Allosteric Properties of Acetylcholinesterase

SEVERAL reports[1-3] have suggested that cholinergic ligands bind to acetylcholinesterase at sites distinct from the active centre. Changeux *et al.*[4] demonstrated that acetylcholine binds to non-catalytic sites as well as to the active centre of the enzyme. Atropine and l-hyoscyamine have also been shown to interact with the enzyme at a region distinct from the active site[5,6]. We have therefore investigated whether acetylcholine and atropine are bound to the same site and whether this site is related to the acetylcholine receptor.

To do this we made a detailed investigation of the kinetic properties of acetylcholinesterase, using acetylthiocholine as substrate and atropine as the inhibitor. We describe here the highlights of this study. Our results suggest that inhibition by excess substrate is due to an allosteric mechanism and that atropine competes with substrate at the allosteric site, preventing substrate inhibition.

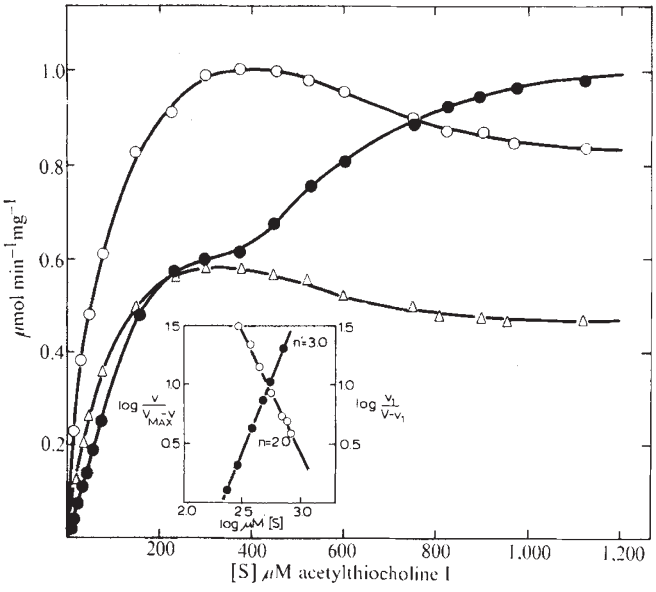

Fig. 1 Plot of initial reaction velocity of acetylthiocholine hydrolysis with respect to acetylthiocholine concentration in the presence of atropine · SO_4 or eserine · SO_4. Acetylcholinesterase was assayed by the method of Ellman *et al.*[10], at 25° C. The reaction mixture (1 ml.) also contained 0.1 M phosphate buffer, *p*H 8.0, 0.01 M dithio-*bis*-nitrobenzoic acid containing bicarbonate, and atropine or eserine. ●, 5×10^{-3} M atropine · SO_4; △, 5×10^{-7} M eserine · SO_4; ○, no inhibitor. The enzyme was isolated from head ganglia of squid (*Loligo opalescens*) and purified by the method of Kremzner and Wilson[11]. It had a specific activity of 220 μmol of acetylthiocholine hydrolysed/mg/h. The inset shows a Hill plot for the saturation of the enzyme with acetylthiocholine in the presence of 5×10^{-3} M atropine · SO_4 (●); and inhibition by excess substrate (○). The ordinate on the left is for the substrate inhibition line; the ordinate on the right is for the substrate saturation line in the presence of atropine. The Hill equation was applied to that portion of the rate versus [S] curve which was sigmoid. The Hill equation when applied to substrate inhibition can be represented in the following form[7]:

$$\log \frac{v}{V_{max} - v} = \log K - n \log S$$

where S is substrate concentration, K is a constant, and n indicates the power of the substrate concentration. V_{max} is the maximum activity obtained in these conditions and v the velocity of the reaction. When applied to the kinetics of inhibition by atropine the Hill equation takes the following form:

$$\log \frac{v_1}{V - v_1} = n' \log S - \log K'$$

where $V = v_1$ maximum, K' is a constant and n' is the order of the reaction with respect to substrate in the presence of inhibitor.

Acetylcholinesterase when assayed at *p*H 8.0 exhibits Michaelis-Menten kinetics with respect to acetylthiocholine. At substrate concentrations above the saturation level for the enzyme, inhibition due to excess substrate was apparent and the curve of rate of hydrolysis of substrate versus substrate concentration became sigmoid (Fig. 1).

The inhibition by atropine follows competitive kinetics at low concentration of substrate ($K_i = 4 \times 10^{-3}$ M). In the presence of atropine, the dependence of velocity on substrate concentration is hyperbolic and seems to level off and reach a saturating level. A further increase in the concentration of acetylthiocholine, however, produces a further increase in the velocity of the reaction and the rate versus [S] plots become sigmoid (Fig. 1). Increasing the concentration of acetylthiocholine still further at a constant level of atropine relieves the inhibition by excess substrate but the rate never exceeds V_{max}.

The rate versus [S] curves in the presence of l-hyoscyamine and gallamine (not shown) are also sigmoidal at high substrate concentrations, whereas with eserine (Fig. 1) or neostigmine the curves exhibit no evidence of this type of sigmoidicity. With eserine inhibition was non-competitive with $K_i = 1.2 \times 10^{-6}$ M.

The rate versus [S] curve in the presence of atropine becomes sigmoidal at that concentration of substrate which saturates the enzyme in the absence of inhibitor. If Monod's model[7] is applied, saturating concentrations of substrate convert the enzyme from the R to the T form, which has a reduced catalytic activity and also binds atropine. Atropine competes with acetylthiocholine at the allosteric site and therefore relieves inhibition by excess substrate. Atropine and acetylthiocholine (and probably ACh) therefore bind at the same non-catalytic sites.

When the kinetics of substrate inhibition were fitted by the Hill equation[8], a straight line with slope = 2.0 was obtained (inset, Fig. 1). This implies that the number of interacting sites is greater than one and that the phenomenon of inhibition by excess substrate may be due to an allosteric mechanism. The Hill equation, when slightly modified (Fig. 1), can be applied to the kinetics of inhibition by atropine. The order of the reaction with respect to substrate (n') in the presence of 5.0 mM atropine sulphate was 3.0.

Cooperativity is generally explained by allosteric effects involving multiple subunits. Acetylcholinesterase is known to have a subunit structure[9]. It would be interesting to see if the catalytic and "regulatory" subunits in this enzyme are distinct molecular entities.

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