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MECHANICAL AND THERMAL CHANGES IN THE *TORPEDO* ELECTRIC ORGAN ASSOCIATED WITH ITS POSTSYNAPTIC POTENTIALS

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Summary: Rapid mechanical and thermal changes in slices of the *Torpedo* electric organ evoked by electric stimulation were investigated. The organ was found to swell simultaneously with the postsynaptic potential. This swelling was followed by prolonged shrinkage of the organ. These findings may be explained from the proven facts that the acetylcholine(ACh)-receptor proteins have a large binding capacity for Ca-ions and that addition of ACh to the medium can cause release of Ca-ions from the receptor proteins.

The electric organ of *Torpedo* is well suited for studying the process of synaptic transmission because of its regularity in structure and richness of cholinergic innervation (1-3). The organ is made of a large number of closely packed prismatic columns which extend vertically from the ventral skin to the dorsal skin. Each column, 2 to 5mm in width, consists of 500 - 1000 superposed flat (5 to 10μm thick) functional units, electrocytes, separated by layers of inert viscous fluid (1,4). These electrocytes are not capable of producing regenerative action potentials (5,6). Electric shocks delivered directly to the organ excite only nerve fibers and nerve terminals, so that electric responses evoked by the shocks represent a summation of postsynaptic potentials generated across the receptor-rich (ventral) membranes of the electrocytes (3).

In this communication, we demonstrate that the *Torpedo* electric organ responds to applied electric shocks with rapid mechanical and thermal changes in the organ. In our preliminary experiments, we have employed large lumps of the organ covered with the skin. We found it relatively easy to record, from such nerve-electric organ preparations, large mechanical and thermal responses to nerve stimulation; however, the records obtained did not give much useful information about the processes occurring in individual electrocytes. In this

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communication, records of mechanical and thermal changes taken from slices of the electric organ are presented.These records demonstrate that the production of an electric response is invariably associated with rapid swelling followed by prolonged shrinkage of the electrocytes. The electric response was shown to be associated also with rapid production of heat followed by slow absorption of heat. A reasonable explanation of these findings is offered based on the facts that the acetylcholine(ACh)-receptor proteins have anionic sites which possess a great binding capcity for Ca-ions (7,8) and that the binding of ACh causes release of Ca-ions from the proteins (9,10).

MATERIAL AND METHODS

Specimens of *Torpedo californica*, 30 to 50cm in diameter, were obtained from Aquatic Research Consultants, CA. After fish were anesthetized by pipetting a 0.15% tricaine methane sulfonate (U.S. Biochem. Corp.) seawater solution into the spiracles (6,11), the electric organs were dissected out together with the covering skin and were then cut transversely into pieces of 2 to 4cm in width. Next, using a tissue-slicer with its large blade moving parallel to the skin, roughly 3mm thick slices of the organ were prepared. These slices were stored in a large volume of aerated and cooled elasmobranch saline solution, of which the composition was (12): 200mM NaCl, 8mM KCl, 1.8mM $MgCl_2$, 3.4mM $CaCl_2$, 5mM $NaHCO_3$, 5.5mM glucose, 300mM urea, 100mM sucrose and 9mM Tris-buffer (pH 7.3).

Changes in the pressure in the electric organ slice, evoked in response to direct electric stimulation, were measured by use of a piezoelectric bender (G-1195, Gulton Industries, N.J.). A piece of slice was placed on a plastic plate provided with two pairs of Ag-AgCl electrodes, one pair for stimulating the slice and the other pair for recording the electric responses (see Fig.1). By the aid of a rack-and-pinion device, the bender was lowered from above and the stylus at the tip of the bender was brought in contact with the surface of the slice. After lowering the stylus 0.2 to 0.5mm below the point of initital contact, the slice was maximally stimulated by delivering a 1ms-long voltage pulses (up to 30V across the electrodes separated by about 25mm). In recording these mechanical responses, precautions were taken to reduce artefacts arising from small horizontal movements of the slice evoked by the stimulating shock (prior to the onset of the response).

Rapid changes in the length of the electric organ slice were recorded by use of a small (35mm long) aluminum lever in conjunction with a dual light-guide (Fig. 2). A slice was trimmed into a piece of about 8mm in width and 25mm in length, which contained two rows of prismatic columns.The slice was introduced into a small plastic chamber (14x25x40 mm^3 in size).The lower end of the slice was fixed to the bottom of the chamber by means of a T-shaped clamp; the upper end of the slice was fastened to a small piece of "Velcro" and was connected, using a thin thread, to the mobile end of the lever. The major portion of the elasmobranch saline solution in the chamber was replaced with a high-density oil (Fluorinert, Sigma Chem. Co.). Direct electric stimulation of the slice was accomplished by using two Ag-AgCl electrodes, one placed in the saline on the top of the chamber and the other attached to the clamp at the bottom of the chamber. Movements of the lever were determined by recording changes in the intensity of the light reflected by the shiny aluminum surface at the end of the lever. The procedure of calibrating this device has been previously described (13). In a series of observations, changes in the tension of the slice evoked by direct stimulation was measured by connecting the thread at the end of the slice to a piezoelectric bender and applying an initial tension of about 0.5g.

Thermal responses of the electric organ slice were recorded by use of the detector employed in our studies of heat production in the eel electric organ

(14). Note that both the polyvinylidene-fluoride(PVDF) heat-sensor and the operational amplifier of the detector used (see Fig.3) were shielded from the electric field produced by the electric organ by inserting a 5μm-thick layer of stainless-steel (covered with a 2μm-thick poly-carbonate film) between the electric tissue and the PVDF sensor. The half-maximum response time of the detector, determined by using a brief pulse of Joule heat, was 2 to 4ms.

All the measurements were carried out at room temperature (21 to 22°C).

RESULTS

In Fig. 1 is presented an example of the records showing changes in the pressure in the slices of *Torpedo* electric organ evoked by direct stimulation. It is seen that the time-course of the observed change was diphasic. The first phase, which started simultaneously with the electric response, represented a rise in the force tending to push the foot-plate of the pressure sensor upwards. This was followed by a phase during which the pressure fell below the initial level. The temporal relation between the observed electric and mechanical responses was reminiscent of that seen in giant axons of the squid (15). The magnitude of the initial rise in pressure was usually in the range 0.6 and 2.5 dyn/cm^2; its fall in the second phase was usually 3 - 20 dyn/cm^2. These pressure changes were found to vary, to some extent, with the initial pressure applied to the slice. Note that, under these conditions, the stylus of the pressure sensor is roughly perpendicular to the surface of the electrocytes in the slice.

Fig. 2 shows mechanical changes in the electric organ slice measured in the direction parallel to the surface of the electrocytes.The upper record in the figure was taken by delivering a single maximal stimulating shock to the slice. Again, the time-course of the observed change was diphasic, the first phase representing, in this case, an increase in the length of the slice. The lower record was taken by delivering 6 shocks at about 25ms

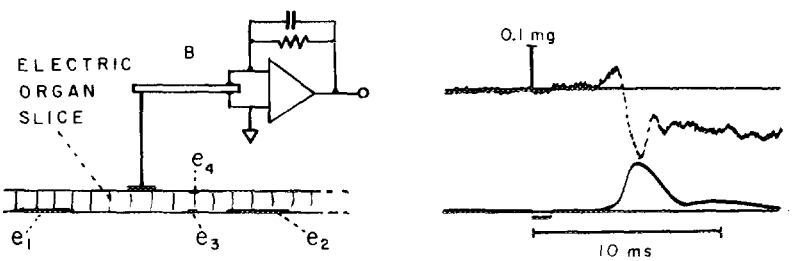

Fig. 1. Left: Experimental setup employed for recording pressure changes in the electric organ slice evoked by direct stimulation. B represents a piezoelectric bender; e_1 & e_2, stimulating electrodes; e_3 & e_4, electrodes for recording electric responses. The foot-plate at the end of the bender stylus was about 3mm in diameter. Right: Pressure changes (top) and postsynaptic potential (bottom) evoked by a single stimulating shock. The amplitude of the potential change observed was 5.7V.

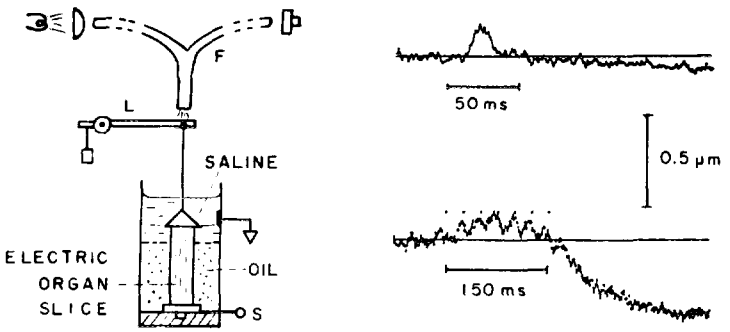

Fig. 2. Left: Setup employed for recording length (isotonic) changes in the electric organ slice evoked by direct stimulation. F represents dual light-guide; L, lever; S, terminal for delivering electric shocks to slice. Right: Records of mechanical responses evoked by single shock (top) and by multiple shocks (bottom). The upward deflections of the trace represent elongation of the slice.

intervals; it is seen that there was a pronounced summation of shortening of the slice during the second phase of the response. At the peak of the response to a single shock, the increase in the length of the slice was 0.15 - 0.9μm. Following repetitive stimulation, a shortening of the slice of greater than 1μm had been encoutered. The results obtained by recording changes in tension of the slice under isometric conditions (see Methods) were quite consistent; the decrease of the tension evoked by a singe shock --corresponding to the elongation of the slice under isotonic conditions-- was in the range 0.1 - 0.4dyn.

Fig. 3 shows an example of the records of temperature changes in the slice evoked by direct stimulation. It is seen that the generation of an electric response was accompanied by a rapid rise in the temperature of the slice. This rise was followed by a prolonged period of temprature fall (active cooling) of the slice. The maximum temperature rise observed was usually in the range between 0.2 and 0.7mdeg.

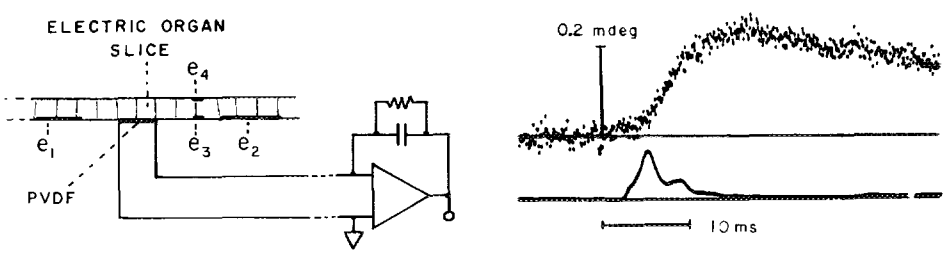

Fig. 3. Experimental setup used for recording temperature changes in the electric organ slice evoked by direct stimulation (left) and an example of the records obtained (right). PVDF represents heat-sensor. The amplitude of the potential change recorded was 2.4V. Note that the Joule heat generated by the stimulating current pulse is very small.

DISCUSSION

The experiments described under Results show that, during the first phase of the mechanical response, the electric organ slice *expands* in the directions both perpendicular and parallel to the surface of the slice. These findings strongly suggest that the generation of a postsynaptic potential in the slice is associated with *swelling* of the electrocytes.

These experimental findings may be interpreted based on the theory of synaptic (chemical) excitation proposed previously by Neumann, Nachmansohn and Katchalsky (9,10). In fact, Eldefrawi et al. (7), Chang and Neumann (8) and others have shown that the isolated acetylcholine(ACh)-receptor proteins have a great binding capacity for Ca^{2+} and also that addition of ACh to a Ca^{2+}-receptor protein solution causes release of Ca^{2+} from the proteins. We now know that, in anionic gels immersed in a salt solution containing both Ca^{2+} and Na^{+} at an appropriate mole ratio, addition of ACh to the solution causes release of Ca^{2+}, resulting in a rise of the water content of the gel (see ref.16). The process of release of Ca^{2+} bound to anionic sites in biopolymers into an aqueous medium is exothermic.It seems therefore that the experimental results described in this communication render strong support to the above-cited theoory of synaptic excitation.

A futher study of the mechanical changes in the *Torpedo* electric organ is required to elucidate the mechanism of synaptic transmission.

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