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Rapid Volume Expansion in the *Torpedo* Electric Organ Associated with Its Postsynaptic Potential

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By electrically stimulating *Torpedo* electric organs enclosed in a sealed plastic chamber, it was demonstrated that the production of a postsynaptic potential is accompanied by a rapid increase in the volume of the organ. This increase started almost simultaneously with the onset of the postsynaptic potential and reached its maximum at about the end of the potential. The volume returned slowly to its resting level. Repetitive stimulation produced a summation of volume changes. The maximum change evoked by a single electric shock was roughly 10^{-6} times the electrocyte volume. The thermal expansion of the organ associated with the production of a postsynaptic potential accounts for roughly 25% or less of this volume increase. The possibility is pointed out that a cation-exchange process involving Ca^{2+} on anionic sites in the electrocyte protoplasm may be at the base of the volume changes. © 1997 Academic Press

The rapid volume changes in the skeletal muscle during its contractile activity have been amply characterized by a number of earlier investigations (1-4). More recently, the occurrence of comparable volume changes during excitation of non-myelinated nerve fibers has been reported (5). In this communication, it is demonstrated that similar volume changes take place in the *Torpedo* electric organ when it is excited by a nerve impulse arriving at the presynaptic terminals.

As is well-known, the *Torpedo* electric organ is well suited for studying synaptic processes because of the richness in its cholinergic nerve terminals and receptors (6-8). The electric responses of the organ, evoked by an electric shock applied either directly to the organ or indirectly to the electric nerve, represent a summation of postsynaptic potentials resulting from the release of the transmitter, acetylcholine, from the nerve terminals (8-11). For the demonstration of rapid volume changes associated with electric responses of the organ, substantially the same devices as those used in previous studies of the volume changes in the muscle and nerve (2,4,5) were employed.

MATERIAL AND METHODS

All the experiments were performed using specimens of *Torpedo californica* obtained from Aquatic Research Consultants, San Pedro, CA. After anesthetizing a fish by pipetting a 0.15% tricaine methanesulfonate sea-water solution into the spiracles (12), the electric organs, covered by the intact skin on both the dorsal and ventral surfaces, were dissected out together with the four electric nerves. The organ was cut into four blocks, each connected to one nerve. The blocks were kept in *Torpedo* saline solution which had the following composition (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 (pH 7.3) 9. In most experiments, rapid volume changes in the electric organ were examined without removing the skin covering the organ. In one series of experiments, approximately 3.5 mm thick slices of the electric organ, prepared by the method described previously (13), were employed. These slices were washed thoroughly with saline solution and were then stored in refrigerated saline solution.

For the detection of rapid volume changes in the organ, a block of electric organ was introduced, together with its nerve, into a large plastic chamber with a thick wall. The capacity of the chamber most frequently used was 4.5 × 7.5 × 5.0-cm and the thickness of the wall varied between 1 and 2.5 cm The chamber was provided with a pair of Ag-AgCl electrodes on the inner surface of the wall; these were used for recording electric responses of the nerve-electric organ preparation. On the top surface of the wall, there was a long, narrow groove (5 × 5-mm in cross-section) connected to the main cavity at one end (see the diagram in Fig. 1, top). A pair of small Ag-AgCl electrodes (connected to pin E_1 and E_2 in the diagram) were glued to the bottom surface of the groove. These electrodes were employed to deliver brief electric shocks (0.2 ms in duration and about 20 V in amplitude) to the nerve introduced into the groove. The flat surface on the top of the chamber wall was coated with a thin layer of silicone rubber. The lid of the chamber was made of a 1.25 cm thick plastic plate. A small hole was bored through the plate. This hole was for transmitting the effect of volume changes inside the chamber to the detector.

Two different methods of detecting rapid volume changes were employed. In the experimental setup illustrated by the diagram in Fig. 1, volume changes were detected in terms of the surface movement of the saline solution in the small hole connected to the cavity of the chamber (4). A high-frequency A.C. from an oscillator (100 kHz, 10V) was led to a metal rod (1.5 mm in diameter) through a resistor (about 1 $M\frac{1}{2}$). The entire detector set, including the metal rod, the resistor, the buffering operational amplifier (OPA 128) and

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the power supply (12 V batteries), was enclosed in an electrically shielded plastic box, with the lower end of the metal rod protruding from the bottom of the box. By means of a rack-and-pinion device, the box could be moved up-and-down with the precision of a few μm. The plastic chamber was placed on a modified microscope table and could be moved in the horizontal plane by use of a mechanical stage. The small hole in the lid of the chamber was made of a metal tubing (2.3 mm i.d. and 4.0 mm o.d.). After completely filling the remaining space in the chamber with saline solution, the chamber was tightly closed by bolting the lid. Precaution was taken to remove visible air bubbles in the chamber by way of the small hole in the lid. By using a screw penetrating the wall of the chamber (S in the diagram), the upper surface of the saline solution in the hole was brought to the top of the metal tubing. Then, the top surface of the metal tubing was covered by a small piece of 2 μm thick metallized polycarbonate film (purchased from Goodfellow Corp., Berwyn, PA). The metal tubing was grounded. The A.C. output of the detector (about 1.5 V) was amplified 2-fold, rectified (using a 2N-914 diode and an RC network) and then led to a recorder (Data-6000 Analyzer, Analogic Corp.). When, by use of the rack-and-pinion device, the smooth end of the metal rod was brought close to the concave surface of the polycarbonate film, a potential shift could be recognized at the input of the recorder. Volume changes in the electric organ were determined by recording transient potential shifts under the condition that the tip of the metal rod was within about 40 μm from the surface of the film. The device was calibrated by use of screw S penetrating the wall of the chamber. To carry out volume measurements by this "capacitance" method, it was essential that the electric organ in the chamber was fully adapted to the temperature of the surrounding saline solution.

In the experimental setup illustrated in Fig. 2, top, a 25 mm long piezoelectric bender (G-1195, Gulton Industries, Metuchen, N.J.) was used for detecting the pressure changes in the chamber. A light wooden stylus, roughly 1 mm in diameter and about 20 mm in length, was attached near one end of the bender. An operational amplifier (OPA128) with a feedback resistor of 10 G$\frac{1}{2}$ and a parallel capacitor (0.1 or 1.0 nF) was connected to the other end. Both the bender and the amplifier (together with its power supply) were enclosed in a shielded plastic box attached to a rack-and-pinion device. [The resonance frequencies of this detector were determined by bringing the tip of the bender stylus in contact with the diaphragm of a microphone; no resonance was observed at frequencies below 4 kHz.] The small hole (2.5-5 mm in diameter) in the plastic lid was closed with a thin polymer film (usually 5 μm thick mylar). Then, the film was pressed down with a thin plastic disk which had a small hole (surrounding by a rubber O-ring) located precisely at the position of the hole in the lid. Finally, the piezoelectric bender was lowered from above, and the stylus was bought in firm contact with the polymer film covering the hole. Under these conditions, an electric shock delivered to the nerve trunk of the preparation evoked a postsynaptic potential accompanied by a simultaneous change in the output of the detector. The responsivity of this piezoelectric device was calibrated by measuring changes in the amplifier output brought about by inserting a small screw into the chamber through the wall. In the case of a small plastic chamber, the responsivity was calibrated also by filling the chamber completely with a 10 mM KCl solution and recording the response of the recorder to a volume change induced by the Joule heat associated with a flow of 5 kHz A.C. between the large electrodes in the chamber; the result of this calibration was consistent with that obtained by driving a screw through the wall.

Rapid volume changes were recorded also from slices of the electric organ (see Fig.3, C). An organ slice was trimmed into a rectangular shape (about 15 × 35-mm) and was held tightly with a frame made of thin plastic plates. The slice was introduced vertically into a small plastic chamber whereby the space inside the chamber was divided into two equal parts. Then, physiological saline solution was poured into the chamber until the entire exposed surface of the slice was immersed in the solution. The remaining upper part of the chamber was filled completely with mineral oil. Finally, the chamber was tightly closed with the lid. Electric shocks were delivered directly to the slice by use of a pair of Ag-AgCl electrodes on the wall of the chamber. The changes in the hydrostatic pressure associated with excitation of the slice was detected by employing the piezo-electric method described above.

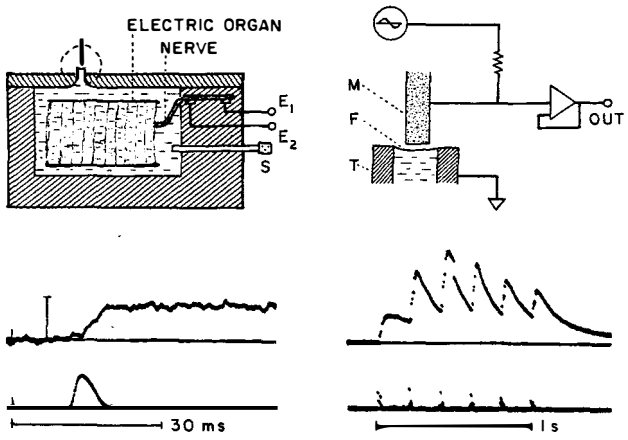

FIG. 1. (Top) Diagram illustrating the "capacitance" method of measuring volume changes in the *Torpedo* electric organ. The encircled portion of the diagram is enlarged and shown on the right. M, metal rod; F, 2 μm thick metallized polycarbonate film; T, metal tubing; S, screw; E_1 and E_2, stimulating electrodes. See Material and Methods. (Bottom left) Volume change evoked by a single shock (top trace) and simultaneously recorded postsynaptic potential (bottom trace); the vertical marker represents an increase of 10^{-4} ml; the amplitude of the potential change was 36 V. (Bottom, right) Summation of volume changes evoked by multiple shocks (5 Hz) applied to the nerve.

Almost all the measurements were carried out at room temperature, 21-22°. On several occasions, volume increases were recorded from electric organs kept at temperatures between 4 and 11°C. Photographic records of the volume changes in the electric organ were taken usually after averaging 2-20 responses to electric shocks repeated at about 4 s intervals.

RESULTS

Figure 1, left, shows an example of the records obtained by the "capacitance" method of detecting rapid volume changes in a block of the *Torpedo* electric organ (upper traces) associated with the production of a postsynaptic potential (lower traces). It is seen that a brief electric shock delivered to the nerve entering into the organ evoked a rapid increase in the volume of the organ. This volume increase started almost immediately or a fraction of ms after the onset of the electric response. In most preparations, the volume reached its maximum at about the end of the postsynaptic potential. The return of the volume to its resting level was very slow and its time course was not always exponential. When the organ responded to a single shock with multiple responses, there was a transient increase in the volume corresponding to each of the responses. When the time course was smooth and roughly expo-

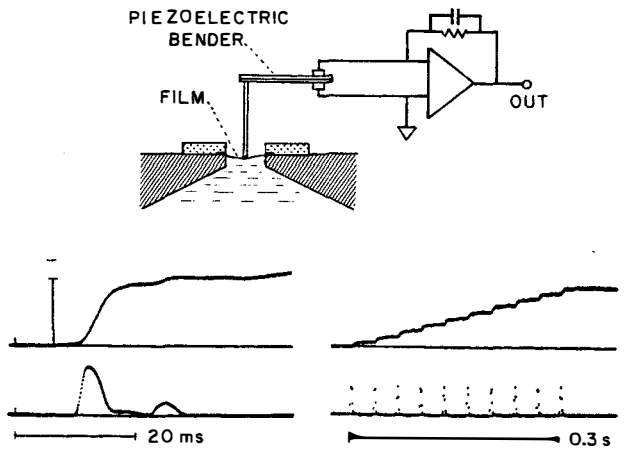

FIG. 2. (Top) Diagram illustrating the "piezoelectric" method of measuring volume changes in the electric organ. Volume changes are detected by recording pressure changes in the plastic chamber in which a block of electric organ is enclosed. Only the setup above the lid of the chamber is shown. See Material and Methods. (Bottom) Examples of the records obtained. Calibration, 2×10^{-4} ml. Postsynaptic potentials, 45 V (left) and 16 V (right).

nential, the time required to fall to half the maximum level was usually between 100 and 500 ms. Reflecting this slow return, there was a summation of volume changes when multiple shocks were delivered to the nerve (Fig. 1, right).

Two examples of the records obtained by the "piezoelectric" method are furnished in Fig. 2. Again, the rapid increase in the volume evoked by a single shock took place during the period between the beginning and the end of the postsynaptic potential. The recovery of the volume was very slow and variable. Even in the absence of multiple postsynaptic potentials, a considerable temporal variation in the rate of fall of the pressure inside the chamber was frequently observed.

At the end of the postsynaptic potential evoked by a single shock, the volume increase determined by the capacitance method at room temperature was $(3.3 \pm 2.2) \times 10^{-5}$ ml (17 preparations). The corresponding values obtained by using the piezoelectric method was $(2.3 \pm 1.6) \times 10^{-5}$ ml (25 preparations). This volume expansion was found to become smaller when the temperature of the organ was lowered. In the range of temperature of 5-11°C, most of the values obtained from 7 preparations were between 0.3 and 1.0 times 10^{-5} ml.

The difference between the values obtained by the two different methods of detection may partly be due to few small air bubbles remaining in the chamber. The large variation in the magnitude among different electric organ preparations may be attributed to (*i*) a variation in the size of the excited portion in the block, (*ii*) injury inflicted upon the organ before and during dissection and (*iii*) the difference in the time elapsed from dissection to measurement.

In the experiments mentioned above, the production of a postsynaptic potential was accompanied by an electric current passing through the electric organ. In the experiments stated below (Fig. 3), volume measurements were carried out under the conditions where there was practically no current through the organ. Record A was taken from an organ preparation from which the covering skin had been removed beforehand. Record B was obtained from a preparation with intact skin. In these experiments, the organ was immersed completely in mineral oil. The magnitudes of the volume changes observed were well within the range described above. Record C was taken from a slice held in a plastic frame that was in direct contact with the chamber wall (see Methods); hence, no current could pass through the slice during excitation. The magnitude of the volume change was roughly 1/20 of the range described above. This is expected from the size of the organ slice used.

The blocks of the electric organ employed most frequently in the present study were between 60 and 80 ml in size. Unquestionably, 70-80% of this volume is taken up by the inert viscous fluid separating individual electrocytes (6,7,10) and the skin. [Note that the fresh tissue consists of about 92% of water (11).] Thus, the observed volume change evoked by a single shock represents an expansion of roughly 10^{-6} times the electrocyte volume.

DISCUSSION

It is asserted at the outset that the observed volume change is not merely a manifestation of the thermal expansion of the organ. Quite recently, the temperature rise in the organ slice evoked by application of a single electric shock was shown to be 0.2-0.7 mdeg (13). In the present study using skinned electric organ-nerve preparations, almost identical results, a rise of 0.3-0.6 mdeg at the bare organ surface, were obtained. Combining this result with the thermal expansion coefficient of water, 0.21×10^{-3} per deg at 20°C, it appears

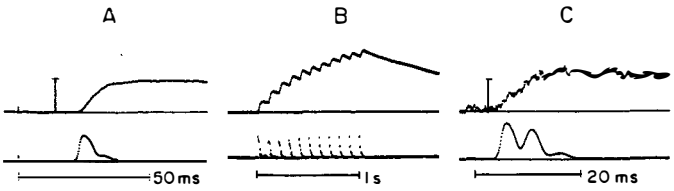

FIG. 3. (A) Volume change evoked by a single shock applied to the nerve entering into a skinned block of electric organ immersed in mineral oil. Calibration, 10^{-5} ml; postsynaptic potential, 32 V. (B) Summation of volume changes recorded from a block of the organ (covered by skin) immersed in mineral oil. Stimulus frequency, 10 Hz; postsynaptic potentials, roughly 20 V in amplitude. (C) Volume change in a slice of electric organ evoked by a single shock, 0.05 ms in duration and 14 V in amplitude, applied directly to the slice. Calibration 10^{-6} ml; postsynaptic potential, 4.5 V.

that the volume expansion attributable to the temperature rise in the organ is not larger than 0.9×10^{-5} ml which is about 1/4 of the observed value.

It was shown quite recently that the mechanical change in the electric organ evoked by a single shock is diphasic, representing the occurrence of swelling followed by shrinkage of the organ (13). The volume change described in this communication showed no clear sign of diphasicity. Since the swelling of the organ is considered to represent translocation of water from the inert fluid phase in the organ to the electrocyte protoplasm (14), this process (by itself) is not expected to bring about an expansion of the total volume of the organ. It is well known that, when a Ca-salt solution is mixed with a solution of anionic macromolecules in their Na^+-or K^+-rich form, there is an increase in the total volume of the mixture, resulting from rearrangements of water molecules associated with the binding of Ca^{2+} (15). Therefore, the experimental findings described above appear to indicate that there is, during the postsynaptic potential, an enhanced influx followed by rapid binding of Ca^{2+} to the anionic sites in the electrocyte protoplasm. The recovery of the volume undoubtedly involves biochemical processes that are essentially inaccessible by the methods employed in the present study. Further studies will be required to elucidate the origin of the volume expansion described in this communication.

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