ACETYLCHOLINE RECEPTOR PROTEIN AND NERVE ACTIVITY. II. CATIONIC GROUP IN LOCAL ANESTHETICS AND ELECTRICAL RESPONSE*

E. Bartels, W. D. Dettbarn, H. Higman+ and P. Rosenberg+

Departments of Neurology and Biochemistry
College of Physicians and Surgeons, Columbia University
New York, N.Y.

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The functional significance of an anionic site of acetylcholinesterase in the interaction with substrates and inhibitors have been extensively analyzed in vitro and in vivo (see e.g., Nachmansohn, 1959). The isolation of the acetylcholine receptor protein in solution (Ehrenpreis, 1959, 1960) permits us to test for the presence of an anionic site in this protein and to correlate molecular forces acting in vitro with the function and response of the intact cell. The binding of the local anesthetic tested (tetracaine) to the receptor protein in solution is indeed stronger at pH 7.5 than at pH 9 (Ehrenpreis and Kellock, preceding communication). The interaction is apparently increased by Coulombic forces acting between the anionic site in the protein and the cationic nitrogen. If the local anesthetics block electrical activity by combining with the active site of the receptor protein, thus competing with acetylcholine, then their cationic (protonated) forms should have a stronger action than the uncharged ones. We have, therefore, tested the effect of local anesthetics as a function of pH on the isolated single electroplax of Electrophorus developed by Schoffeniels (1959)

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which is a monocellular preparation and extremely sensitive in its response.

Experimental. The electroplax was exposed to Ringer's solution containing procaine, tetracaine or dibucaine in concentrations affecting electrical activity at a pH varying from about 6 to 9. Representative experiments are shown in Fig. 1 and Table I. The lower the pH, the stronger is the effect on both the direct and indirect electrical responses. Both types of response stimulation were found to be similarly affected.

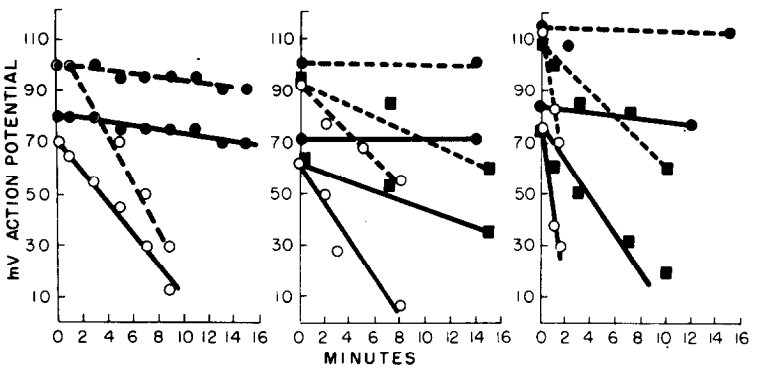

Fig. 1. Effect of local anesthetics as a function of pH. The blocking effect on the isolated single electroplax increases markedly at low pH when the compounds are in the cationic form. The action on synaptic and conducting membrane is strikingly parallel. A. Procaine: 135 μg/ml. B. Tetracaine: 10 μg/ml. C. Dibucaine: 5 μg/ml. Response to direct (——) and indirect (----) stimulation. ● pH 9; o pH 6-6.5; ■ pH 7.

TABLE I

Effect of pH on the action of procaine and dibucaine on the electrical response of the isolated single electroplax to direct and neural stimulation. The minimal active concentration (M.A.C.) is that producing at least 50% decrease of spike height within 30 min. or less.

Compound	pH	# of exp.	M.A.C. μg/ml.	min. of exposure
Procaine	6.0-6.5	3	135	6-9
	9.0-9.5	3	270	10-15
Dibucaine	6.0-6.2	2	5-10	15-25
	8.5-9.5	3	20	20-30

Discussion. It was previously shown (Rosenberg, Higman and Nachmansohn, 1960; Rosenberg and Higman, 1960) that the tertiary nitrogen derivatives, physostigmine, dimethylaminoethyl acetate (the tertiary analogue of acetylcholine) and the tertiary analogue of Prostigmine have a stronger action on electrical activity of the isolated single electroplax at pH 6 than at pH 9. These compounds have pK's between 8 and 9 and the increased effectiveness at low pH may be attributed to the charged form being attracted by Coulombic forces to an anionic group in the acetylcholine receptor protein. Their interaction with the receptor protein in solution as function of pH has not yet been studied. However, the binding strength of tetracaine to the receptor protein tested by equilibrium dialysis has been shown to be greater at pH 7.5 than at pH 9. Thus, the stronger action of local anesthetics on the electrical response at low pH parallels the stronger interaction with the receptor protein in vitro.

Although the parallelism is satisfactory in a qualitative way, no straight quantitative relationship between in vivo and in vitro effect exists nor can one be expected. Additional factors must influence the effect on the intact cell. The pH of the outside fluid may affect the intracellular and intramembraneous pH to some extent, but there most probably still will be quite a considerable difference in pH between the outside fluid and the membrane. Structural barriers will decrease the penetration of the charged molecule more than the uncharged one. In some preparations this may reverse the effect of pH, as is apparently the case with frog sciatic nerve fibers (Skou 1954) where the lipid barriers are probably quite strong.

We have demonstrated that at pH 7.0 tetracaine and dibucaine do not depolarize the membrane. Thus these findings are of particular interest since they show the similarity of the effect of the compounds on

synaptic junctions and on the conducting membrane as indicated by the response to neural and direct stimulation. This is a new support for the unified concept of Nachmansohn proposing a basically similar mechanism and similar chemical forces controlling the ion movements during activity across membranes of axons and those at synaptic junctions.

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