INHIBITORY EFFECT OF *AGROBACTERIUM TUMEFACIENS* LIPOPOLYSACCHARIDES ON SYNAPTOSOMAL MEMBRANE-BOUND ACETYLCHOLINESTERASE ACTIVITY

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ABSTRACT

Agrobacterium tumefaciens lipopolysaccharides inhibit the acetylcholinesterase activity *in vitro* in mice brain synaptosomal membrane. Arrhenius plot shows that the transition temperatures of membrane-bound acetylcholinesterase is reduced significantly in the presence of lipopolysaccharides, although activation energies above and below the transition temperature remain unaltered. The results indicate that an alteration in the fluidity of the membrane may be responsible for the membrane-specific effect of lipopolysaccharides on acetylcholinesterase activity.

INTRODUCTION

LIPOPOLYSACCHARIDES (LPS) of the outer membrane of gram-negative bacteria represent the bacterial endotoxins and are of considerable interest both from the biochemical and clinical point of view[1-4]. LPS administration changes the fluidity of hepatic plasma membrane[5] and there is a close relationship between membrane enzyme activities and membrane fluidity[6]. The acetylcholinesterase (AChE, 3.1.1.7), an important brain enzyme, is involved in impulse transmission at cholinergic synapses[7]. The activity of this peripheral-extrinsic enzyme is influenced significantly by membrane phospholipids[8]. LPS administration decreases the membrane lipid fluidity and thus plays a significant role in the development of the pathophysiology of toxic action at the cellular level[9]. There are also reports that LPS initiates its biological responses through alteration of membrane lipids[10].

Agrobacterium tumefaciens, a gram-negative bacteria, causes crown gall tumours in most dicotyledonous plants and the whole cells of the bacteria have an acute lethal effect on mice[11]. The LPS of *A. tumefaciens* have been isolated and characterized recently from this laboratory[12] and it has been found that LPS of *A. tumefaciens* are not lethal but have potential toxic effects on mice, which are similar to those of other endotoxic LPS[13]. It was also established that *Agrobacterium* LPS can alter the antioxidant defence system of mice liver[14] and erythrocyte[15]. In this communication our studies on the possible changes in lipid-protein interactions of the enzyme AChE in the isolated mice brain synaptosomal membrane under treatment of *A. tumefaciens* LPS are reported.

MATERIALS AND METHODS

Organism

A. tumefaciens TIP Kerr 14, agrocin sensitive, virulent variety strain was kindly provided by Prof. J. Schell, Director, Laboratorium Voor Genetika, Rijsks Universiteit, Ghent, Belgium. The LPS was extracted and purified according to the method described earlier[13].

Membrane preparation and enzyme assay

Male swiss albino mice weighing 20 ± 3 g maintained on a basal diet[16] and water *ad libitum* were

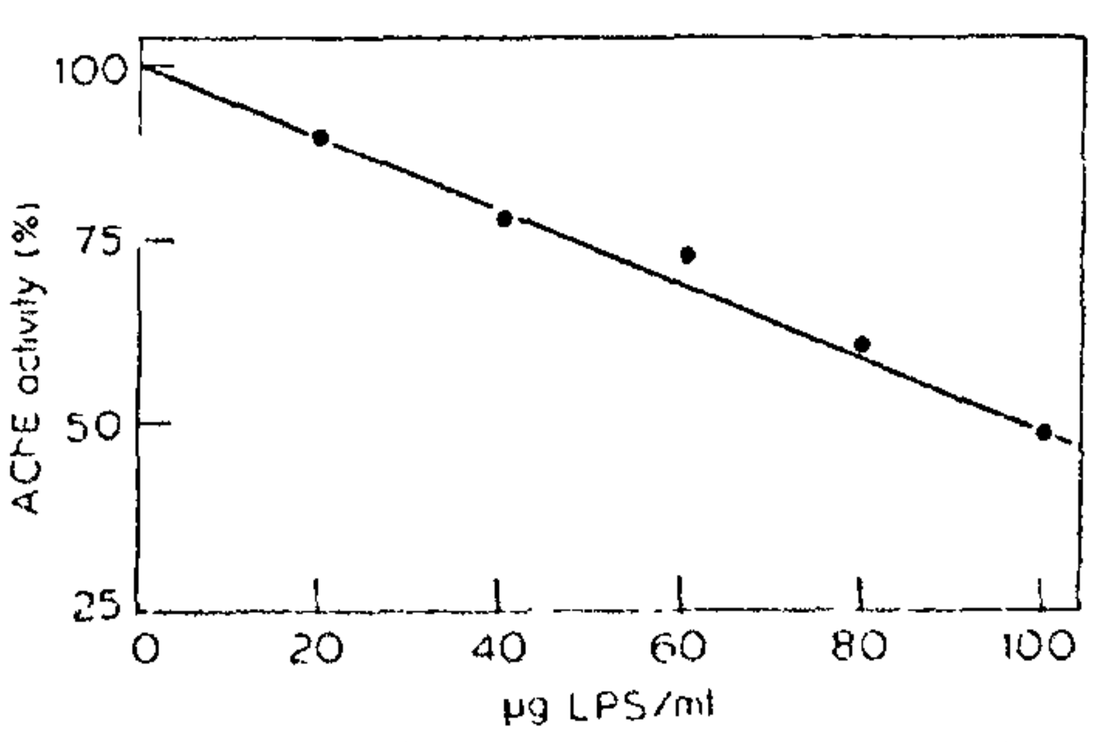

Figure 1. Effect of *A. tumefaciens* LPS (●—●) on the brain synaptosomal acetylcholinesterase activity.

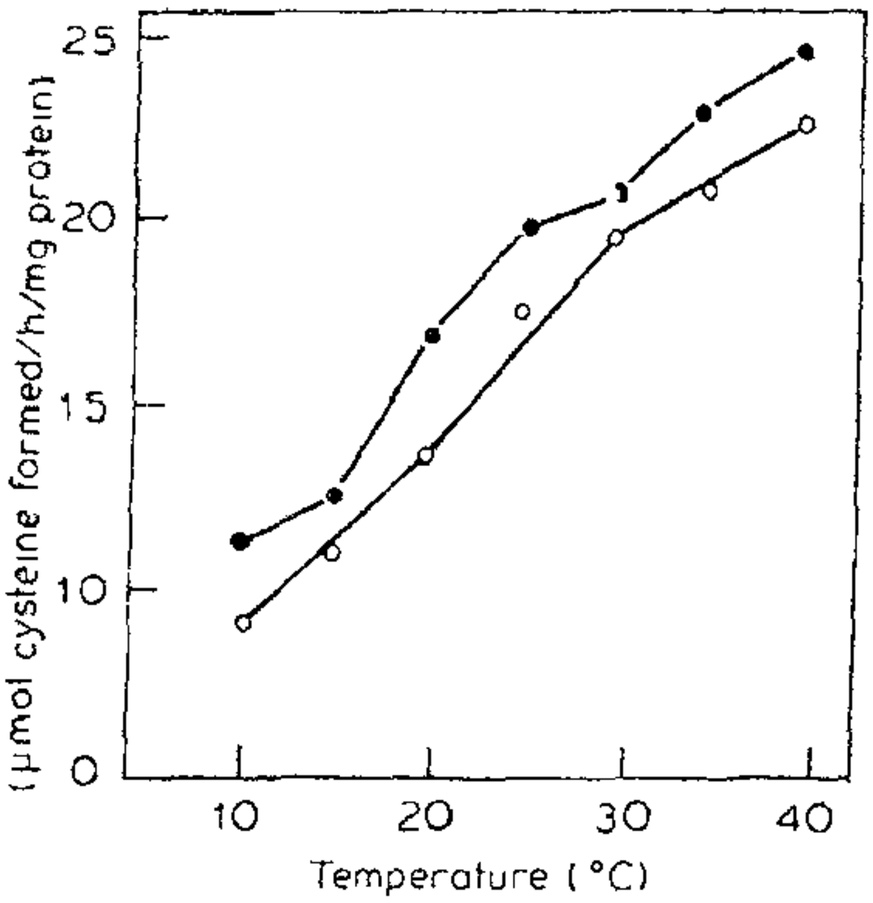

Figure 2. Effect of temperature on the brain synaptosomal acetylcholinesterase activity in the absence (●—●) and presence (○—○) of *A tumefaciens* LPS.

used. Mice brain synaptosomal membrane was prepared according to the differential method of Gray and Whittaker[17]. AChE activity was measured spectrophotometrically according to Ellman *et al*[18]. To study the *in vitro* effect of LPS, the membrane preparation was preincubated with 100 μg/ml of LPS at 37°C for 15 min prior to the addition of substrate. Assays were performed at various temperatures between 10 and 40°C at 3–5 degree intervals. The protein content was estimated according to Lowry *et al*[19] using bovine serum albumin as the standard.

Arrhenius plots and activation energy

To construct the Arrhenius plots, logarithms of the corrected specific activity values at each temperature were plotted against the reciprocal of absolute temperature. The value of transition temperature (T_c) was read directly from the plot and the Arrhenius equation was utilized to estimate activation energies of the enzyme above and below the T_c.

The statistical significance of differences between mean values of experimental and control was determined by Student's t test.

RESULTS

Figure 1 shows that LPS of *A. tumefaciens* inhibits *in vitro* the AChE activity in a concentration-dependent manner and 50% inhibition of the enzyme activity was achieved with 100 μg/ml of LPS.

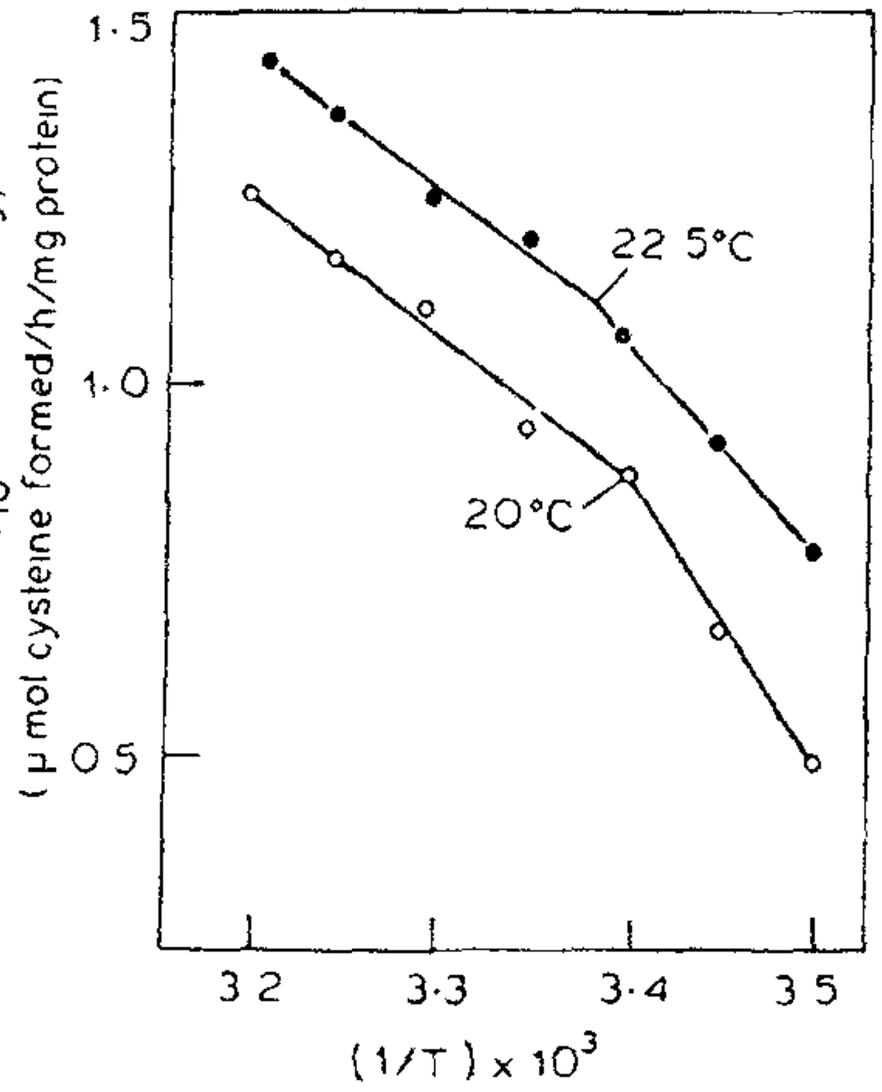

Figure 3. Arrhenius plots of brain synaptosomal acetylcholinesterase activity in the absence (●—●) and presence (○—○) of *A. tumefaciens* LPS.

The data from a typical experiment on the effects of temperature on synaptosomal membrane-bound AChE activity in the absence of and presence of LPS are shown in figure 2. The enzyme activity of both control and treated groups was measured at various temperatures between 10 and 40°C. The activity increased with rise in temperature with a distinct inhibition of the activity under the LPS treatment.

Arrhenius plots of the data depict the discontinuities in the slope irrespective of the absence of or presence of LPS (figure 3). An examination of the plot shows that the normal T_c of synaptosomal AChE is 22.5°C which is lowered to 20°C on being treated with *A. tumefaciens* LPS *in vitro*.

The apparent activation energy above and below the T_c of membrane-bound AChE activity is listed in table 1.

DISCUSSION

The lipid bilayer is believed to be the site of action of LPS and other lipophilic compounds on biomembranes[20]. AChE is a peripheral extrinsic phospholipoprotein and phospholipids are vital for its activity[21]. The enzyme is known[22] to undergo a dramatic change in activation energy between 10 and 40°C. Although many explanations have been offered for this phenomenon, lipid protein interaction has often been suggested as of major conse-

Table 1 *In vitro effect of A. tumefaciens LPS on transition temperature and apparent activation energy of synaptosomal membrane-bound acetylcholinesterase activity*

	Control	LPS of *A. tumefaciens* (100 μg/ml)
Transition temperature	22.5	20.0
Activation energy (kcal/mol)		
Below T_c	12.94 ± 0.52	12.13 ± 0.50
Above T_c	2.5 ± 0.21	2.54 ± 0.18

Activation energy was calculated from the slope of Arrhenius plots; Each result represents a mean value ± SD of 5 separate determinations.

quence. The present data suggest that the lipid phase change can be correlated to the Arrhenius plot breaks. The work of Haque *et al*[22] and Kimelberg[23] on membrane system containing AChE and (Na^+–K^+) ATPase lends support to the above assumption. The sharp discontinuity in the Arrhenius plot is found at the T_c which is the crystalline to liquid crystalline phase T_c of membrane lipids. The T_c of synaptosomal membrane-bound AChE is found to be 22.5°C and the LPS induced lowering of T_c in membrane indicates that membrane lipid phase transition takes place at low temperature in the presence of *A. tumefaciens* LPS. Hence it is reasonable to assume that variation of lipid composition of membrane[24] as well as their structural organization[25] may be responsible for the membrane-specific effect of LPS on AChE activity.

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