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LIGNOCAINE: INHIBITORY EFFECT ON SYNAPTOSOMAL AND ERYTHROCYTE MEMBRANE-BOUND ACETYLCHOLINESTERASE ACTIVITY

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Abstract—Lignocaine (5–20 mM) reversibly inhibits (*in vitro*) AChE activity of rat brain synaptosome (33–66%) and erythrocyte membrane (10–54%) in a concentration dependent manner. Lineweaver–Burk plots indicate that lignocaine-induced inhibition of AChE activity in synaptosome is competitive, whereas in erythrocyte membrane inhibition of AChE is non-competitive in nature. Arrhenius plots show that transition temperatures of both synaptosomal and erythrocyte membrane-bound AChE are significantly reduced in the presence of lignocaine. These results suggest that lignocaine increases the lipid fluidity of synaptosomal and erythrocyte membrane which may be a cause of inhibition of membrane-bound AChE activity.

Lignocaine [2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide], a lipophilic compound, unlike other local anesthetics, has recently been extensively used as a local anesthetic. Local anesthetics are known to affect various physico-chemical properties of biological membranes, e.g. fluidity [1–4], conductivity [5–7], elasticity [8, 9], etc. It is known that the changes in the membrane-microenvironment alter the activities of various membrane-bound enzymes [10–14] and it seems likely that lignocaine-membrane interactions may lead to the changes in the activities of membrane-bound enzymes. Recently, Haque *et al.* [4] and Fowler *et al.* [15] have shown that lignocaine inhibits the activity of membrane-bound monoamine oxidase. In the present investigation authors have studied the inhibitory effect of lignocaine on rat brain synaptosomal and erythrocyte membrane-bound acetylcholinesterase (AChE, EC 3.1.1.7) activity and its possible mode of action in the light of Arrhenius parameters.

MATERIALS AND METHODS

Adult male albino rats (Charles Foster strain) weighing 125–150 g, maintained on a standard laboratory diet and water *ad libitum* were used in the present study. Acetylcholine chloride and bovine serum albumin were purchased from Sigma Chemical Co. (U.S.A.). Lignocaine hydrochloride was a gift from MIT Laboratories, Bangalore, India. Other experimental reagents were of analytical grade.

Rat brain synaptosome was prepared in accordance with the differential centrifugation method of Gray and Whittaker [16], modified by Bradford *et al.* [17]. Whole blood was taken from rat and erythrocyte membrane was prepared according to the method of Dodge *et al.* [18]. The AChE activity was determined following the colorimetric method of Hestrins [19], modified by Kaplay [20]. The incubations were carried out for 20 min at or above 37° and for 10 min at temperatures below 37° using 0.4 mg synaptosomal protein or 0.8 mg erythrocyte membrane protein as enzyme source. Preliminary experiments were performed to ensure that enzyme activity is linear with respect to incubation time and the enzyme concentration employed. For kinetic studies substrate concentrations were varied from 0.25 to 4.0 mM. To study the *in vitro* effect of lignocaine the enzyme was preincubated with lignocaine at 37° for 20 min prior to addition of substrate. The values of transition temperature (TT) were recorded directly from the Arrhenius plots and the values of AE below and above TT were determined by using the Arrhenius equation.

The protein content of the enzyme preparation was estimated according to Lowry *et al.* [21] using bovine serum albumin as standard. The statistical significance of differences between mean values of experimental and control was determined by two-tailed Student *t*-test.

RESULTS

In the present study it has been noted that lignocaine (5–20 mM) inhibits (*in vitro*) the AChE activity of rat brain synaptosome (33–66%) and erythrocyte membrane (10–54%) in a concentration dependent manner.

Results presented in Table 1 show that removal of lignocaine by 4 hr dialysis reverses its (lignocaine) inhibitory effect on AChE activity of both synaptosome and erythrocyte membrane.

It appears from the Lineweaver–Burk plot (Fig. 1(a)) of synaptosomal AChE activity in the presence of varying concentrations of lignocaine (0–20 mM) that lignocaine increases Km (95–316%) without any change of V_{max}. The Lineweaver–Burk plot of AChE activity in erythrocyte membrane (Fig. 1(b)), on the other hand, shows that lignocaine decreases V_{max}

Table 1. Reversibility of AChE inhibition by lignocaine

Tissue	System	Per cent activity of AChE		
		Before dialysis	After dialysis for 2 hr	After dialysis for 4 hr
Synaptosome	Control	100.00 ± 2.35	97.84 ± 2.41	99.35 ± 2.67
	Control lignocaine (10 mM)	43.05 ± 1.23	88.45 ± 1.76	96.80 ± 2.36
Erythrocyte membrane	Control	100.00 ± 3.68	102.94 ± 3.53	98·97 ± 4.12
	Control lignocaine (20 mM)	44.12 ± 3.38	80.45 ± 1.74	101.03 ± 3.82

Each value represents the mean ± SEM of three separate determinations. Enzyme was preincubated with or without lignocaine for 20 min at 37° and dialysed against 0.5 mM Tris-HCl buffer, pH 7.4 for 2 and 4 hr at 4°.

(12.6–46.2%) without any appreciable change in Km values.

Figure 2 is the Arrhenius plots of AChE of synaptosome and erythrocyte membrane in the presence of varying concentrations of lignocaine (0–20 mM). It is noted from the Arrhenius plots (Fig. 2(a)) that a transition temperature (TT) of synaptosomal AChE is 31.0° and this value of TT changes to 20.7° and 19.0° in the presence of 10 mM and 20 mM lignocaine respectively. The apparent activation energies (AE) of synaptosomal AChE below and above TT are significantly increased (31% and 30% respectively) in the presence of higher concentration of lignocaine (20 mM). It is evident from Fig. 2(b) that TT of erythrocyte membrane is reduced from 28.7° to 22.4° and 19.9° in the presence of 10 mM and 20 mM lignocaine respectively. AE of erythrocyte membrane-bound AChE below TT is significantly increased (38–83%), while at temperatures above TT, AE is not significantly affected in the presence of lignocaine (10 and 20 mM).

DISCUSSION

Results of dialysis experiment (Table 1), Ackermann–Potter plots [22] and dilution experiment (not shown) suggest that lignocaine-induced inhibition of AChE activity of both synaptosome and erythrocyte membrane preparations is reversible in nature. No appreciable variation in the degrees of inhibition of AChE activity of synaptosome (44–57%) and erythrocyte membrane (26–37%) on increasing the preincubation time (0–30 min) of the enzyme with lignocaine (10 mM) (not shown) further indicates that lignocaine is a reversible inhibitor AChE [23]. It has been noted (Table 1) that AChE of erythrocyte membrane is less sensitive to lignocaine than synaptosomal AChE. AChE is thought to be a peripheral-extrinsic [14] phospholipoprotein [12] and the role of lipid, especially phospholipid is vital for this enzyme activity [12]. The lipid bilayer is believed to be the site action of lignocaine and other local anesthetics on biomembranes [1–4].

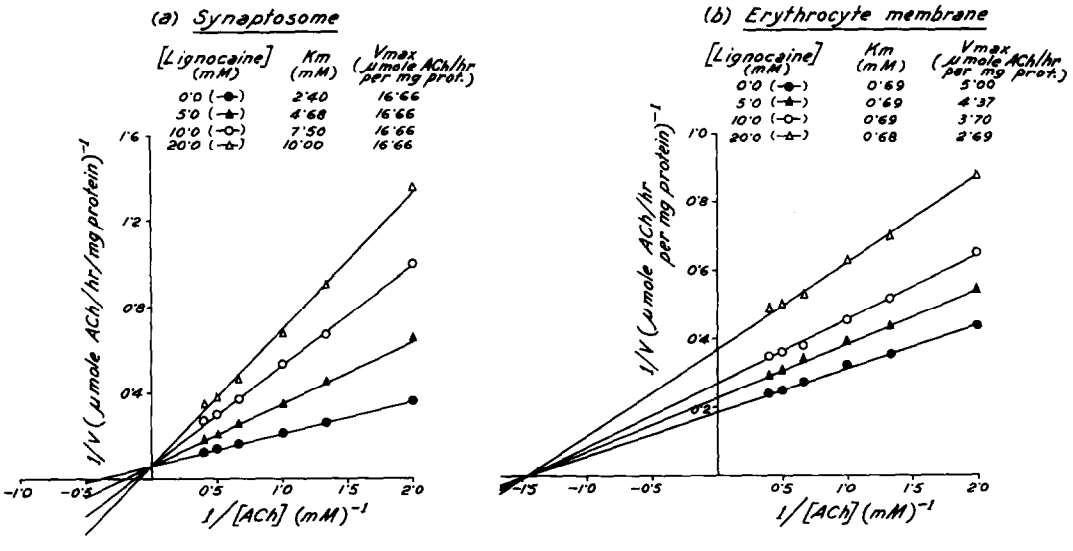

Fig. 1. Lineweaver–Burk plots of AChE activity of rat (a) brain synaptosome and (b) erythrocyte membrane in the absence and presence of lignocaine (5–20 mM). Other experimental details are described in the text. Each point represents the mean of four separate determinations.

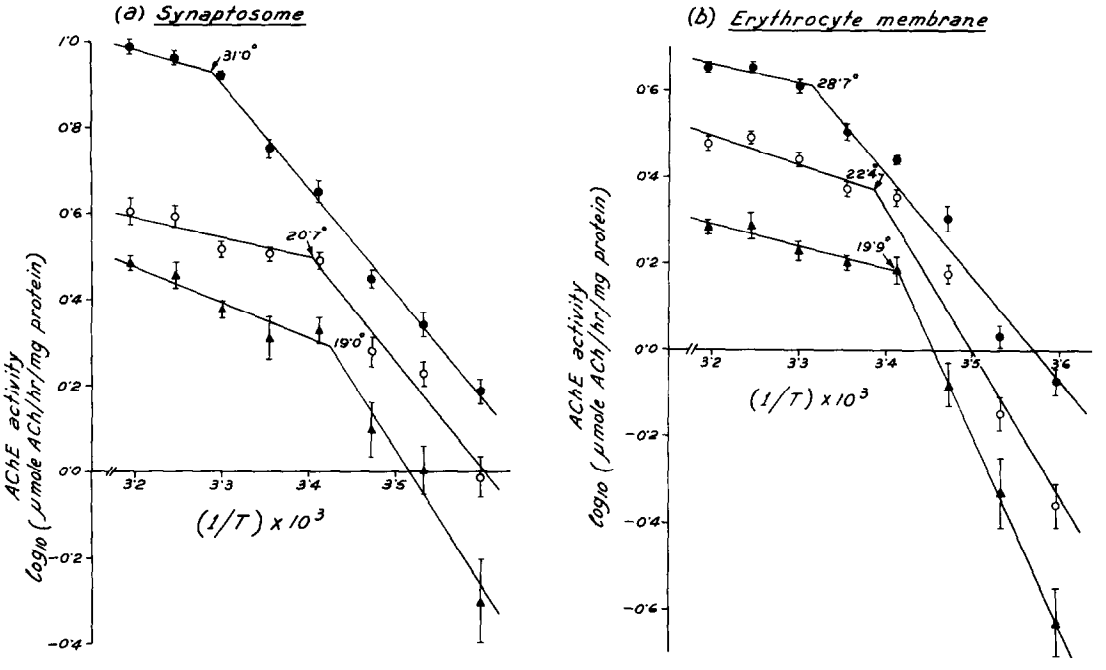

Fig. 2. Arrhenius plots of AChE of rat (a) brain synaptosome and (b) erythrocyte membrane in the absence (●) and presence of 10 mM (○) and 20 mM (▲) lignocaine. Each point represents the mean ± SEM of four separate determinations.

Hence it is not unreasonable to assume that variation of lipid composition of different membranes [24] as well as their structural organization [25] may be responsible for the membrane specific effect of lignocaine on AChE activity.

The results of Lineweaver–Burk plots suggest that lignocaine at different concentrations (5–20 mM) inhibits (i) competitively synaptosomal AChE activity (Fig. 1(a)) and (ii) non-competitively AChE activity of erythrocyte membrane (Fig. 1(b)). Decrease of substrate affinity (Km^{-1}) in the presence of lignocaine (5–20 mM) without any change in the catalytic property (V_{max}) of synaptosomal AChE suggests that lignocaine 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 [26].

Lignocaine (5–20 mM)-induced noncompetitive inhibition of erythrocyte membrane-bound AChE activity indicates that lignocaine produces a conformational change of the enzyme [27].

A sudden change in activation energy of membrane-bound enzymes at a particular temperature, known as transition temperature (TT), is generally exhibited by a discontinuity in Arrhenius plot. The crystalline-to-liquid-crystalline phase transition of membrane lipids takes place at this temperature (TT) [28, 29]. The TT of synaptosomal AChE is found to be 31° (Fig. 2(a)). Lignocaine-induced decrease in TT indicates that lipid phase transition of synaptosomal membrane takes place at lower temperature in the presence of lignocaine. The TT of erythrocyte membrane is around 28.7° and this value similarly decreases in the presence of ligno-

Table 2. *In vitro* effect of lignocaine on transition temperature (TT) and apparent activation energy (AE)* of synaptosomal and erythrocyte membrane-bound AChE

Lignocaine concentration (mM)	Synaptosome			Erythrocyte membrane		
		AE (kCal/mole)			AE (kCal/mole)	
	TT°	Below TT	Above TT	TT°	Below TT	Above TT
0.0	30.95 ± 0.22	11.17 ± 0.29	2.87 ± 0.10	28.66 ± 0.26	10.85 ± 0.26	2.05 ± 0.20
10.0	20.68[a] ± 0.41	11.40 ± 0.63	2.10 ± 0.33	22.42[a] ± 0.51	14.96[b] ± 0.78	2.96 ± 0.32
20.0	18.97[a] ± 0.36	14.68[b] ± 0.53	3.74 ± 0.27	19.91[a] ± 0.43	19.88[a] ± 0.51	2.28 ± 0.12

Each value represents mean ± SEM of four separate determinations.
* AE is calculated from the slope of lines.
Significantly different from control: [a]$P < 0.001$, [b]$P < 0.005$.

caine. It is known that the thermal transition around 20° in mammalian membrane-bound enzymes indicates a change in the lipid fluidity of membrane [30, 31] and rise in TT is suggested to be due to condensation, i.e. decrease in fluidity of phospholipid mono- or bilayer [14]. Thus the lowering of TT (Table 2) of synaptosomal and erythrocyte membrane-bound AChE in the presence of lignocaine suggests that lignocaine increases the lipid fluidity of the membrane [1–4] and thereby produces an inhibition of AChE activity.

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REFERENCES

1. M. K. Jain, N. Yen-Min Wu and L. V. Wrong, *Nature, Lond.* **255**, 494 (1975).
2. A. G. Lee, *Biochim. biophys. Acta* **448**, 34 (1976).
3. A. G. Lee, *Nature, Lond.* **262**, 545 (1976).
4. S. J. Haque, B. Roy Choudhury and M. K. Poddar, *Indian J. Biochem. biophys.* (in press) (1983).
5. M. P. Blaustein and D. E. Goldman, *J. gen. Physiol.* **49**, 1043 (1966).
6. M. P. Blaustein and D. E. Goldman, *Science* **153**, 429 (1966).
7. J. Bojta and F. Antoni, *Biochem. Pharmac.* **28**, 2441 (1979).
8. P. Seeman, W. O. Kwant and T. Sauks, *Biochim biophys. Acta* **183**, 499 (1969).
9. S. Roth and P. Seeman, *Nature, New Biol.* **231**, 284 (1971).
10. M. D. Houslay and K. F. Tipton, *Biochem. J.* **135**, 173 (1973).
11. K. Sihotang, *Eur. J. Biochem.* **63**, 519 (1976).
12. G. Beauregard and B. D. Roufogalis, *Biochem. biophys. res. Commun.* **77**, 211 (1977).
13. M. Nemat-Gorgani and E. Meisami, *J. Neurochem.* **32**, 1027 (1979).
14. L. M. Gordon, R. D. Sauerheber, J. A. Esgate, I. Dipple, R. J. Marchmont and M. D. Honslay, *J. biol. Chem.* **255**, 4519 (1980).
15. C. J. Fowler, B. A. Callingham, T. J. Mantle and K. F. Tipton, *Biochem. Pharmac.* **29**, 1177 (1980).
16. E. G. Gray and V. P. Whittaker, *J. Anat.* **96**, 79 (1962).
17. H. F. Bradford, G. W. Bennett and A. J. Thomas, *J. Neurochem.* **21**, 495 (1973).
18. J. T. Dodge, C. Mitchel and O. J. Hanahan, *Archs. biochem. Biophys.* **100**, 119 (1963).
19. S. Hestrins, *J. biol. Chem.* **180**, 249 (1949).
20. S. S. Kaplay, *Indian J. biochem. Biophys.* **14**, 389 (1977).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. W. W. Ackermann and V. R. Potter, *Proc. Soc. exp. biol. Med.* **72**, 1 (1949).
23. J. R. Lagnado, M. Okamoto and M. B. H. Youdim, *FEBS Lett.* **17**, 177 (1971).
24. M. C. Glick, in *Mammalian Cell Membranes* (Eds. G. A. Jamieson and D. M. Robinson), Vol. 1, pp. 61–63. Butterworths, London (1976).
25. J. G. R. Elferink, *Biochem. Pharmac.* **26**, 2411 (1977).
26. J. C. Miller and K. W. Miller in *Physiological and Pharmacological Biochemistry* (Ed. H. K. F. Blaschko), Vol. 12, p. 66. Butterworths, London (1975).
27. W. D. McElory, *Quart. Rev. Biol.* **22**, 25 (1947).
28. P. Overath and H. Trauble, *Biochemistry* **12**, 2625 (1973).
29. C. M. Grisham and R. E. Barnett, *Biochemistry* **12**, 2635 (1973).
30. R. Tanaka and A. Teruya, *Biochem. biophys. Acta* **323**, 584 (1973).
31. H. K. Kimelberg and D. Papahadjopoulos, *J. biol. Chem.* **249**, 1071 (1974).