*ON THE EXCITABILITY AND COOPERATIVITY OF THE ELECTROPLAX MEMBRANE**

By Jean-Pierre Changeux† and Thomas R. Podleski†

DEPARTMENTS OF BIOCHEMISTRY AND NEUROLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY

Communicated by David Nachmansohn, January 15, 1968

Regulatory enzymes and excitable membranes possess certain important properties in common: (1) they are "excitable" in the sense that they respond to the binding of specific ligands by a characteristic modification of their biological activity (in one case, the catalytic or binding properties of an enzymatic site; in the other, the permeability toward ions[1]); and (2) they exhibit complex cooperative phenomena which, together with other physiological consequences, render their biological activity dependent upon threshold concentrations of regulatory ligands and thus confer on these macromolecular assemblies the function of biological amplifiers. Both properties have been attributed to (1) the presence of regulatory sites endowed with the stereospecific recognition of excitatory ligands and the ability of the macromolecular structure, carrying these sites, to undergo reversible changes of conformation; and (2) the organization of the macromolecular repeating units of the system into highly *ordered* structures: either an oligomeric structure or an infinite lattice structure, depending on the size of the cooperative assembly.[1-5]

Although the available information on the macromolecular structure and organization of excitable membranes is still fragmentary, it is of interest to reinvestigate some of the characteristic properties these membranes share with regulatory proteins. The synaptic membrane of the isolated electroplax was selected for its simplicity and reliability.[6, 7] In this paper we present experimental observations on the changes in membrane potentials observed in the presence of several acetylcholine congeners known as receptor activators. We shall concentrate on: (1) the sigmoid shape of the dose-response curve of receptor activators; (2) the effect of various receptor activators and inhibitors upon this shape; and (3) the amplitude of the maximal response measured at saturating levels of activators. The data are consistent with the predictions of current models of allosteric interactions.

Methods.—The isolated monocellular electroplax is mounted as previously described.[7] When the innervated side of the cell is perfused by a solution of receptor activator, the membrane potential decreases and reaches a steady-state value which, in most instances, is stable within the time limits of the experiment. As long as the concentration of activator is not exceedingly large (less than 2–5 times its apparent dissociation constant), successive application of the same solution of activator is followed by the same steady-state depolarization. (In the present experimental condition, therefore, the "receptor desensitization" described with several neuromuscular preparations does not have to be considered.) Almost identical responses are observed when different cells are used. In the present studies the decrease of membrane potential ($E - E_0$) from its resting value (E_0) to the final steady-state value (E) will be considered as a measure of the quantitative response of the cell to the applied concentration of activator. In a typical dose-response curve $E - E_0$ is plotted as a function of increasing concentrations of effector: the limits of the curve are 0 and $E_0 - E_{max}$, where E_{max} is the potential corresponding to infinite

concentration of ligand and is determined by linear extrapolation of the data plotted in the coordinates of Lineweaver and Burk. The response curves can be normalized within the limits 0 and 1 when $E - E_0/E_{max} - E_0 = \langle r \rangle_{rel}$ is plotted instead of $E - E_0$. The Hill coefficient n_H is defined as the maximal value of the derivative $d \log [(E - E_0)/(E_{max} - E)]/d \log F$, F being the free concentration of ligand. When $\langle r \rangle_{rel} = 1/2$, $F = F^1/_2$.

Results.—(1) *The sigmoid shape of the dose-response curve:* In their early studies with the isolated electroplax, Higman, Podleski, and Bartels[7] mentioned that the dose-response curve of carbamylcholine (CCh) deviates from a simple hyperbola; it resembles the S-shaped binding curve of oxygen to hemoglobin. The recent emphasis given to such cooperative effects in regulatory systems prompted us to reinvestigate this point in more detail. Titration curves were thus obtained within a large range of concentrations of activator, particularly in regions close to the origin of the curve where the deviation from the hyperbola

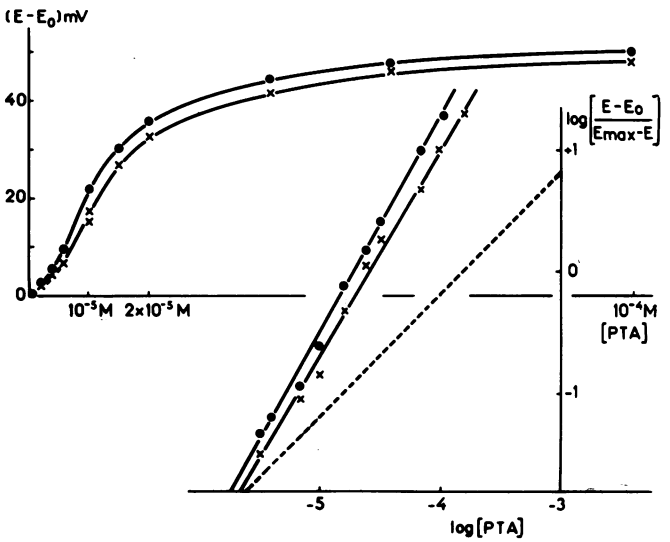

FIG. 1.—Response curve of the electroplax to Pta. Two different experiments performed on different cells are plotted on the same graph. The resting potential of the cell E_0 was in one case −81 mv (● - ●), in the other −80 mv (x − x).

Upper figure: Standard plot of response as a function of increasing concentrations of ligand; *lower figure:* Hill plot: $n_H = 1.7 \pm 0.1$ (see Table 1), *broken line:* $n_H = 1.0$ (theoretical).

was expected to be significant. Figures 1–3 and Table 1 show that a sigmoid shape of the dose-response curve is repeatedly observed with the three activators tested: CCh, phenyltrimethylammonium (Pta), and decamethonium (Dk). In all these cases the Hill coefficient n_H which we use as an index of the sigmoid, or cooperative, character of the curve is significantly different from 1 (hyperbola) and is close to 2 (Table 1). The same values for n_H are observed with different cells even when the initial resting potentials are different, and n_H does not change when the ionic environment of the cell is shifted from eel Ringer's solution to a high potassium medium (15 mM KCl, 150 mM NaCl) (Table 1). The

TABLE 1. *Characteristics of the response curves to various receptor activators.*

	n_H	$F^1/_2$	$E_{max} - E_0$	E_0	E_{min}
CCh	2.0 ± 0.1	2.6×10^{-5} M	69.5	−86	
	1.8 ± 0.1	3.0×10^{-5} M	60.0	−65	
CCh in high K^+ medium	1.6 ± 0.1	4.1×10^{-5} M	36	−58	
CCh + 1.5×10^{-7} M Dk	1.0 ± 0.1	(1.5×10^{-5} M)	58.5	−80	−80
CCh + 7.5×10^{-7} M Dk	0.9 ± 0.1	(4.0×10^{-6} M)	50	−85	−73
CCh + 1.0×10^{-6} M Flaxedil	2.1 ± 0.1	1.2×10^{-4} M	60	−77	−77
CCh + 10^{-5} M Pta	$0.6 < n_H < 1.0$	(3.0×10^{-6} M)	60	−83	−62
Dk	1.63 ± 0.02	1.2×10^{-6} M	50	−85	
Pta	1.77 ± 0.05	1.2×10^{-5} M	50	−80	
	1.66 ± 0.05	1.3×10^{-5} M	50	−81	

Abbreviations, see text. E_{min} is the basal response measured in the presence of the ligand the concentration of which is fixed. Composition of the high K^+ medium, see Fig. 4. Potentials in millivolts.

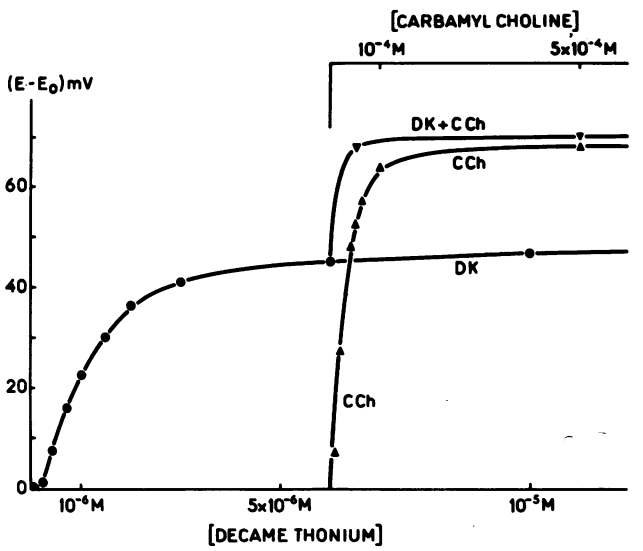

FIG. 2.—Response curve to Dk and CCh and effect of CCh on the maximal response to Dk. Lower scale relative to Dk, upper scale to CCh. Dk + CCh: the response to CCh is followed in the presence of 6×10^{-6} M Dk. E_0, − 85 mv.

sigmoid shape of the dose-response curve is thus a reproducible and characteristic feature of the interaction of the activator with the membrane.

(2) *Effect of receptor activators and inhibitors on the cooperative response of the electroplax to carbamylcholine:* It has been consistently observed with regulatory proteins that the cooperative (homotropic) interactions for the binding of a given ligand are modified by the presence of a different (heterotropic) ligand. Such conversion of shape was considered as a proof that the interactions between both classes of ligands are indirect or allosteric interactions.[2, 8–10] Therefore, we studied the effect of reversible activators and inhibitors on the cooperative response of

the electroplax to a given activator, keeping in mind that the changes of membrane potential are not necessarily stoichiometric to the amount of ligand bound to the membrane. Figure 3 and Table 1 show that in the presence of typical receptor inhibitors such as *d*-tubocurarine and Flaxedil the n_H of the dose-response curve of CCh does not change or slightly increase while the curve is

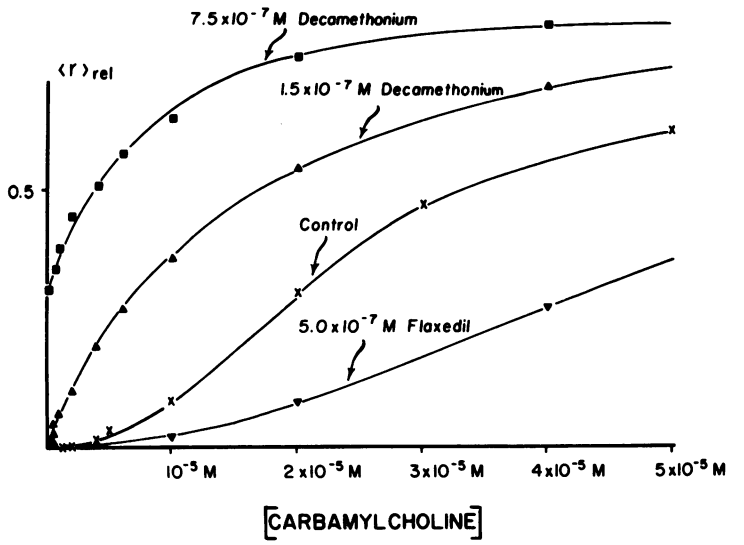

FIG. 3.—Effect of a receptor activator (Dk) and of a receptor inhibitor (Flaxedil) on the shape and position of the dose-response curve of CCh. Each curve is obtained from a different cell and the maximal response to saturating levels of CCh is taken as 1. On a given cell neither Dk nor Flaxedil changes the maximal response to CCh. See also Table 1.

shifted to the right. However, if the response to CCh is tested in a medium supplemented with a constant concentration of a receptor activator, Dk or Pta, the response curve to CCh is displaced towards the left and simultaneously its sigmoid shape is lost (Fig. 3). This displacement cannot be considered as a simple translation of the response curve of CCh along the concentration axis. The effects of CCh and Dk are not simply additive, but cooperative. A conversion of shape of the dose-response curve occurs. This observation, which strikingly parallels similar observations on regulatory enzymes, suggests that: (1) Dk and CCh bind to at least partially different areas; and (2) the same molecular transition accounts for both the process of excitation and the associated cooperative effects.

(3) *The maximal response to receptor activators:* One of the important parameters of a dose-response curve is the value of the maximal response measured at saturating levels of activator.

Figure 4 and Table 1 show that with the three activators tested, CCh, Pta, and Dk, different maximal responses are observed. This maximal response is thus directly related to the structure of the ligand. Let us consider the response to

Dk: the absolute value of the potential measured at saturating levels of Dk lies around −30 to −40 mv (with CCh the limit is −15 to −20 mv). If, at a concentration of Dk for which this limit is reached, the perfusion medium is now supplemented with large quantities of CCh, then the membrane potential is driven to −15 to −20 mv (Fig. 2). The −40 mv potential barrier for Dk can be overcome by an excess of CCh and thus does not constitute an intrinsic electrical parameter of the membrane. In order to test the hypothesis that the

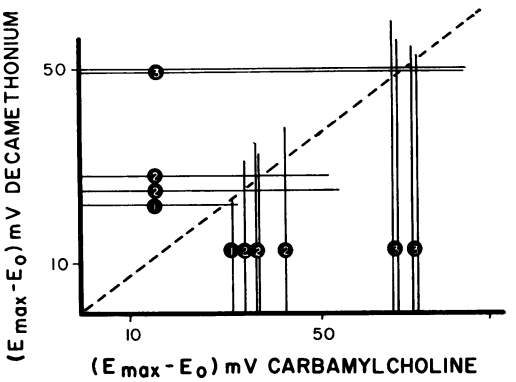

FIG. 4.—Comparative effects of ions on the maximal response to CCh and Dk. The saline solution contains in addition to 0.3 mM NaH_2PO_4, 1.2 mM Na_2HPO_4, 2 mM $MgCl_2$, 2 mM $CaCl_2$: (1) *high K^+ medium:* 150 mM NaCl, 15 mM KCl; (2) *low Na^+ medium:* 50 mM NaCl, 5 mM KCl, 220 mM sucrose; (3) *physiological Ringer's medium:* 160 mM NaCl, 5 mM KCl. E_0 is in (1) −63 ± 5 mv; in (2) −85 ± 10 mv; in (3) −75 ± 10 mv.

different maximal responses are due to the triggering of different ionic mechanisms, comparative estimates of the maximal response to CCh and Dk were obtained in various ionic environments. Figure 4 and Table 1 show that the *absolute* value of the maximal depolarization varies with the ionic composition of the medium but that the *relative* difference between Dk and CCh maxima remains constant. In other words, the same ionic processes account for the action of both Dk and CCh. The differential amplitude of the maximal response is thus determined by the structure of the compound tested and by its elementary interaction with the membrane receptor site. These results strikingly resemble the "partial" inhibitory or activatory effect observed with regulatory enzymes in their response to different regulatory ligands.[9-11]

Discussion.—The response of the electroplax to specific ligands is characterized by: (1) the specific recognition of agonists (or receptor activators) the binding of which triggers the membrane depolarization and also of their antagonists (or receptor inhibitors); (2) the deviation of the dose-response curve of several activators from the Langmuir isotherm: the sigmoid shape of these curves ($n_H \sim 2$) indicates that some type of cooperative interaction intervenes in the response to activators; (3) the change in shape of the dose-response curve of a given activator provoked by the presence of a different activator: the response is potentiated while the S-shape of the curve disappears; (4) a differential maximal response to saturating amounts of various activators which is determined by the structure of the activator: as shown with Dk and CCh, the *relative* extent of maximal depolarization is independent of the ionic environment of the cell.

In the present stage of our work it is difficult to propose a definitive inter-

pretation of these data. Several problems are still unsolved. (1) Various categories of membranes, presynaptic, postsynaptic, and conductive, are present on the innervated side of the electroplax. The specific contribution of each of them in the response to activators is not known. (2) The changes of membrane potential upon activator binding are not simply correlated to structural modifications of the membrane macromolecules.[12] A plausible interpretation can nevertheless be suggested on the basis of the proposed analogy of excitable membranes with regulatory enzymes.[2-5] Concerning the mechanisms of *activation* we postulate that: (1) receptor activators and inhibitors which are, in the present situation, structural analogues, bind to the same macromolecular component of the membrane: the ACh-*protomer*. (2) The ACh-protomer is integrated in the membrane structure and can be present under at least two different conformations which are in an equilibrium ($P \rightleftharpoons D$): (P) corresponds to the polarized state and (D) to the depolarized state of the membrane. (3) The affinity of the ACh receptor-site toward activators is altered by the $P \rightleftharpoons D$ transition: the receptor inhibitors bind preferentially to the P state, the receptor activators to the D state. (4) The relative changes of membrane potentials are determined by the fraction of protomers which undergo the transition to the D state and are better related to a state function rather than to a binding function.

Assuming the P state is favored in the resting membrane, triggering of the membrane depolarization would arise from the transition of the ACh protomer from the P to the D state. The observation that various related drugs provoke different maximal responses would then be due to the preferential rather than exclusive affinity of the activator for the D state: the ratio of the microscopic dissociation constants to the P and D states determines the "intrinsic activity"[13] of the activator. The antagonism between activators and inhibitors would be the consequence of a differential stabilization of either the P or D conformation. Such an antagonism would be similar to that observed between various stereospecific ligands of threonine deaminase[9] or aspartate transcarbamylase.[10] To account for the *cooperative response* we postulate that mutual interactions are established between ACh-protomers: the conformational transition of one protomer favoring the transition of its neighbors. However, the topology of the association between ACh-protomers in the membrane is not yet known. Two extreme situations would account for the low cooperativity observed. (1) The ACh-protomers are unequally distributed on the membrane surface and clustered by small numbers. They strongly interact within a cluster but not between clusters (oligomeric model). (2) The ACh-protomers are part of an infinite statistical ensemble with weak interactions between nearest neighbors (lattice model).

These alternative situations cannot be distinguished on the basis of the present data. Both of them predictably account for the critical change of shape of the dose-response curve which we observed in the presence of two activators. Future studies should enable us to distinguish between these two models and yield further information on the macromolecular events which occur in the course of the membrane response to specific regulatory ligands.

The authors are greatly indebted to Dr. David Nachmansohn for his stimulating encouragement and helpful advice. J.-P. C. would also like to express his appreciation for the generous hospitality extended to him.

* This work has been supported by the National Science Foundation grant NSF-GB-4844, and by the U.S. Public Health Service grant NB-03304.

† Present address: Institut Pasteur, Paris, France.

[1] Nachmansohn, D., *Ann. N.Y. Acad. Sci.*, **137**, 877 (1966).

[2] Monod, J., J. Wyman, and J. -P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

[3] Changeux, J. -P., *J. Mol. Pharmacol.*, **2**, 369 (1966).

[4] Changeux, J. -P., J. Thiery, Y. Tung, and C. Kittel, these PROCEEDINGS, **57**, 335 (1967); Changeux, J. -P., and J. Thiery, in *Regulatory Functions of Biological Membranes*, S. Juselius Symposium, Helsinki, November 1967.

[5] Karlin, A., *J. Theoret. Biol.*, **16**, 306 (1967).

[6] Schoffeniels, E., and D. Nachmansohn,, *Biochim. Biophys. Acta*, **26**, 1 (1957).

[7] Higman, H. B., T. R. Podleski, and E. Bartels, *Biochim. Biophys. Acta*, **75**, 187 (1964).

[8] Douglas, C. G., J. S. Haldane, and J. B. S. Haldane, *J. Physiol.*, **44**, 275 (1912).

[9] Changeux, J. -P., *J. Mol. Biol.*, **4**, 220 (1962).

[10] Gerhart, J. C., and A. B. Pardee, *J. Biol. Chem.*, **237**, 891 (1962). Gerhart, J. C., in *Brookhaven Symposia in Biology*, No. 17 (1964), p. 232. Changeux, J. -P., and J. C. Gerhart, *Federation European Biochem. Soc., Symp., 4th*, Oslo, July 1967.

[11] Rubin, M., and J. -P. Changeux, *J. Mol. Biol.*, **21**, 265 (1966).

[12] Goldman, D. E., *J. Gen. Physiol.*, **27**, 37 (1943). Martin, A. R., *J. Gen. Physiol.*, **130**, 114 (1965). Karlin, A., these PROCEEDINGS, **58**, 1162 (1967).

[13] Ariens, E. J., *Molecular Pharmacology* (New York: Academic Press, 1964), vol. 1.