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Autoinhibition of Acetylcholine Binding to *Torpedo* Electroplax; A Possible Molecular Mechanism for Desensitization

(equilibrium dialysis/acetylcholinesterase/receptors)

M. E. ELDEFRAWI AND R. D. O'BRIEN

Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14850

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ABSTRACT **Binding of [^{3}H]acetylcholine (measured by equilibrium dialysis) to a particulate preparation of *Torpedo* electroplax exhibits autoinhibition at concentrations higher than 1 μM. It is suggested that autoinhibition results from acetylcholine binding to regulatory sites on its receptor macromolecules. This binding causes the change to a new and inactive conformation and rejection of acetylcholine bound to the larger number of active sites. The relationship to the physiological phenomenon of desensitization is discussed.**

We previously showed that a particulate preparation from *Torpedo* electroplax bound four cholinergic ligands reversibly and with multiple high affinities (1, 2). Recently, we showed that the same preparation, after treatment with 0.1 mM Tetram (*O,O*-diethyl *S*-diethylaminoethyl phosphorothiolate) inhibited all the acetylcholinesterase (C 3.1.1.7) present, and bound acetylcholine (ACh) reversibly with two high affinities. This binding was blocked at both sites by nicotinic cholinergic drugs (3, 4). Binding macromolecules were phospholipoproteins and the evidence suggested that they were acetylcholine receptors (AChR), and not acetylcholinesterase. Very recently (5), a fraction was isolated from the same electroplax, after solubilization with the detergents Triton X-100 and sodium dodecyl sulfate. Irreversible binding of the snake venom, α-bungarotoxin, by this fraction was found to be lower when nicotinic ligands were present. It was suggested that the binding macromolecules were AChR, and the concentration of binding sites turned out to be equal to those we found for muscarone, nicotine, and ACh in this electroplax (1, 2, 4). In the present study, we report on the autoinhibition of ACh binding to AChR of *Torpedo* electroplax by high concentrations of ACh.

METHODS

Binding was measured by equilibrium dialysis (at 4°C for 16 hr) in 100 volumes of a modified Krebs–Ringer solution (6), pH 7.4 and ionic strength 0.2, containing various concentrations of [^{3}H]ACh (specific activity 50 Ci/mol; from New England Nuclear). After dialysis, excess radioactivity detected in equal samples of dialysis-bag contents over bath contents represented the amount of bound ACh. Three samples were counted for each ACh concentration used, and every experiment was run in triplicate. Details of the procedure and preparation of the lyophilized pellet (at 12,000 × *g*) of *Torpedo* electroplax (9.3 mg protein/*g* of electroplax, and used at 0.5 g of electroplax/ml) were described (1, 2).

RESULTS

Binding of ACh reached saturation at a concentration just below 1 μM, and when the concentration of ACh was increased, binding was reduced (Fig. 1). This autoinhibitory effect on binding increased gradually at concentrations from 1 to 4 μM, and was followed by a rapid increase at higher concentrations (Fig. 2). Not only was this effect observed when dialysis was at 4°C, but also at 37°C. This inhibitory effect was reversible, for after repeated dialysis in 100 volumes of Kreb's–Ringer solution containing 0.1 μM ACh, high binding was restored.

To investigate whether this autoinhibition was an artifact of the technique, we first checked binding for extended periods (up to 40 hr), and found that equilibrium was completed at all ACh concentrations within 16 hr. Hydrolysis of ACh was not detected at any concentration used throughout the dialysis, hence, there was no recovery of active acetylcholinesterase. Furthermore, a preparation of *Torpedo* AChR, solubilized in Triton X-100 or Lubrol XW, also exhibited this autoinhibition*, thus excluding the possibility that reduction in binding was caused by vesicular exclusion or by the presence of permeability barriers at high ACh concentrations. The same phenomenon was also present when the effect of several chemical modifiers [1,4-dithiothreitol, *p*-chloromercuribenzoate, and *p*-(trimethylammonium)-benzenediazonium fluoroborate] on the binding of ACh was studied†.

DISCUSSION

The autoinhibition of ACh binding to AChR is analogous to excess substrate inhibition of acetylcholinesterase. This enzyme is totally inhibited in the electroplax preparation, and there are differences between the two phenomena. The inhibition of acetylcholinesterase is revealed in the catalytic rather than the binding step and is due to the blockade of deacetylation by high ACh concentrations (7). Also, inhibition of the enzyme occurs at concentrations higher than 1 mM rather than at micromolar concentrations of ACh. It therefore seems unlikely that the enzyme plays any role in the events described herein.

Pharmacologically, the autoinhibitory effect of a drug on a receptor is believed to result from the presence of two receptor

Abbreviations: ACh, acetylcholine; AChR, acetylcholine receptors.

* Eldefrawi, M. E., A. T. Eldefrawi, and R. D. O'Brien, unpublished data.

† Edlefrawi, M. E., and A. T. Eldefrawi, unpublished data.

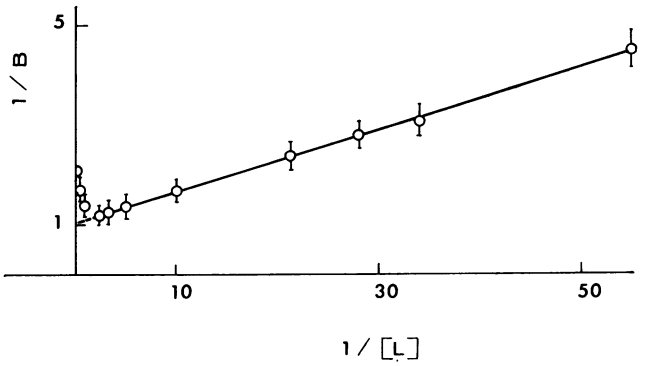

FIG. 1. Lineweaver-Burk plot of ACh binding by *Torpedo* electroplax (*1/B*, in nmol/g electroplax^{-1}; *1/L*, concentration of ACh in μM^{-1}). The vertical bars represent the standard deviation of nine test points at each concentration.

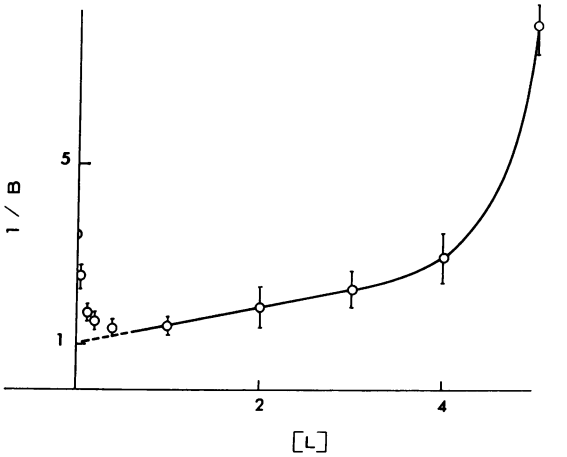

FIG. 2. Plot of *1/B* (in nmol/g electroplax)$^{-1}$ against *L* (concentration in μM). Vertical bars as in Fig. 1. Points on the line represent autoinhibition of ACh binding.

conformations, one having high-affinity sites, whose occupation produces the observed physiological response, and a second with lower-affinity sites, whose occupation leads to elimination (or reduction) of the physiological response (8). ACh is known to exhibit this autoinhibitory effect physiologically. The phenomenon is better known as desensitization of the cholinergic receptor, whereby high concentrations of ACh cause blockade of the response at neuromuscular junctions (9, 10), *Torpedo* electroplax (11), or D- and H-neurones in molluscan ganglia (12). The onset of such desensitization occurs faster with higher ACh concentrations (10). Repetitive nerve stimulation, at frequencies well within the physiological range experienced under tension in the rabbit and cat (13), also leads to desensitization (14).

It is generally accepted that desensitization is the result of inactivation of AChR (10). Thus, we are tempted to speculate that the autoinhibition phenomenon observed here with ACh binding may be directly related to the physiological desensitization phenomenon. To explain the reduction in binding of ACh, we must make the assumption that there exist on any one ACh-binding macromolecule one or more low-affinity binding sites (regulatory sites), whose occupation leads to the elimination (or reduction) of binding to a greater number of high-affinity sites (active sites). We must also assume that each receptor molecule has fewer regulatory sites than active sites, and that if one increases the concentration of ACh above that necessary to saturate the binding sites, a conformational change in the receptor molecule results. In the new conformation, the affinity of the active sites for ACh is decreased greatly, causing elimination of bound ACh molecules from these sites.

It should be pointed out that if one assumes that the only molecule that can bind to the receptor is ACh, it will be hard to explain the energetics by which the receptor, once it is saturated with ACh, can be driven to an even more stable state by adding ACh and reducing the bound ACh. The explanation may involve a promoted binding of other ligands present in the medium (such as Ca^{++} or other cations), which can only occur in the configuration produced by very high ACh concentrations, and which stabilize this configuration.

Of the several models proposed for desensitization of the cholinergic receptor (15), the cyclic model of Katz and Thesleff (10) is widely accepted. This model suggests that the receptor exists in an active conformation at low ACh concentrations and an inactive conformation at high ACh concentrations. It also assumes that the affinity of the active sites to ACh is reduced when in the inactive conformation. However, it does not propose a mechanism to explain how the conformational change and reduction in affinity of those sites is brought about. We suggest that binding of ACh to the proposed regulatory sites could bring about such changes. Accordingly, the autoinhibitory effect of ACh binding brought about by binding to the proposed regulatory sites, and the subsequent conformational change, could represent the molecular regulatory mechanism underlying desensitization. It will be feasible to test this hypothesis once the pure AChR is available and techniques of fast kinetics are used.

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