ACETYLCHOLINE RECEPTOR PROTEIN AND NERVE ACTIVITY. I. SPECIFIC REACTION OF LOCAL ANESTHETICS WITH THE PROTEIN*

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Local anesthetics are known to block axonal conduction and synaptic transmission. This action has been attributed to a "tightening" of the membrane which interfers with ion movements. On the basis of the similarity of the structure of these anesthetics with acetylcholine, Nachmansohn has maintained for many years that the compounds block activity by competing with acetylcholine for the acetylcholine receptor protein. The competitive nature of the action of procaine with acetylcholine has been demonstrated in experiments on the intact electroplax (Schoffeniels and Nachmansohn, 1957). Tetracaine and dibucaine are, on this preparation and on axons, more than 10 to 20 fold more potent than procaine (2).

The recent isolation and identification of the acetylcholine receptor protein (Ehrenpreis, 1959, 1960a, b) offered the possibility of studying directly the interaction of procaine and related local anesthetics with the protein in solution. A strong and quite specific interaction has been found which was strikingly parallel to the graded action of the

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compounds on conducting membranes of both nerve and electroplax as well as on synapses of electroplax.

Experimental. Two types of receptor protein preparations were used: one was obtained by precipitating the protein with d-tubocuraine (curare) directly from the extract of electric organ, the other by precipitating it from the 30% ammonium sulfate fraction (Ehrenpreis, 1960a, b). These two protein solutions are almost identical as to binding of curare (Ehrenpreis, 1960a, b). Interaction of procaine, tetracaine (Winthrop-Stearns) and dibucaine (Ciba Pharmaceutical Products) was determined both by equilibrium dialysis (ionic strength 0.1) and by precipitability. Binding of the first two compounds was determined in phosphate or at pH 7.5 and tris at pH 9. Dibucaine, which is extremely insoluble in phosphate, was used only in tris buffer. The strength of interaction was compared with that of curare. Concentrations of the various compounds were determined by U.V. absorption.

Results and discussion. Tetracaine and dibucaine precipitate the receptor protein at fairly low concentration (Table I); procaine failed to precipitate even at extremely high concentration. This finding immediately suggests that the binding of procaine is significantly weaker than that of the other two local anesthetics used, in agreement with the results on intact tissues. Chondroitin sulfate, nucleic acid and casein failed to precipitate with tetracaine or dibucaine. Studies on the precipitation of these three macromolecules by curare have been presented elsewhere (Ehrenpreis, 1960a, b, c). Precipitation by tetracaine and dibucaine appears to be more specific although somewhat less sensitive than that by curare (Table I). Tetracaine at pH 9, where it is present mostly in its uncharged form, failed to precipitate the receptor; this again parallels the

effect on electrical activity of the isolated electroplax which is much more strongly affected by the protonated, cationic, form at pH 6-7 (Bartels, et. al., 1960) than by the neurtral molecule.

TABLE I

Precipitation of various macromolecules by local anesthetics; curare is included for comparison . Tris buffer, pH 7.5

Compound	Conc. Used M/l	Receptor protein, 1% μ=0.075	Casein 1% μ=0.015	Chondroitin Sulfate 0.7% μ=0.075	Yeast Nucleic Acid, 3% μ=0.015
Procaine	2.5×10^{-2}	clear	clear	clear	clear
Tetracaine	1×10^{-2}		Turbid	clear	clear
	7×10^{-3}*	Precipitate	clear		
Dibucaine	4.5×10^{-3}		clear	clear	clear
	3.7×10^{-3}*	Precipitate			
Curare	5×10^{-3}*			Precipitate	
	1.8×10^{-3}*	Precipitate	Precipitate		Precipitate

*Minimum concentration required to cause precipitation.

In equilibrium dialysis, the affinity of both tetracaine and dibucaine for the receptor protein was found to be significantly greater than that of procaine (Fig. 1). Tetracaine is much more weakly bound at pH 9 than at pH 7.5. Although chondroitin sulfate interacts with procaine and tetracaine in solution (unpublished results) in both instances the binding is weaker than to the receptor protein, i.e., complex formation was absent at a concentration of 5×10^{-4} M despite the fact that the concentration of anionic groups was extremely high, about 2×10^{-3} M. This finding once again indicates that the isolated receptor protein combines with a variety of acetylcholine analogues in a highly specific way and is strong evidence that binding in solution does not reflect complex formation purely by Coulombic forces.

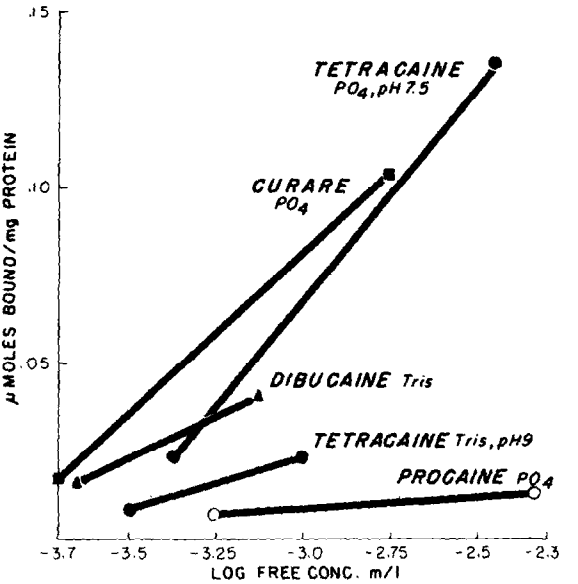

Fig. 1 Binding of local anesthetics and curare to the receptor protein, ionic strength = 0.1, 0° C. Tetracaine binding was determined at pH 7.5 and 9.0, the others only at pH 7.5. The buffers used are indicated in the figure.

The results are pertinent in two directions: 1) They offer evidence for the contention that the effects of local anesthetics on the axon are due to a specific chemical reaction with a component of the acetylcholine system, the receptor protein. These results thus supplement the previously shown essentiality of cholinesterase in conduction (Nachmansohn, 1959). 2) These findings support the unified concept of Nachmansohn as to events occurring in axon and synapse. It becomes increasingly apparent that the same specific chemical reactions take place at both in axon and synapse and influence both types of electrical events in a similar way.

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