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Interaction of lanthanum chloride with human erythrocyte membrane in relation to acetylcholinesterase activity

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Abstract. Lanthanum chloride (1 mM) inhibits the activity of acetylcholinesterase *in vitro* in the human erythrocyte membrane. Lineweaver-Burk analysis indicates that lanthanum chloride induced inhibition of acetylcholinesterase activity is competitive in nature. The Arrhenius plot shows that the transition temperature of erythrocyte membrane-bound acetylcholinesterase is significantly reduced in the presence of lanthanum chloride. These results suggest that lanthanum chloride increases the fluidity of the erythrocyte membrane and this may be a cause of inhibition of membrane-bound acetylcholinesterase activity.

Keywords. Human erythrocyte membrane; lanthanum; acetylcholinesterase; Arrhenius plot.

Introduction

Lanthanum, a member of the light lanthanides, exists in the ionic form at low concentration. It binds to the phospholipid component of the erythrocyte membrane and acts at the outer periphery of the membrane, without penetrating it (Venugopal and Luckey, 1978). It is known that changes in the membrane microenvironment alter the activities of various membrane-bound enzymes (Beauregard and Roufogalis, 1977; Nemat-Gorgani and Meisami, 1979; Gordon *et al.,* 1980) and it seems likely that lanthanum-erythrocyte membrane interactions may lead to changes in the activities of membrane-bound enzymes. Weiner and Lee (1972) have shown that lanthanum inhibits the activity of erythrocyte membrane-bound Ca-activated ATPase. Another light lanthanide, holmium, inhibits the erythrocyte membrane-bound (Ca+Mg)-ATPase (Schatzmann and Tschabold, 1971). In the present investigation the possible changes in lipid-protein interactions of the human erythrocyte membrane bound acetylcholinesterase (AChE) as a result of treatment with lanthanum have been discussed in the light of Arrhenius parameters.

Materials and methods

The chemicals used in this study were commercially available analytical grade material. Acetylthiocholine iodide, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) and

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Abbreviations used: AChE, Acetylcholinesterase; ACD, acid citrate dextrose; LaCl3, lanthanum chloride; TT, transition temperature.

bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Lanthanum chloride ($LaCl_3$) (99·98% purity) was obtained from Indian Rare Earths Ltd., Rare Earth Division, Udyogmandal, India.

Membrane preparation and incubation

Whole blood was collected by venepuncture in acid citrate dextrose (ACD) from male healthy donors between 25 and 40 years of age. The whole blood was centrifuged at 600 *g* for 10 min at 4°C and the plasma and buffy coat were removed by aspiration. Packed erythrocytes were suspended in 0·9% saline. The erythrocyte count was taken by light microscopy and the concentration was adjusted to 1×10^6 erythrocytes/ml. The erythrocyte suspension was incubated with 1 mM $LaCl_3$ solution at 37°C for 1 h. A control incubation was carried out with an equal volume of 0·9% saline instead of 1 mM LaCl3. Incubation was stopped by the addition of ice-cold 0·9% saline and the erythrocytes washed thrice with cold saline to remove $LaCl_3$. Erythrocyte membranes were prepared from control and $LaCl_3$-treated erythrocytes according to the method of Kunimoto and Miura (1985).

Enzyme assay

AChE (EC 3.1.1.7) activity was measured spectrophotometrically in control and $LaCl_3$-treated erythrocyte membranes according to the method of Ellman *et al.* (1961). The final assay medium (3 ml) consisted of 0·29 mM DTNB, 0·5 mM acetylthiocholine iodide and 0·05 ml of the enzyme preparation in phosphate buffer. The rate of change of colour was measured at 412 nm. Assays were performed at temperatures varying from 10°–40°C with 2–5 degree intervals. For kinetic studies substrate concentrations were varied from 0·2–0·8 mM. The protein content of the enzyme preparations was estimated according to Lowry *et al.* (1951) using bovine serum albumin as standard.

Arrhenius plots

To obtain the Arrhenius plots, square root analysis of the data was first carried out and then the logarithms of the corrected specific activity values at each temperature were plotted against the reciprocal of absolute temperature. The value of transition temperature (TT) was read directly from the plot.

The Arrhenius equation was utilised to estimate the activation energies of the enzyme above and below the transition temperature.

The Statistical significance of difference between the mean values of test and control reactions was determined by Student's *t* test.

Results

From the Lineweaver-Burk plot (figure 1) of erythrocyte membrane AChE activity in the presence of 1 mM LaCl3 it appears that the lanthanide increases K_m by 1·9–fold without affecting V_{max}.

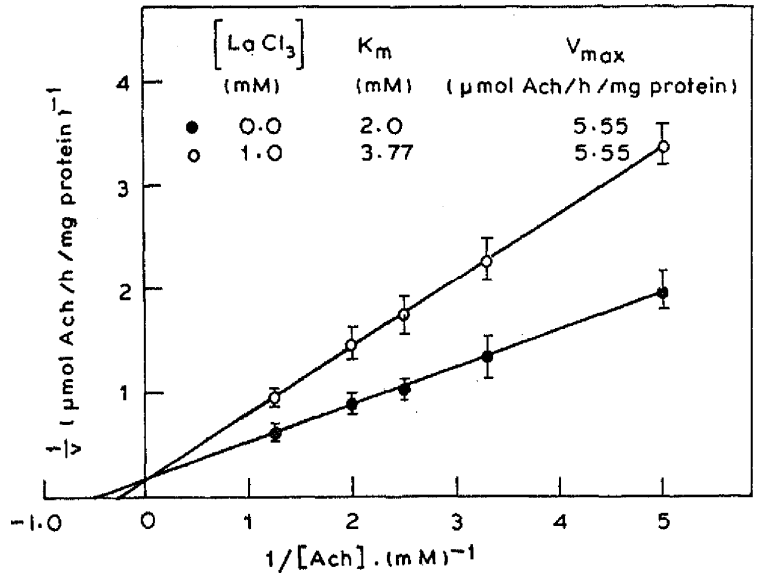

Figure 1. Lineweaver-Burk plots of AChE activity of human erythrocyte membrane in the absence and presence of $LaCl_3$. Each point represents mean of 5 independent experiments; bars are SD.

The activity of erythrocyte membrane-bound AChE was measured at various temperatures (10°–40°C). It was found that the activity increased linearly with rise in temperature in both $LaCl_3$-treated erythrocyte membrane and untreated erythrocyte membrane (figure 2). However, the activity of AChE in $LaCl_3$-treated erythrocyte membrane was consistently lower than the activity in untreated erythrocyte membrane for all the temperatures at which the enzyme activities were measured. When the results were transformed into Arrhenius plots discontinuities in the slope became apparent in the case of both $LaCl_3$-treated membrane and untreated membrane. Figure 3 shows the Arrhenius plot of AChE activity. Examination of the plots shows that the TT of human erythrocyte membrane-bound AChE is 21·3°C and this is lowered to 18·5°C on treatment of membranes with 1 mM $LaCl_3$ *in vitro*. Table 1 gives the apparent activation energies above and below the TT.

Discussion

AChE is thought to be a peripheral extrinsic (Gordon *et al.,* 1980) phospholipoprotein (Beauregard and Roufogalis, 1977) and the role of lipid, especially phospholipid, is vital for enzyme activity (Beauregard and Roufogalis, 1977). AChE forms the link between lecithin and protein in the erythrocyte membrane and contributes to the maintenance of membrane integrity (Kutty *et al.,* 1976). These workers extended the fluid mosaic concept of membranes by proposing lipoprotein-protein interaction and active participation of AChE in the maintenance of membrane stability and function. Further it was proposed that the polar head groups of lecithin form ionic bonds with the esteratic and anionic sites of the enzyme and the free protein or the non-active site of the enzyme forms a protein-protein bond with structural proteins. The phospholipid component of biomembranes is

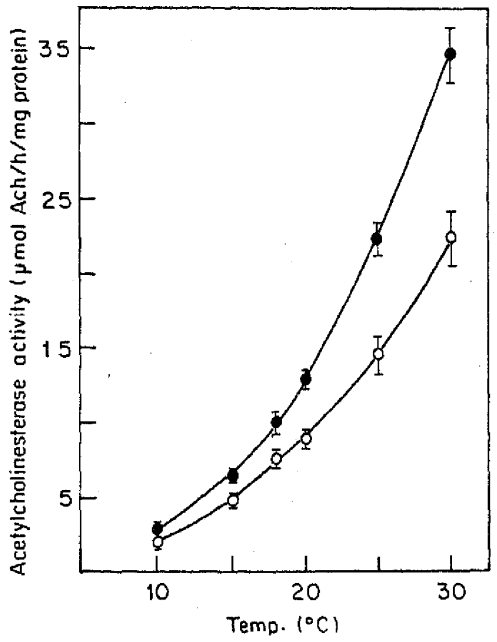

Figure 2. Effect of temperature on human erythrocyte membrane-bound AChE activity. (1), Control; (O), 1 mM $LaCl_3$.

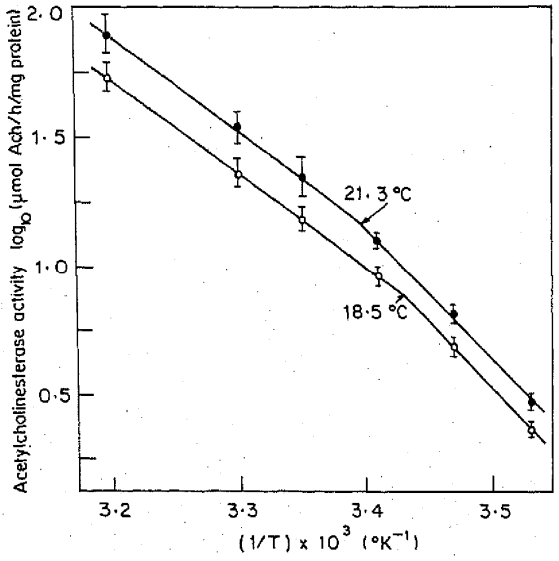

Figure 3. Arrhenius plots of human erythrocyte membrane-bound AChE activity. (1), Control; (O), 1 mM $LaCl_3$.

believed to be the site of action of lanthanides (Venugopal and Luckey, 1978). Hence, it is not unreasonable to assume that variation of lipid composition of different

Table 1. *In vitro* effect of $LaCl_3$ on transition temperature and apparent activation energy of human erythrocyte membrane bound AChE activity.

$LaCl_3$ concentration (mM)	Transition temperature (°C)	Activation energy[a] (Kcal/mol)	
		Below TT	Above TT
0·0	21·3 ± 1·31	24·18 ± 1·20	15·55 ± 0·81
1·0	18·5 ± 1·10[b]	27·80 ± 1·22[b]	16·41 ± 0·92

Each result is the mean ± SD of 5 independent experiments.
[a]Calculated from the slopes of the lines in figure 3.
[b]Mean value significantly different from that of control, $P < 0·01$.

membranes (Glick, 1976) as well as their structural organization (Elferink, 1977) may be responsible for the membrane specific effect of lanthanum on AChE activity.

The results of Lineweaver-Burk analysis suggest that lanthanum competitively inhibits AChE activity in the erythrocyte membrane (figure 1). Decrease of substrate affinity (K_m^{-1}) in the presence of lanthanum without any change in the catalytic property (V_{max}) of erythrocyte membrane-bound AChE suggests that lanthanum binds at or close to the substrate binding site of the enzyme in such a way as to prevent the conformational change that normally occurs during catalysis (Miller and Miller, 1975). AChE, like many other membrane-bound enzymes undergoes a dramatic change in apparent activation energy between 10° and 40°C. Although a number of explanations have been presented for this phenomenon, lipid-protein interactions have been often suggested as playing a major role (Ray *et al.,* 1987). A sudden change in activation energy of membrane-bound enzymes at a particular temperature, the TT, is generally indicated by a discontinuity in the Arrhenius plot. The crystalline-to-liquid-crystalline phase transition of membrane lipids takes place at this temperature (Overath and Trauble, 1973; Grisham and Barnett, 1973). The TT of erythrocyte membrane-bound AChE was found to be 21-3°C (figure 3). The lanthanum-induced decrease in TT indicates that the lipid phase transition of erythrocyte membrane takes place at a lower temperature in the presence of lanthanum. It is known that the thermal transition around 20°C in mammalian membrane-bound enzymes indicates a change in the lipid fluidity of the membrane (Tanaka and Teruya, 1973; Kimelberg and Papahadjopoulos, 1974). A rise in TT is suggested to be due to condensation, *i.e.* decrease in fluidity of the phospholipids mono- or bilayer (Gordon *et al.,* 1980). Thus the lowering of TT (table 1) of human erythrocyte membrane-bound AChE in the presence of lanthanum suggests that lanthanum increases the lipid fluidity of the membrane and thereby produces an inhibition of AChE activity.

Acknowledgements

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