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Rapid Changes in Light-Scattering in the Prism of *Torpedo* Electric Organ Slice Associated with the Production of Postsynaptic Potentials

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Using a bifurcated lightguide placed on the surface of a prism of the electric organ slice of *Torpedo,* the generation of postsynaptic potentials by direct stimulation was found to be associated with readily detectable, disphasic optical changes. The changes consisted of a decrease in light-scattering during the rising phase of the electric potential, followed by a prolonged increase in scattering. The effects of varying the calcium-ion concentration in the medium strongly support the view that replacement of transmitter molecules for bound Ca-ions in the receptor proteins is at the base of these changes. The effects of synaptically active reagents on these optical signals were demonstrated.

The electric organ of *Torpedo* is composed of closely packed prismatic columns which extend, vertically, from the dorsal surface to the ventral surface of the fish. Each column, 3–5mm in width and separated from its neighbors by a 5- or 6-sided boundary, is made up of a stack of flat (about 10μm thick) cells, electrocytes, separated from each other by layers of inert viscous fluid (1,2). An electric shock, delivered directly to a prismatic column in the organ, excites the nerve fibers which terminate on the ventral surface of the electrocytes and elicits a postsynaptic potential almost simultaneously from all the innervated electrocytes (3–5).

In a recent communication (6), experimental findings were presented demonstrating that the process of postsynaptic potential production in slices of the *Torpedo* electric organ is associated with diphasic mechanical changes, swelling followed by shrinkage of the slices. In the present communication, new experimental findings are described demonstrating that, again in the *Torpedo* electric organ slice, the production of a postsynaptic potential is accompanied by a readily-detectable, diphasic optical change. The amplitude and the duration of this change could be significantly increased by raising the Ca^{2+} concentration in the medium. Several synaptically active reagents were shown to profoundly affect the magnitude and the time-course of these optical changes. These experimental findings are interpreted based on the previously reported finding that Ca^{2+} bound to the anionic sites in the receptor proteins are released by the chemical transmitter, acetylcholine (7,8).

MATERIAL AND METHODS

Approximately 3 mm thick slices of the electric organ of *Torpedo californica* were prepared by the procedure described previously (6) and were stored, until making the measurements, in a large volume of aerated and cooled elasmobranch saline solution. The composition of the solution was (in mM): NaCl 200, KCl 8, $MgCl_2$ 1.8, $CaCl_2$ 3.4, $NaHCO_3$ 5, glucose 5.5, urea 300, sucrose 100, and Tris-buffer 9 (pH 7.3). The effects of synaptically active reagents were examined by dissolving the reagents in normal saline solution.

Fig. 1, left, schematically illustrates the experimental setup employed to record light-scattering changes in the electric organ prism. An organ slice was trimmed down to a piece of about 25 mm square and was introduced into a shallow plastic chamber (about 40mm square) provided with a 6 mm wide Ag-AgCl electrode at the bottom (e_3). The peripheral region of the slice was fixed to the chamber bottom. Then, by inspecting the upper surface of the organ slice, a large prism located above of the electrode at the chamber bottom was chosen as the object for subsequent optical measurements. Next, a pair of large stimulating Ag-AgCl electrodes, e_1 and e_2, were placed on the slice, one on each side of the chosen prism and firmly

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pressing the slice. Finally, a bifurcated lightguide (6), held by means of a rack-and-pinion device, was lowered from above and was brought in firm contact with the center of the chosen prism.

The bifurcated lightguide is composed of two bundles of fine optical fibers mixed randomly at one (sensing) end. One of the bundles was used for transmitting white light from a 100W quartz-iodine lamp, L, through heat- and color-filters to the prism. The other bundle was for carrying light scattered inside the prism to a photodiode (UDT 4555UV), D in the figure. Due to the limited numerical aperture of the optical fibers, the observed optical changes represent variations aperture of the optical fibers, the observed optical changes represent variations in the portion of light scattered predominantly by the layers more than about 50μm away from the tip of the lightguide. The stainless steel ferrule (2mm i.d., 3mm o.d.) was used, in combination with e_3, for recording the post-synaptic potentials. The optical records were obtained by use of a Data-6000 analyzer (Analogic Corp.), often averaging 2–20 responses. All the measurements were carried out at room temperature, 20–21°C.

RESULTS

Fig. 1, right, shows a typical example of the records taken from the *Torpedo* electric organ slice under steady illumination with bluish white light from the tip of the lightguide. It is seen that a single electric shock, delivered to the top surface of a freshly excised slice, evoked, with a latency of about 2 ms, a large postsynaptic potential (bottom traces). Simultaneously, there was a rapid, diphasic change in the intensity of light transmitted from the prism to the photodiode (top traces). The first phase of this optical change, which represented a *decrease* in light intensity, was found to start at about, or a fraction of a ms prior to, the onset of the postsynaptic potential and reached its minimum roughly at the peak of the electric response. The amplitude of the change was usually in the range between 0.03–0.13% of the intensity of the steady background light. The second phase of the optical change, representing a prolonged *increase* in the light intensity, was found to reach its peak at about the end of the postsynaptic potential, the peak amplitude being usually in the range between 0.04 and 0.20%. Its decay was slow and appeared to be roughly exponential, the time-constant being of the order of 0.5s. Because of this slow decay, there was a marked summation of optical changes when a train of shocks repeated at a short interval was delivered to the prism.

In most of the present studies, bluish white light was employed to observe optical changes. However, optical changes with similar magnitudes and time-courses could be elicited from the prism under steady illumination with quasi-monochromatic light, 620, 550 and 450 nm in wave-length. There is little doubt, therefore, that the observed *optical responses* represent transient changes in the intensity of light scattered by the cellular elements in the prism which, in the room light, appears almost transparent. The ventral plasma membrane of the electrocyte is known to be densely populated with intramembrane particles which are generally regarded as representing the receptor protein molecules (2,5). These particles are expected to create a small spatial variation in the optical density inside the prism. Therefore, the observed optical responses are interpreted as

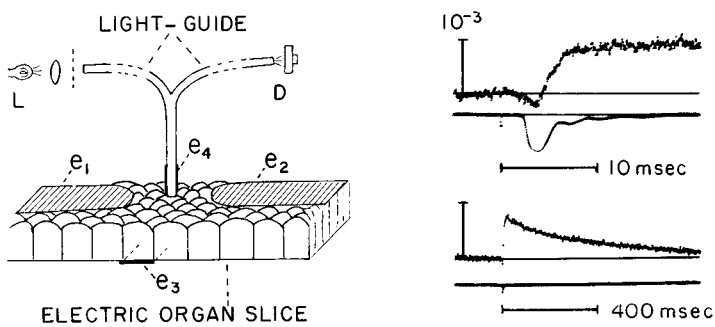

FIG. 1. Left: Schematic diagram illustrating the experimental setup employed for recording light-scattering changes in the prism of electric organ slices by use of a bifurcated lightguide. L represents a 100W lamp; D, photodiode; e_1 & e_2, stimulating Ag-AgCl electrodes. Electrode e_3 and the lightguide ferrule e_4 were used for recording electric responses. Right: Records showing light-scattering changes (upper traces) and electric responses (bottom traces) evoked by single electric shocks (0.15 ms, about 25V) delivered to a freshly excised electric organ slice. The electric response observed was about 5V in amplitude.

reflections of rapid, reversible changes in the optical density of these and other elements in the prism.

As is well known, diffusion of chemical substances into the electric organ slices is a very slow process (3,4). In fact, in order to demonstrated clear effects of synaptically active reagents on optical responses, it was necessary to keep the slices in test solutions for a period of about 4 hr or longer. Note that the optical responses are derived from several membrane layers at some distance away from the surface of the prism. Note also that, after a period of 20 hr or longer, the postsynaptic potentials generated by slices kept in normal saline solution start to deteriorate.

When the Ca^{2+} concentration in the medium bathing an electric organ slice was raised, there was a gradual increase in the amplitude and duration of the optical response of the slice during the following 4–12 hr period. Fig. 2 shows examples of the optical responses taken from slices kept overnight in either Ca^{2+}-rich or Ca^{2+}-deficient saline solution. In a series of measurements a comparison was made between the optical responses recorded from two sets of 11 slices each, one set kept for 20–24 hr in the saline solution containing 10 mM Ca^{2+} and the other set (taken from the same electric organ) bathed in the normal saline solution (containing 3.4 mM Ca^{2+}) for the same period of time. The amplitude of the first phase of the optical response observed in the first set of slices, $(1.5 \pm 0.5) \times 10^{-3}$, was on the whole larger than that seen in the second set, $(1.0 \pm 0.4) \times 10^{-3}$. This enhancement of the response amplitude was accompanied by a clear increase in the duration of the first phase of the response. The most conspicuous effect of an increase in the Ca^{2+}-concentration in the medium was on the rate of decay of the optical response during the second phase: the rate of decay in the slices kept in the saline containing 10 mM Ca^{2+} was roughly 1/3 of the rate in the slices bathed in the normal saline. The small response observed in slices kept in the Ca^{2+}-free medium (Fig. 2, right) is considered to be a reflection of incomplete depletion of Ca^{2+} in the tissue.

The time required for observing pharmacological effects of synaptically active reagents varied considerably from reagent to reagent (see Fig. 3). Suppression of optical responses by d-tubocurarine (0.2–0.8 mM) was incomplete even after 24 hr in the drug solution. Prolongation of responses by eserine (0.01–0.02 mM) could be readily demonstrated after immersion of the slices in the drug solution for 4–5 hr. Carbachol (1 mM) was found to suppress optical responses of the slices after 4–6 hr immersion. Iodoacetate (1 mM) almost completely suppressed optical responses within about 4 hr (not shown). In all the cases examined, the drug effects on the optical response were seen to proceed in parallel with those on the electric response. When the electric response amplitude became progressively smaller during repetitive stimulation of the slices poisoned with d-tubocurarine or eserine, there was a parallel fall in the amplitude of the optical response. This finding indicates that these two kinds of responses have a common origin.

DISCUSSION

According to the interpretation of the process of synaptic excitation given by Neumann, Nach-

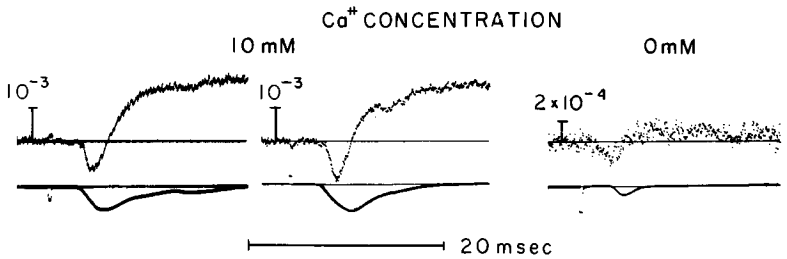

FIG. 2. Effects of varying the Ca-ion concentration in the medium on the light-scattering changes in the electric organ prism (top traces). The records were taken from three different slices immersed for 20 hr in elasmobranch saline solutions containing 10 or 0 mM Ca-ion. The electric responses were 5.2V (left), 6V (middle) and 1.5V (right) in amplitude.

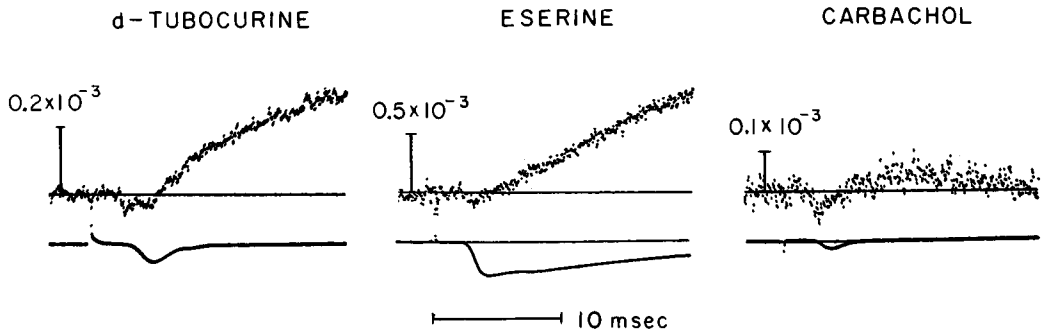

FIG. 3. Records showing the effects of d-tubocurarine chloride (0.2 mM, 23hr), eserine sulfate (0.01mM, 4hr) and carbachol (1mM, 4.5 hr) on the optical (top traces) and electric responses (bottom traces) of electric organ slices to single shocks. The electric responses were 0.07V (left), 1.9V (middle) and 0.9V (right).

mansohn and Katchalsky (7), the receptor proteins in the plasma membranes of the electrocytes at rest are in a Ca^{2+}-rich state. Acetylcholine molecules, released from the nerve terminals upon arrival of a nerve impulse replace a portion of the Ca^{2+} bound to the negatively charged sites in the proteins (8). It is known that such a divalent-monovalent cation-exchange in anionic polymers can evoke a large, often discontinuous (i.e. all-or-none) volume transition in anionic polymers (9–12). The first phase of the Ca^{2+} concentration-dependent light-scattering changes described under Results may then be regarded as the consequence of a fall of the optical density of the receptor proteins associated with a rise in the water content of the receptor proteins (see Discussion in ref. 6). The increase in light-scattering in the second phase of the optical changes is attributable to the uptake of Ca^{2+} by the receptor and cytoplasmic proteins as the consequence of an enhanced Ca^{2+}-influx into the electrocytes during the first phase.

It is reasonable to assume that, in the normally metabolizing electrocytes, the Ca^{2+} concentration is maintained at a level close to the threshold for a transition from the compact to the swollen state of the receptor proteins. Under these circumstances, the dominant rapid physicochemical change associated with a transition is the enrichment of the plasma membrane of the electrocytes with water molecules transferred from the surrounding inert fluid layer. The importance of the movements of water molecules in the process of synaptic excitation is emphasized.

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