieved through feeding rats an EFA-deficient diet, linearized the Arrhenius plot or shifted the breakpoint temperature towards a higher value; interestingly, no difference could be observed in the temperature-dependence of the enzyme solubilized with Triton X-100 from erythrocytes of rats kept on an EFA-sufficient or -deficient diet. These results support the view that discontinuities in the temperature-dependence of AChE activity from rat erythrocytes are driven primarily by a direct effect of the temperature on the enzyme; however, they also suggest that changes of the composition of the membrane environment may somehow affect the phenomenon. Studies performed on other systems are, conversely, consistent with the possibility that the effect of temperature on AChE is mainly mediated by fluidity changes on the membrane environment. Non-linear Arrhenius plots have been reported for native membrane AChE from *Torpedo* [13], sheep platelets [15] and dog synaptosomes [16]. Solubilization of *Torpedo* AChE with papain or proteinase K and of the other two enzymes with Triton X-100 has been demonstrated to linearize the plot. In the case of dog synaptosomal AChE, it has also been observed that there is a close correspondence between the breakpoint temperature

of the enzyme Arrhenius plot and the temperature at which synaptosomal membranes undergo lipid phase separation [16]. Frenkel *et al.* [17] demonstrated that purified AChE from human erythrocytes displays a linear Arrhenius plot, whereas reconstitution of the purified enzyme in Triton X-100 micelles yields a non-linear plot, with activation energy gradually decreasing with increasing temperature, but with no evident breakpoint. When the enzyme was reconstituted in liposomes made of dimyristoyl phosphatidylcholine (DMPC), a break in the plot was observed at 22°, corresponding to the temperature of phospholipid phase transition (Table 1); conversely, a linear plot was observed for the enzyme incorporated into vesicles made of egg phosphatidylcholine, which do not experience a phase transition within the temperature range studied. The Arrhenius plot of the enzymatic activity incorporated into DMPC turned linear again when liposomes were enriched with cholesterol, which abolishes DMPC phase transition (Table 2). The authors suggested that AChE conformational changes are driven primarily by a direct effect of the temperature but a major role in the phenomenon is played by the constraint imposed on the enzyme by protein–protein interaction as well as by its lipid environment. When the constraint is almost totally relieved by detergent solubilization, the enzyme can freely undergo gradual conformational changes. Cholesterol hemisuccinate enrichment of membranes can easily be achieved '*in vitro*' and has been employed in some instances to assess whether the lipid environment has any role in determining the non-linear slopes observed in the temperature-dependence of AChE activity. Studies on the human erythrocyte enzyme in the native state demonstrated a break at 24° in the Arrhenius plot and an abrupt stop of the increase of the enzymatic activity at temperatures above 36°. According to previous observations carried out by ESR spectroscopy [21], the temperature-dependence of the human erythrocyte lipid order parameter, as assessed by 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence depolarization, showed an inflection around 30° which could be related to membrane phase transition; while this inflection was abolished when the cholesterol to phospholipid molar ratio was raised from 0.95 to 1.40, no change in the slope of the enzyme Arrhenius plot was observed after membrane cholesterol enrichment [18]. It was concluded that the breaks in the Arrhenius plot of human erythrocyte AChE activity are unrelated to sudden changes of membrane physical state but arise from a direct effect of the temperature on enzyme conformation. Indeed, an increase of the activity with temperature was found above 36°, when the enzyme from either control membrane or cholesterol-loaded membrane was solubilized; furthermore, a difference in the K_i for substrate between membrane-bound and soluble enzyme was also observed at 40° [18]. In sheep platelet membranes [19], native AChE was demonstrated to display a discontinuity at 17° in the Arrhenius plot; upon raising the cholesterol/phospholipid molar ratio from 0.66 to 1.40, such a discontinuity was no longer observed [19]. Cholesterol enrichment of platelet membranes led to a small but significant increase of enzyme K_m [19], a circumstance that was not observed in the case of the human erythrocyte enzyme [18]. In the case of rat erythrocyte AChE, the effect of keeping animals on different fat-supplemented diets (with or without cholesterol) can be seen clearly in Table 2. Two breakpoint temperatures in the Arrhenius plot of erythrocyte AChE from rats fed corn oil plus cholesterol were reported; the Arrhenius plot of the enzyme from lard-fed rats showed a break around 20° as it did in corn oil-fed rats. The behaviour of erythrocyte AChE from lard-fed rats was not altered when the diet was supplemented with cholesterol. The enzyme from rats fed a diet supplemented with hydrogenated fat exhibits a break around 28°. When the diet was supplemented with cholesterol, a shift to 19° in the breakpoint was observed. The heterogeneous response was obtained either '*in vivo*' when the cholesterol was supplemented to the diet or in '*in vitro*' loaded membranes with cholesterol [20].

Hill plot studies

Cooperative enzymes show sigmoid activity curves in the presence of allosteric effectors (activators or inhibitors). The Hill coefficient (h), which combines the number of interacting sites ("n") and the strength of interactions and that, for cooperative enzymes, has a value different from 1.0, is determined graphically using the Hill equation: $\log[v/(V - v)] = \log K - h \log[E]$. In this equation v is the reaction velocity, V is the rate of the reaction in the absence of the effector $[E]$, and K is a constant. For systems where cooperativity is complete, the Hill plot is equal to the number of binding sites. The effect of membrane fluidity in the regulation of cooperative enzymes from mammalian and bacterial membranes has been reviewed [22, 23]. A possible clue as to how the membrane structure acts on a cooperative enzyme was given in a study performed with Ca^{2+}-ATPase from bacterial membrane in which it was suggested that membrane fluidity may affect the equilibrium "compact conformation" (non-cooperative) ⇔ "expanded conformation" (cooperative) of the enzyme [24]. The cooperative behaviour of rat erythrocyte AChE is modulated by membrane fluidity [22, 23]. Studies were carried out using the nutritional approach to change the membrane lipid (fatty acid and cholesterol) composition. The values of "n" for the inhibition by F^- increased from 1.0 to 1.6 with the increase of the double bond index/saturated fatty acid ratio, obtained by feeding rats different fat-supplemented diets [25]. The double bond index/saturated fatty acid ratio was taken as an indicator of membrane fluidity; this assumption was supported by studies performed with physical methods [26, 27]. Decreasing membrane fluidity by cholesterol incorporation into membranes yielded confirmatory results of this regulatory mechanism since in rats fed a cholesterol-supplemented diet the values of "n" shifted from 1.6 to 1.0, as predicted. AChE cooperative behaviour to F^- inhibition was also observed in synaptosomes [16]. Frenkel *et al.* [17] reported a Hill coefficient of 1.0 for the inhibition by F^- of AChE purified from human erythrocytes and incorporated into DMPC

liposomes; this value remained unchanged when the fluidity of the lipid matrix was varied through changes of the assay temperature (temperature below and above the breakpoint observed in the Arrhenius plot), and it was concluded "that changes in lipid fluidity do not induce changes in the allosteric properties of the enzyme". Similar conclusions were drawn by Sánchez-Yagüe *et al.* [15] for AChE from sheep platelet plasma membranes. In connection with the above results [15, 17], it should be observed that if the Hill coefficient for the enzyme in a given membrane is the maximum or the minimum one possible, a further modification of the interaction energy would not be detected [28]. Lenz *et al.* [29] showed differences in the kinetic properties of soluble and membrane-bound AChE from electric eel when the inhibition by F^- was studied in the presence of pesticide. This is a confirmation of early observations [30, 31] on the relationship between pesticide action and membrane fluidity changes.

AChE and amphiphilic drugs

Many compounds of pharmacological interest are amphiphilic in nature and could affect AChE activity either by direct interaction or through perturbation of the membrane lipid environment. Spectroscopic studies on a wide range of systems have demonstrated that amphiphilic drugs are able to enhance membrane fluidity [21, 32–34]; furthermore, owing to the asymmetric distribution of membrane lipids between the two halves of the bilayer, it has also been suggested that anionic and cationic amphiphiles interact preferentially with the membrane exofacial and endofacial leaflet, respectively [35]. The effect of ketamine, a general anaesthetic, on synaptosomal AChE has been thoroughly investigated: Mazzanti *et al.* [36] demonstrated that synaptic membranes from anaesthesized rats display both an increased fluidity, as assessed by ESR spectroscopy, and a decreased specific enzymatic activity when compared with membranes from the control animals. These results suggested the possibility that changes in AChE activity could be mediated by the effect of the ketamine on the physical state of the membrane; however, a cause-to-effect relationship between the two phenomena could not be unequivocally established. Indeed, in an earlier study [37], it was demonstrated that native and solubilized rat synaptosomal AChE is sensitive to the same extent to ketamine inhibition; ketamine was also reported to inhibit with the same potency synaptosomal bovine enzyme in the native state and after purification [37]. A study of the effects of ketamine on purified bovine brain AChE solubilized with Triton X-100 or reconstituted in soybean lecithin liposomes has also been carried out [38]. It was observed that the reconstituted enzyme is slightly more sensitive to ketamine inhibition than the soluble one; however, since the inhibition kinetics of ketamine are of the mixed type and the observed difference in enzyme inhibition was restricted to the competitive component, the authors indicated as unlikely that the effect of ketamine is mediated by the fluidity of liposomal membranes [38]. This notion was substantiated by further studies indicating that ketamine enantiomers inhibit the enzyme with a slightly different potency [39]. Deliconstantinos and Tsakiris [16] studied the differential effect of anionic and cationic amphiphiles on dog synaptosomal AChE. It should be remembered that membrane-bound AChE faces the extracellular medium; therefore, if the enzyme is modulated by its lipid milieu, it is expected to be more sensitive to anionic drugs (which interact preferentially with the outer membrane leaflet) than to cationic drugs (which interact predominantly with the inner leaflet). It was reported that the anionic drugs phenobarbital and barbituric acid—as well as the neutral drug primidone—increase enzyme activity at millimolar concentrations, dramatically affecting also the temperature-dependence of AChE activity and the enzyme cooperative behaviour to F^- inhibition; among the cationic drugs, perphenazine and trifluoperazine, but not imipramine, had effects on AChE activity. Inhibition of AChE by cationic amphiphilic drugs (procaine, lidocaine, tetracaine, dibucaine and chlorpromazine) has also been demonstrated in rat synaptosomes, but the possibility that drug effects could be mediated by membrane physical state perturbation was not explored [40]. Curatola *et al.* [41] investigated the effect of *n*-butanol and ketamine on the activity of the human erythrocyte enzyme. While both compounds were effective in inhibiting AChE in the native state, their inhibitory action was lost after enzyme solubilization by sonication. Restoration of the inhibitory effect of the two molecules was achieved by enzyme reconstitution in a lipid environment, thus demonstrating that their effect occurs through mediation of the lipid environment. Benzyl alcohol, a local anaesthetic, has been demonstrated to enhance membrane fluidity and to affect the activity of intrinsic membrane enzymes [42]. A relationship has been suggested between the membrane-perturbing properties of the anaesthetic and its inhibitory effect on human erythrocyte AChE [43]. The basis on which these conclusions have been drawn, however, is unclear: it was, in fact, reported that benzyl alcohol is also able to fully inhibit soluble AChE [43]. In human erythrocytes, procaine and tetracaine were found to inhibit AChE at concentrations at which they were ineffective in perturbing membrane physical state whereas imipramine and lidocaine inhibited AChE activity, eliciting concomitantly a decrease of DPH steady-state fluorescence polarization [34]. Membrane integrity, however, was not an essential condition for imipramine and lidocaine inhibitory action: the two drugs fully retained their inhibitory effect when assayed using Triton X-100-solubilized AChE, in the presence of detergent concentrations immediately above its critical micellar concentration. Higher detergent concentrations were able to partially reduce drug inhibitory potencies; this effect, however, could be explained by amphiphile partition in to detergent micelles, leading to a decrease of the effective concentration in the aqueous phase [34]. Unequivocal demonstration of human erythrocyte AChE inhibition by direct molecular interaction was achieved in the case of chlorpromazine [44]; this drug, in fact, inhibited with equal potency native AChE and the fraction of enzyme that is solubilized by PI-PLC

treatment. Interestingly, the presence of 0.5% Triton X-100 was able to fully reverse the drug effect not only in the case of the native enzyme but also after its solubilization with PI-PLC: the sequestering effect of detergent was demonstrated in this study by rapid gel filtration on Sephadex G-15. Recent studies support the notion that phenothiazines may directly interact with AChE both as monomers and micellar aggregates [44] producing different inhibitory effects. Studies with chlorpromazine demonstrated that the type of inhibition kinetics displayed by this drug is concentration dependent, turning from non-competitive to mixed type at a concentration at which chlorpromazine has been demonstrated previously to undergo micellar aggregation [45]. A similar behaviour was demonstrated for perphenazine and trifluoperazine [46]. An explanation for the appearance of the inhibition component was attempted on the basis of the recent elucidation of the three-dimensional structure of AChE from *Torpedo californica* [1]. It was proposed that amphiphile aggregates may block the opening of the enzyme "gorge" at the bottom of which the active site is located [1], thus preventing substrate access.

Methodological considerations

From the results analyzed two aspects deserve methodological considerations. The first deals with Arrhenius plot evaluation and the second with the study of amphiphile effects.

(a) Artifacts arising upon obtaining enzyme Arrhenius plots have been discussed extensively [47, 48]: the most trivial deal with changes that may occur with temperature, concerning either intrinsic kinetic parameters (K_m or K_i for substrate) or extrinsic factors (pH modification of the assay buffer). In the case of AChE from human red blood cells the change in pH and the change in substrate concentration strongly modify the Arrhenius plots [18]. Changing the pH of the enzyme assay from 7.0 to 8.0 changed the breakpoint temperature from 30° to 20° in rat erythrocyte membranes (Table 1). On the other hand, another important intrinsic factor that, in the case of membrane enzymes, could affect enzyme response is the membrane fatty acid composition, which in turn depends on the dietary fatty acid composition. As shown in Tables 1 and 2, a strong influence of this parameter was found. The modification of the lipid environment through feeding conditions leads to some particular regulatory pattern for several membrane-associated enzymes (AChE falls in this group) [49]. Control of dietary fat effect was carried out only for rat synaptosomal AChE [50]; thus, from the above observations, precaution is necessary even when studies on the same membrane system are performed. In the case of synaptosomal AChE, another intrinsic problem could be present, since age-dependent changes in the breakpoint temperatures of Arrhenius plot were reported [50].

(b) With respect to the study of the mechanism by which amphiphilic molecules affect AChE activity, it should be noted that most amphiphiles interact equally well with proteins and with the lipid matrix [33, 51]. A major consequence of this circumstance is that concomitant occurrence of drug effects on AChE activity and membrane physical state does not unequivocally prove a cause-to-effect relationship: the latter should take into account amphiphile effect on the enzyme in the native state and after solubilization in a lipid- as well as a detergent-free environment. Another aspect that must be considered is the ability of amphiphilic drugs to form micelles, when present in the aqueous medium above a certain critical concentration. Although this issue has received relatively little attention, it must be pointed out that drug comicellation with membrane lipids may lead to severe damaging of membranes and to protein solubilization [45].

Concluding remarks

The body of experimental data herein reviewed does not fully fit into a general regulative pattern of membrane-bound AChE by the membrane environment; in fact, evidence supporting a major effect of the membrane environment on enzyme activity has been provided in some systems but not in others. The circumstance that experimental data are, in some instances, even conflicting calls for a strong complexity of the phenomenon in which type of membrane anchoring, membrane composition and intrinsic properties of the protein structure could possibly be involved. The question arises as to the mechanism through which membrane fluidity could affect AChE activity. For a "true" integral enzyme it can be expected that, owing to the continuity of the polypeptide chain, the constraint imposed on the hydrophobic domain by the physical state of lipid environment may lead to conformational changes of the hydrophilic moiety, and hence of the active site; furthermore, the notion that the fluidity state of the lipid matrix affects the equilibrium position at the water–lipid interface of integral membrane proteins implies that changes in membrane fluidity may affect protein conformation by exposing hydrophobic domains of the protein to the aqueous phase or hydrophilic domains to the lipid phase [12]. In the case of those forms of AChE that are anchored to the membrane by a hydrophobic peptide, membrane fluidity could affect protein conformation through similar mechanisms, much depending on whether AChE catalytic subunits and the hydrophobic peptide, which are connected through disulfide bridges, also interact through other domains on their polypeptide chains. Moreover, the occurrence of vertical displacement of the anchoring peptide could shift the position of the enzyme catalytic subunits with respect to the polar headgroups of the membrane. The ability of membrane fluidity to modulate protein lateral and rotational diffusion has been, in some instances, considered central to the mechanism by which the lipid environment affects those processes in which proper association of different membrane proteins is required. The high diffusion coefficient in the plane of the membrane is a feature of PI-anchored proteins, including AChE [52]; if this feature has a functional counterpart, for example if *in vivo* modulation of AChE also occurs through rapid association and dissociation of enzyme units, as might be predicted by the cooperative behaviour of the enzyme observed in some instances,

then the physical state of the environment could modulate enzyme activity by controlling its diffusion and association. At the moment, however, any assertion on how membrane fluidity could concur to modulate AChE activity remains purely speculative.

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REFERENCES

1. Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L and Silman I, Atomic structure of acetylcholinesterase from *Torpedo californica*: A prototypic acetylcholine-binding protein. *Science* **253**: 872–879, 1991.
2. Massoulié J and Bon S, The molecular form of cholinesterases and acetylcholinesterases in vertebrates. *Annu Rev Neurosci* **5**: 57–106, 1982.
3. Silman I and Futerman AH, Modes of attachments of acetylcholinesterase to the surface membrane. *Eur J Biochem* **170**: 11–22, 1987.
4. Inestrosa NC and Perelman A, Association of acetylcholinesterase with the cell surface. *J Membr Biol* **118**: 1–9, 1990.
5. Low MG and Finean JB, Non-lytic release of acetylcholinesterase from erythrocytes by a phosphatidylinositol-specific phospholipase CD. *FEBS Lett* **82**: 143–146, 1977.
6. Futerman AH, Low MG, Michaelson DM and Silman I, Solubilization of membrane-bound acetylcholinesterase by a phosphatidylinositol-specific phospholipase C. *J Neurochem* **45**: 1487–1494, 1985.
7. Roberts WL, Myher JJ, Kuksis A, Low MG and Rosenberry TL, Lipid analysis of the glycoinositol phospholipid membrane anchor of human erythrocyte acetylcholinesterase. Palmitoylation of inositol results in resistance to phosphatidylinositol-specific phospholipase C. *J Biol Chem* **263**: 18766–18775, 1988.
8. Inestrosa NC, Roberts WL, Marshall TL and Rosenberry TL, Acetylcholinesterase from bovine caudate nucleus is attached to membranes by a novel subunit distinct from those of acetylcholinesterase in other tissues. *J Biol Chem* **262**: 4441–4444, 1987.
9. Ott P, Membrane acetylcholinesterases: Purification, molecular properties and interactions with amphiphilic environments. *Biochim Biophys Acta* **822**: 375–392, 1985.
10. Shinitzky M and Barenholz Y, Fluidity parameters of lipid regions determined by fluorescence polarization. *Biochim Biophys Acta* **515**: 367–394, 1978.
11. Stubbs CD, Membrane fluidity: Structure and dynamics of membrane lipids. *Essays Biochem* **19**: 1–29, 1983.
12. Shinitzky M, Membrane fluidity and cellular functions. In: *Physiology of Membrane Fluidity* (Ed. Shinitzky M), Vol. 1, pp. 1–51. CRC Press, Boca Raton, 1984.
13. Barton PL, Futerman AH and Silman I, Arrhenius plots of acetylcholinesterase activity in mammalian erythrocytes and in *Torpedo* electric organ. Effect of solubilization by proteinases and by a phosphatidylinositol-specific phospholipase C. *Biochem J* **2312**: 237–240, 1985.
14. Bloj B, Morero RD and Farias RN, Effect of essential fatty acid deficiency on the Arrhenius plot of acetylcholinesterase from rat erythrocytes. *J Nutr* **104**: 1265–1274, 1974.
15. Sánchez-Yagüe J, Cabezas JA and Llanillo M, Subcellular distribution and characterization of acetylcholinesterase activities from sheep platelets: Relationships between temperature-dependence and environment. *Blood* **76**: 737–744, 1990.
16. Deliconstantinos G and Tsakiris S, Differential effect of anionic and cationic drugs on the synaptosome-associated acetylcholinesterase activity of dog brain. *Biochem J* **229**: 81–86, 1985.
17. Frenkel EJ, Roelofsen B, Brodbeck U, Van Deenen LLM and Ott P, Lipid–protein interactions in human erythrocyte-membrane acetyldcholinesterase. Modulation of enzyme activity by lipids. *Eur J Biochem* **109**: 377–382, 1980.
18. Spinedi A, Rufini S, Luly P and Farias RN, The temperature-dependence of human erythrocyte acetylcholinesterase activity is not affected by membrane cholesterol enrichment. *Biochem J* **255**: 547–551, 1988.
19. Sànchez-Yagüe J, Cabezas JA and Llanillo M, Effect of cholesterol supplementation on acetylcholinesterase activity from sheep platelet plasma membrane. *Biochem Pharmacol* **42**: 2037–2040, 1991.
20. Bloj B, Galo MG, Morero RD and Farias RN, Heterogeneous effect of dietary cholesterol on acetylcholinesterase and ATPases of rat erythrocytes: Arrhenius plots. *J Nutr* **109**: 63–69, 1979.
21. Ogiso T, Iwaki M and Mori K, Fluidity of human erythrocyte membrane and effect of chlorpromazine on fluidity and phase separation of membrane. *Biochim Biophys Acta* **649**: 325–335, 1981.
22. Farias RN, Bloj B, Morero RD, Sineriz F and Trucco RE, Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. *Biochim Biophys Acta* **415**: 231–251, 1975.
23. Farias RN, Membrane cooperative enzymes as a tool for the investigation of membrane structure and related phenomena. *Adv Lipid Res* **17**: 251–282, 1980.
24. Sineriz F, Moreno H and Farías RN, Membrane-bound cooperative enzymes. Stokes' radii, Hill plots and membrane fluidity in the regulation of adenosinetriphosphatase from *Escherichia coli*. *Biochim Biophys Acta* **513**: 78–88, 1978.
25. Bloj B, Morero RD, Farias RN and Trucco RE, Membrane lipid fatty acids and regulation of membrane-bound enzymes. Allosteric behaviour of erythrocyte Mg^{2+}-ATPase, $(Na^+ + K^+)$-ATPase and acetylcholinesterase from rats fed different fat-supplemented diets. *Biochim Biophys Acta* **311**: 67–79, 1973.
26. Lipiello PM, Holloway CT, Garfield SA and Holloway PW, The effects of estradiol on stearyl-CoA desaturase activity and microsomal membrane properties in rooster liver. *J Biol Chem* **254**: 2004–2009, 1979.
27. Castuma CE and Brenner RR, Effect of fatty acid deficiency on microsomal membrane fluidity and cooperativity of the UDP-glucoronyl transferase. *Biochim Biophys Acta* **729**: 9–16, 1983.
28. Sineriz F, Farias RN and Trucco RE, The convenience of the use of allosteric "probes" for the study of lipid–protein interactions in biological membranes: Thermodynamic considerations. *J Theor Biol* **52**: 113–120, 1975.
29. Lenz DE, Maxwell DM and Walden MD, Kinetic properties of soluble and membrane-bound acetylcholinesterase from electric eel. *Life Sci* **34**: 219–224, 1984.
30. Domenech CE, Machado de Domenech EE, Balegno HF, de Mendoza D and Farias RN, Allosteric behaviour of rat erythrocyte membrane-bound acetylcholinesterase in the presence of organophosphorous compounds. *FEBS Lett* **74**: 243–246, 1977.
31. Machado de Domenech EE, Domenech CE, Balegno HF, de Mendoza D and Farias RN, Pesticide action: Different response of erythrocyte membrane acetylcholinesterase to inhibition by organopho-

sphorous compounds under varied dietary conditions. *Pest Biochem Physiol* **14**: 1–4, 1980.
32. Ondrias K, Balgavy P, Stolc S and Horvath LI, A spin label study of the perturbation effect of tertiary amine anesthetics on brain lipid liposomes and synaptosomes. *Biochim Biophys Acta* **732**: 627–635, 1983.
33. Minetti M and Di Stasi AMM, Involvement of erythrocyte skeletal proteins in the modulation of membrane fluidity by phenothiazines. *Biochemistry* **26**: 8133–8137, 1987.
34. Spinedi A, Pacini L and Luly P, A study of the mechanism by which some amphiphilic drugs affect human erythrocyte acetylcholinesterase activity. *Biochem J* **261**: 569–573, 1989.
35. Sweet WD and Schroeder F, Charged anaesthetics alter LM-fibroblast plasma-membrane enzymes by selective fluidization of inner and outer membrane leaflets. *Biochem J* **239**: 301–310, 1986.
36. Mazzanti L, Pastuszko A and Lenaz G, Effects of ketamine anesthesia on rat-brain membranes: Fluidity changes and kinetics of acetylcholinesterase. *Biochim Biophys Acta* **861**: 105–110, 1986.
37. Cohen ML, Chan SL, Bhargava HN and Trevor AJ, Inhibition of mammalian brain acetylcholinesterase by ketamine. *Biochem Pharmacol* **23**: 1647–1652, 1974.
38. Puu G and Koch M, Comparison of kinetic parameters for acetylthiocholine, soman, ketamine and fasciculin towards acetylcholinesterase in liposomes and solution. *Biochem Pharmacol* **40**: 2209–2214, 1990.
39. Puu G, Koch M and Artursson E, Ketamine enantiomers and acetylcholinesterase. *Biochem Pharmacol* **41**: 2043–2045, 1991.
40. Sidek HM, Nyquist-Battie CD and Vanderkooi G, Inhibition of synaptosomal enzymes by local anesthetics. *Biochim Biophys Acta* **801**: 260–31, 1984.
41. Curatola G, Mazzanti L, Lenaz G and Pastuszko A, General anesthetics inhibit erythrocyte acetylcholinesterase only when membrane-bound. *Bull Mol Biol Med* **4**: 139–146, 1979.
42. Gordon LM, Sauerheber RD, Esgate JA, Dipple I, Marchmont RJ and Houslay MD, The increase in bilayer fluidity of rat liver plasma membranes achieved by the local anesthetic benzyl alcohol affects the activity of intrinsic membrane enzymes. *J Biol Chem* **255**: 4519–4527, 1980.
43. Palmieri DA, Rangachari A and Butterfield DA, Effects of domain-specific erythrocyte membrane modulators on acetylcholinesterase activity and NADH:cytochrome b_5 reductase activities. *Arch Biochem Biophys* **280**: 224–228, 1990.
44. Spinedi A, Pacini L, Limatola CD, Luly P and Farias RN, A study of human erythrocyte acetylcholinesterase inhibition by chlorpromazine. *Biochem J* **278**: 461–463, 1991.
45. Luxnat M and Galla H-J, Partition of chlorpromazine into lipid bilayer membranes: The effect of membrane structure and composition. *Biochim Biophys Acta* **856**: 274–282, 1986.
46. Spinedi A, Pacini L, Limatola CD, Luly P and Farias RN, Phenothiazines inhibit acetylcholinesterase by concentration-dependent-type kinetics: A study with trifluoperazine and perphenazine. *Biochem Pharmacol* **44**: 1511–1514, 1992.
47. Dixon M and Webb EC, *Enzyme Kinetics*. Longman, London, 1964.
48. Silvius JR, Read BD and McElhaney RN, Membrane enzymes: Artifacts in Arrhenius plots due to temperature dependence of substrate-binding affinity. *Science* **199**: 902–904, 1978.
49. Farias RN and Trucco RE, Membrane structure and function with different lipid-supplemented diets. *Adv Exp Med Biol* **83**: 591–607, 1977.
50. Foot M, Cruz TF and Clandinin MT, Effect of dietary lipid on synaptosomal acetylcholinesterase activity. *Biochem J* **211**: 507–509, 1983.
51. Yamaguchi T, Watanabe S and Kimoto E, ESR spectral changes induced by chlorpromazine in spin-labeled erythrocyte ghost membranes. *Biochim Biophys Acta* **820**: 157–164, 1985.
52. Low MG and Saltiel AR, Structural and functional roles of glycosylphosphatidylinositol in membranes. *Science* **238**: 268–275, 1988.