*PHOTOREGULATION OF BIOLOGICAL ACTIVITY BY PHOTOCHROMIC REAGENTS, III. PHOTOREGULATION OF BIOELECTRICITY BY ACETYLCHOLINE RECEPTOR INHIBITORS**

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Abstract.—The photochromic compounds *N-p*-phenylazophenyl-*N*-phenylcarbamylcholine chloride and *p*-phenylazophenyltrimethylammonium chloride inhibit the carbamylcholine-produced depolarization of the excitable membrane of the monocellular electroplax preparation of *Electrophorus*. The *trans* isomer of each predominates in the light of a photoflood (420 mμ) lamp; they are stronger inhibitors than the *cis* isomers, which predominate under ultraviolet (320 mμ) irradiation. The potential difference across the excitable membrane may be photoregulated by exposing an electroplax in the presence of a solution of carbamylcholine and either of the two compounds to light of appropriate wavelengths, since light shifts the *cis-trans* equilibrium. The system may be considered as a model illustrating how one may link a *cis-trans* isomerization, the first step in the initiation of a visual impulse, with substantial changes (20–30 mv) in the potential difference across an excitable membrane.

The electrical currents which propagate nerve impulses are carried by ion movements resulting from changes in the ionic permeabilities of excitable membranes. It has been proposed that such permeability changes are effected by a series of reactions in which acetylcholine released by stimulation acts as a trigger. According to this hypothesis, combination of acetylcholine with the acetylcholine receptor leads to excitation, perhaps through regulation of the ionic permeabilities of the excitable membrane by calcium ions (known to be associated with excitability) released by a conformational change of the receptor. Rapid hydrolysis of acetylcholine by acetylcholinesterase would allow the return of the receptor to its resting state and the reestablishment of the barrier to ion movements.[1, 2]

Initiation and propagation of nerve impulses can result from the response of sensory receptors to specific stimuli, such as light, sound, and touch. A great deal is known about the physiology of the receptor cells, and information is being accumulated concerning the elementary reactions occurring in the reception of stimuli. Vision, for example, is based on the *cis-trans* isomerization of retinal.[3] *Cis*-retinal reacts in the dark with the protein opsin to form rhodopsin. Light-induced isomerization of the retinal to the all-*trans* conformation leads to nerve excitation. Absorption of only a few quanta of light is sufficient to produce a measurable response in the retina.[4] However, the mechanism by which isomerization leads to excitation remains open to speculation. A model system is presented here in which the potential difference across the excitable membrane of the

monocellular electroplax of *Electrophorus* is photoregulated by use of light-sensitive molecules like those described in the preceding paper.[5]

The monocellular electroplax preparation developed by Nachmansohn and coworkers[6, 7] is especially suited for study of the interactions of ligands with the proteins in the excitable membrane. The large size and rectangular shape of the cell contributes to its usefulness; however, the outstanding feature is its specialization for bioelectrogenesis. The rate of chemical activity associated with electrical activity is very high compared with the rate of other metabolic processes. The innervated (excitable) membrane is reversibly depolarized by exposure to compounds which interact with the acetylcholine receptor; e.g., acetylcholine, carbamylcholine, and decamethonium. Changes induced by such depolarizing agents (receptor activators) and the effects of their antagonists (receptor inhibitors), such as curare, may be monitored by intracellular electrodes, and the resulting dose-response curves used to evaluate structure-activity relationships. Studies using such methods have provided a considerable amount of information about the receptor and the groups associated with its active site. Details of the methods used in the present study are similar to those described previously.[6, 7]

In order to photoregulate the potential across the cell membrane, compounds are required which are structural analogs of receptor activators or inhibitors and whose structures change under irradiation. In this respect, light causes a reversible shift in the *cis-trans* equilibrium about the nitrogen-nitrogen double bond of the compounds used in the present study, *N-p*-phenylazophenyl-*N*-phenylcarbamylcholine chloride (azo-CarCh) and *p*-phenylazophenyltrimethylammonium chloride (azo-PTA). The *trans* isomer of each predominates in the light of a photoflood lamp (or daylight); the *cis* predominates in ultraviolet (320 mμ) light.[5, 8] Azo-CarCh and azo-PTA are derivatives of potent depolarizing agents, carbamylcholine and phenyltrimethylammonium, respectively. Both reversibly inhibit acetylcholinesterase activity and, on the electroplax, act at low concentrations (azo-CarCh at 1 μM, azo-PTA at 10 μM) as receptor inhibitors to block the depolarization produced by CarCh. Such inhibition by the *trans* isomer of azo-CarCh is illustrated in Figure 1. At higher concentrations (100 μM) each compound blocks both the post-synaptic potential and the conducted spike.

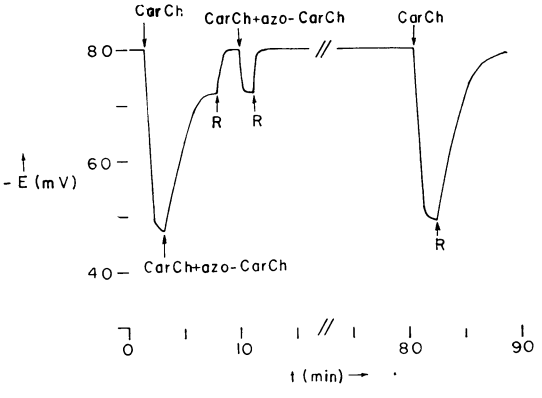

FIG. 1.—Reversible inhibition of carbamylcholine depolarization by 1 μM azo-CarCh (*trans* isomer). The potential is measured across the innervated membrane. CarCh, 20 μM carbamylcholine; R, eel Ringer's solution.

The effect of each isomer of each compound on the dose-response curve for carbamylcholine is shown in Figures 2 and 3.

The *trans* isomer of each compound is readily seen to be a stronger inhibitor than the *cis;* that is, at a given concentration of carbamylcholine, the depolarization in the presence of the *trans* isomer is less than that in the presence of the same concentration of the *cis*. Consequently, it is possible to regulate the potential difference across the innervated membrane by exposing a cell in the presence of a solution of carbamylcholine and either of the two compounds to light of appropriate wavelengths. Irradiation does not affect the membrane potential of a cell in the presence of carbamylcholine alone. Such regulation is shown in Figures 4 and 5. Prolonged exposure to ultraviolet radiation decreases the membrane potential to that expected from the data in Figures 2 and 3 for carbamylcholine in the presence of the *cis* isomer; exposure for intermediate lengths of time produces a potential between those for the *cis* and the *trans*. Subsequent exposure of the cell to the light of a photoflood lamp restores the potential to that for the *trans*.

It is interesting that limited changes in structure such as the light-induced *cis-trans* isomerization can cause changes in membrane potential of 20 to 30 millivolts. Effects of the same magnitude are observed in the visual process, in which the *cis-trans* isomerization of retinal leads to a neural impulse. Vision is a far more efficient process, since absorption of only a few quanta of light suffices to excite a visual cell within a millisecond. Such a great difference in efficiency is not surprising. The components of the retina are highly specialized, and the light-sensitive molecule is an integral part of the retinal cells. It is common to find that the efficiency of even a biologically active compound is low when applied externally to a cell. To illustrate, the electroplax barely responds to 1 μM

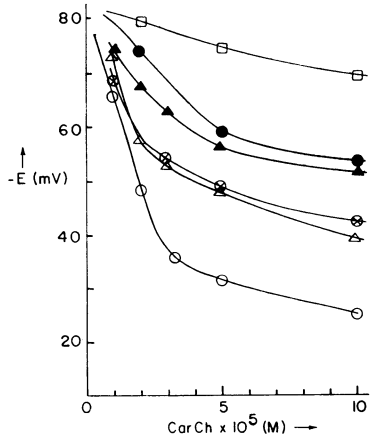

FIG. 2.—Effect of azo-CarCh on carbamylcholine dose-response curve: O, no inhibitor; Δ, 1 μM *cis*; ⊗, 0.2 μM *trans*; ▲, 3 μM *cis*; ●, 1 μM *trans*; □, 2 μM *trans*.

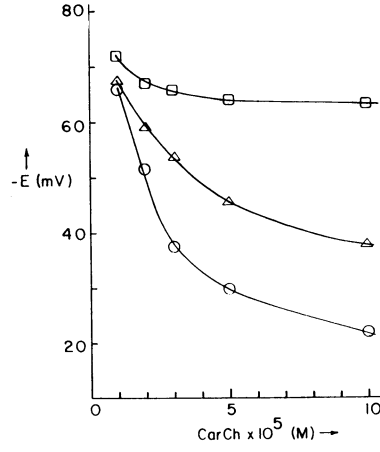

FIG. 3.—Effect of azo-PTA on carbamylcholine dose-response curve: O, no inhibitor; Δ, 20 μM *cis*; □, 20 μM *trans*.

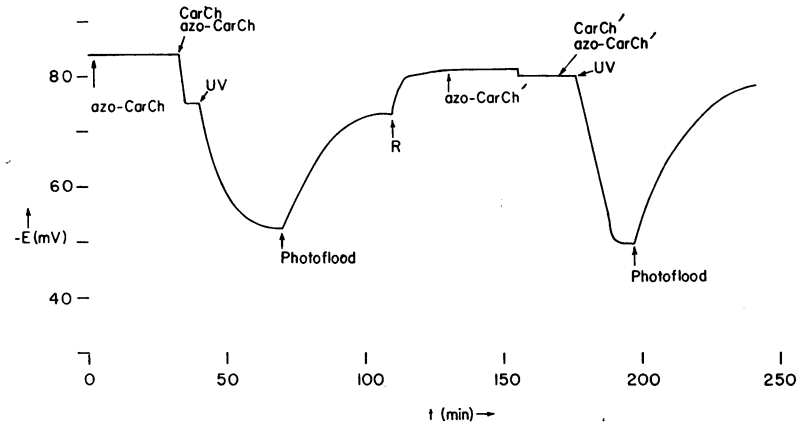

FIG. 4.—Photoregulation of the potential across the innervated membrane. Ultraviolet (Spectroline model B-100) and photoflood lamps were turned on at the times indicated. CarCh, 20 μM; azo-CarCh, 1 μM; azo-CarCh', 3 μM; CarCh', 50 μM.

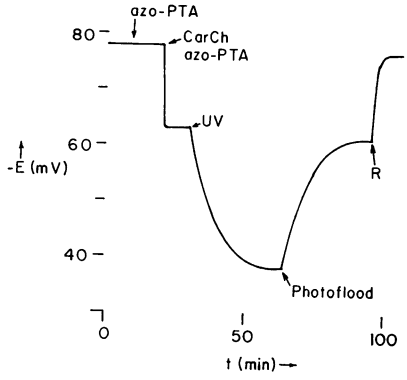

FIG. 5.—Photoregulation of the potential across the innervated membrane. azo-PTA, 20 μM; CarCh, 100 μM.

acetylcholine (and then only when an acetylcholinesterase inhibitor such as physostigmine is present), while in nerve activity the amounts of acetylcholine released are at least six to eight orders of magnitude smaller.[1, 2]

The system presented here may be considered as a model illustrating how one may link a *cis-trans* isomerization, the first step in the initiation of a visual impulse, with substantial changes in the potential difference across an excitable membrane. Details of the events associated with the isomerization of retinal, perhaps involving a conformational change of opsin,[3] remain to be explored. Their analysis will be necessary before the connection between the light-induced *cis-trans* isomerization of retinal and nerve conduction is fully understood.

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